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ABSTRACTS

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Plenary Lecture 1

MINIATURIZED MID-INFRARED SENSORS - HOW SMALL IS STILL USEFUL?

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State-of-the-art biodiagnostic platforms increasingly take advantage of miniaturized and integrated sensing and assay technologies ideally providing direct access to molecule-specific information. With point-of-care and personalized medicine becoming more prevalent, detection schemes that do not require reagents or labeled constituents facilitate on-site analysis providing close to real-time information, or may be used in a continuous monitoring mode e.g., in intensive care scenarios.

However, decreasing the analytically probed volume may adversely affect the analytical figures of merit such as the signal-to-noise-ratio, the representativeness of the sample, or the fidelity of the obtained analytical signal. Yet, probing minute samples volumes facilitates rapid analysis, minimally intrusive or even non-invasive sampling, and a rapid diagnostic response. Consequently, the guiding paradigm for the miniaturization of diagnostic devices should be creating analytical platforms that should be as small as still useful, rather than as small as possible by smartly balancing the benefits and disadvantages of small device dimensions.

Optical/spectroscopic sensor technology taking advantage of the mid-infrared (MIR) spectral range (3-20 µm) is increasingly adopted in bioanalytics due to the inherent molecular specificity enabling discriminating constituents at ppm-ppb concentration levels in condensed and vapor phase media. Recently emerging strategies take advantage of innovative waveguide technologies such as MIR transparent hollow waveguides and planar semiconductor waveguide structures in combination with compact FT-IR spectrometers or highly efficient broadly tunable quantum cascade lasers, thereby facilitating highly miniaturized yet robust MIR diagnostic platforms providing sensitive and selective access to (bio)molecular signatures [1-7].

Selected application examples and novel optical sensing strategies will highlight the most recent advances in miniaturized IR sensor technology toward establishing next-generation optical sensing platforms in biodiagnostics.

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Plenary Lecture 2

DIVING DEEP INTO THE CHEMISTRY OF THE HUMAN BRAIN

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During the last decade we have been able to probe deeper and deeper into the chemistry of the human brain. One of the windows into the living brain is the analysis of the fluid surrounding the central nervous system – cerebrospinal fluid obtainable by lumbar puncture. We have in collaboration with other groups managed to map the soluble protein content in this fluid both in health and disease and currently hold the world record in this field. We see an enormous complexity in the analytical information obtained but also appreciate the important biological and clinical leads that are generated from such initiative. The analytical tools developed and tailored for the investigations are also in themselves generating technology leaps useful for any other area dealing with complex samples.

We are currently exploring novel technology for the isolation and enrichment of the non-soluble portion of the proteome – the membrane bound proteins from various tissues. Among the methods developed in-house a temperature induced phase fractionation also known as cloud-point extraction (CPE) with the non-ionic surfactant Triton X-114 has been used to simultaneously extract hydrophobic and hydrophilic proteins from brain tissue. Various protein precipitation/delipidation procedures have been investigated to efficiently remove lipids and detergents, while retaining maximum protein recoveries. Different “bottom up” proteomic approaches for protein analysis of the brain have been compared, i.e. 1D-gel electrophoresis followed by in-gel digestion and reversed phase nanoliquid chromatography (RP-nanoLC) followed by electrospray ionization (ESI) high-resolution tandem mass spectrometry (MS/MS) analysis or alternatively direct digestion of the proteins followed by RP-nanoLC separation in combination with matrix assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF/TOF MS), or as an alternative the combination of in-solution high-resolution isoelectric focusing (hr-IEF) with ESI-nanoLC-MS/MS of the IEF separated peptides. The results indicate that cloud point extraction is a very promising sample preparation tool, which allows simultaneous in depth studies of brain derived membrane proteins as well as other hydrophilic proteins. This panel of techniques can be very useful when studying human central nervous system (CNS) tissue or animal models of neurological diseases.

Plenary Lecture 3

METABOLOMIC FINGERPRINTING / PROFILING EMPLOYING HIGH RESOLUTION MASS SPECTROMETRY: A CHALLENGING STRATEGY IN FOOD ANALYSIS

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At the time of its emergence, metabolomics was mainly viewed as an advanced, specialized tool of analytical biochemistry enabling innovative research on plants and other organisms. Recently, this “omics” strategy centred around detection of the broadest possible range of small molecules (<1500 Da) in complex biological matrices, using a single or small number of analyses, has also emerged as a field of interest in food analysis. Within the research conducted at the ICT, metabolomics is used either for “fingerprinting” of food samples to perform comparative analyses aimed at detection of differences or for “profiling” in which individual, differential sample components (including secondary components originating during food processing) are identified for further analyses. In addition to food quality assessment / safety control, authentication is another challenging application area. Mass spectrometry (MS) represents one of the key techniques employed for obtaining comprehensive information on metabolites contained in respective matrix; moreover, identification of marker compounds is possible when needed. In any case, sophisticated chemometric tools are applied for handling of generated data and development of relevant models for sample classification. In most studies, either liquid (LC) or gas chromatography (GC) is coupled with MS. Recently, ambient mass spectrometry employing novel ion source - Direct Analysis in Real Time (DART) hyphenated with high resolution mass analyzers such as high resolution time of flight (HRTOF) or Orbitrap has become a challenging option for high throughput food analyses. In this presentation, various examples metabolomic-based food analyses will be demonstrated.

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Plenary Lecture 4

PEPTIDE AND OLIGONUCLEOTIDES APTAMERS AS NEW LIGANDS FOR ANALYTICAL CHEMISTRY

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So far, several bio-analytical methods have used nucleic acid probes to detect specific sequences in RNA or DNA targets through hybridisation. More recently, specific nucleic acids, aptamers, selected from random sequence pools, have been shown to bind non-nucleic acid targets, such as small molecules or proteins. The development of *in vitro* selection and amplification techniques has allowed the identification of specific aptamers, which bind to the target molecules with high affinity. Many small organic molecules with molecular weights from 100 to 10000 Da have been shown to be good targets for selection. Moreover, aptamers can be selected against difficult target haptens, such as toxins or prions. The selected aptamers can bind to their targets with high affinity and even discriminate between closely related targets.

Aptamers can thus be considered as a valid alternative to antibodies or other bio-mimetic receptors, for the development of biosensors and other analytical methods. The production of aptamers is commonly performed by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process, which, starting from large libraries of oligonucleotides, allows the isolation of large amounts of functional nucleic acids by an iterative process of in vitro selection and subsequent amplification through polymerase chain reaction.

Aptamers are suitable for applications based on molecular recognition as analytical, diagnostic and therapeutic tools. In this review, the main analytical methods which have been developed using aptamers, will be discussed together with an overview on the aptamer selection process.

Recently Aptamers from peptides libraries have been realized with similar techniques in order to obtain structures with much varied receptors points and higher analytical capabilities.

Some experimental results will be reported and discussed.

S1: Bioanalysis I

Keynote Lecture 1

SELECTED APPLICATIONS OF VIBRATIONAL SPECROSCOPY IN BIOCHEMICAL ANALYSIS

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In this presenation, selected new developments for biochemical analysis based on vibrational spectroscopic detection methods will be discussed. Infrared and Raman spectroscopies provide direct molecular specific information. In combination with other enabling technologies such as microfluidic systems, standing ultrasound waves and separation systems, vibrational spectroscopy can facilitate novel approaches with which to study biochemical interaction processes, perform in-line bioprocess monitoring and for the sensitive identification of separated biomolecules.

In the first example, a microfluidic mixing device has been used, in conjunction with infrared micro-spectroscopy, to study the duplex formation between two single stranded RNA molecules, Poly I and Poly C in the aqueous phase in the sub and low ms time range. Spectral changes accociated with ring stretching vibrations, as well as the sugar-phosphate backbone were observed and show completion of duplex formation, as indicated by the A-helical marker band, is complete by 0.36 ms.

The second example shows how standing ultrasound waves can be used, in combination with a fibre optic ATR probe, to selectively measure spectra of micro-organisms (yeast cells) in fermentation broth. This can be achieved by controlled manipulation of the micro-organisms in front of the planar ATR element of the fibre optic probe, allowing separate measurement of cells and surrounding solution.

Finally, using a novel SERS (surface enhanced Raman scattering) substrate, based on silver colloids and ZnS capped CdSe quantum dots, nucleic acid bases were selectively detected at low concentrations after having been separated with a capillary liquid chromatography system. A flowthrough microdispenser was employed as an interface between the cap-LC system and the SERS substrate, thus allowing optimum conditions for both separation and detection.

ANALYTICAL NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY *IN VIVO*: QUANTIFICATION OF ALCOHOL-INDUCED CHANGES OF BRAIN METABOLITE LEVELS IN MICE

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The non-destructive nature of nuclear magnetic resonance (NMR) makes it one of the most versatile methods in biomedical research and clinical medicine. Whereas NMR imaging (MRI) is an indispensable tool in medical diagnostics, the analytical ability of *in vivo* high-resolution NMR spectroscopy (MRS) - quantitative analysis of metabolite levels and turnover rates - is less known. Here we present one use of volume selective, high-resolution, proton NMR spectroscopy for quantitative analysis of brain metabolites in live mice. We quantified various brain metabolites in the nucleus accumbens, which regulates addictive behaviors, during withdrawal from chronic ethanol vapor inhalation as well as following acamprosate (Campral), a clinically used medication to assist alcoholic patients maintain abstinence. Ethanol withdrawal significantly increased Glx (glutamate+glutamine), glutamate, glutamine, *N*-acetylaspartic acid and lactate in the nucleus accumbens compared to basal levels in wild-type mice. Interestingly, acamprosate treatment (i.p.; 400 mg/kg/day) for 5 consecutive days during ethanol withdrawal normalized the altered metabolite levels in the nucleus accumbens. This study could be useful to better understand the molecular basis of alcohol use disorders and to determine the pharmacological effect of acamprosate or other clinically available medications.

CHARACTERIZATION OF IN VITRO METABOLIC PROFILES OF CINITAPRIDE OBTAINED WITH LIVER MICROSOMES OF HUMANS AND VARIOUS MAMMAL SPECIES USING UPLC AND CHEMOMETRIC METHODS FOR DATA ANALYSIS

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Metabolism studies are important in preclinical development of new drug candidates to check the occurrence of possible toxicological effects and the potential pharmacological activity of metabolites. The rich data resulting from chromatographic and spectroscopic measurements are often treated using mathematical tools as a way to obtain more feasible information from complex metabolic systems.

In this communication, an interspecies study is presented comparing the metabolic profiles of cinitapride (4-amino- N- [1-(3-cyclohexen-1-yl-methyl) -4-piperidinyl] -2-ethoxy -5-nitrobenzamide). Cinitapride is a gastrointestinal stimulant with prokinetic activity, developed and marketed in Spain and Mexico by Almirall, S.A. The drug is used to treat dyspepsia and gastroesophageal reflux disease. The biotransformation of this molecule is complex. It is mainly metabolized to one dihydroxylated and two hydroxylated molecules, although a large number of minor metabolites are also formed.

In this study, an ultra-high pressure liquid chromatographic method has been utilized to obtain the metabolic profile of cinitapride in humans and various mammal species such as rats, mice, mini pigs, dogs and monkeys. Metabolites have been generated incubating cinitapride with hepatic microsomes and using NADPH as a cofactor. Cinitapride and its metabolites have been separated by reversed-phase mode using ammonium formate aqueous solution (pH 6.5) and acetonitrile as components of the mobile phase. Concentrations of metabolites in the incubation samples have been utilized as multivariate data to extract metabolic information. Statistic parameters and principal component analysis have been used to compare the in vitro metabolism of humans with the other species. Conclusions on correlations between metabolites and species have been also obtained.

NON-LINEAR ANALYSIS IN METABOLOMICS DATA OF MUTIPLE SCLEROSIS DISEASE

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The composition of biofluids carries invaluable information about the biochemical status of a living organism. Cerebrospinal Fluid (CSF) is the biofluid, which is in closest interaction with the Central Nervous System (CNS). It is therefore the biofluid that best mirrors the biochemical status and processes in brain and CNS. The chemical composition of CSF may thus provide insights about metabolic pathways in the CNS. The comprehensive analysis of CSF may define the fingerprint of neurological diseases such as the Multiple Sclerosis (MScl).
Our objective is to detect molecular biomarkers for MScl in CSF. For this an innovative approach, i.e. ^{1}H Nuclear Magnetic Resonance (^{1}H -NMR) spectroscopy in combination with chemometric analysis was used.
The research results presented here are obtained from a clinical study on MScl. We examined the human CSF metabolomics profile of MScl group. It was then compared to group representing the pre-stage of MScl, i.e. clinically isolated syndrome of demyelination (CIS). This human dataset is very complex, because the biological variations and environmental variations are comparable in size (or even larger) than the variance of interest. The challenge is then to still extract the relevant information, which requires special, more sophisticated approaches than linear methods. For this purpose we have applied a kernel-PLS-DA. In addition we implemented variable selection procedure which is based on SVM Recursive Feature Elimination for nonlinear kernel function [1]. A novel method presented by Krooshof *et al.* [2] has been applied for variables visualization and interpretation in K-PLS-DA space. Using this complete procedure we obtained discrimination between MScl group and CIS group with high prediction accuracy and identified metabolites responsible for class separation.

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SENSORS FOR BIOACTIVE COMPOUNDS

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Sensors have found many applications in pharmaceutical, clinical and environmental laboratories and are being explored for numerous other applications due to low cost and short time of analysis.

In this study, the construction and general performance characteristics of potentiometric plastic-membrane sensors for amino acids, antibiotics and neurotransmitters are described. Borane anions are useful materials able to form ion-pair complexes with nitrogen containing compounds. The chemical compositions of the compounds obtained were elucidated by: NMR-^{1}H, ^{11}B, ^{13}C, MALDI-TOF-MS, FTIR.

Also, a comparative study between ion selective electrodes and carbon paste electrodes obtained for bioactive compounds was performed.

The properties of the prepared electrodes were studied, slope, concentration range, detection limit, lifetime, and selectivity.

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A NOVEL BIOSENSOR FOR THE DETECTION OF *CHLAMYDOPHILA ABORTUS* IN VETERINARY DIAGNOSTICS

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One of the biggest challenges in the field of veterinary diagnostics is the on-site analysis of disease relevant parameters.
Only with on-site analysis, the early detection of pathogenic agents can be guaranteed. This is a crucial criterion in preservation of healthy animal stocks.
Chlamydophila abortus, an obligate intracellular pathogen, is one of the main causes of Ovine Enzootic Abortion (OEA) and leads to massive financial losses in sheep farming countries.
C. abortus is transmitted by sexual contact and feces and spreads within a herd undetected until the first abortions. To avoid herd-wide expansion after onset, a fast and cost-effective monitoring of chlamydial infection status is essential. [1]
We face this challenge using a label-free biosensor based on Reflectometric Interference Spectroscopy (RIfS). RIfS is a direct optical, time resolved method, which measures interference of polychromatic light caused by reflection at thin layers. The binding of an analyte (e.g. antibody) to a sensor surface immobilized ligand (e.g. pathogen) changes the interference spectra and thus can be quantified. For on-site applications, RIfS can be easily miniaturized and automated. [2]
To validate this approved tool in veterinary diagnostics, we developed a RIfS based bioassay sensitive on *C. abortus* antibodies.
The new assay uses surface-immobilized chlamydial LPS as ligand and was fully characterized and calibrated in buffer. Thereby, we achieved high sensitivity to chlamydial antibodies with LOD of 0,71 mg/L and LOQ of 2,44 mg/L. Recovery rates were between 97 % and 120 %. The sensor was used more than 50 times and stored for at least a week at room temperature without loss of sensitivity. This confirms the assay as an ambitious solution for on-site Chlamydia monitoring.
To accomplish this goal, measurements in full blood samples and on-site will be performed.

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Keynote Lecture 2

THE USE OF FUSED SILICA CAPILLARIES AS VERSATILE TOOLS FOR ELECTROANALYSIS AND SEPARATION TECHNIQUES HYPHENATED TO MASS SPECTROMETRY

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Fused silica capillaries are well established in gas chromatography and capillary electrophoresis (CE) and can readily be connected to various injection and detection systems without introducing significant band broadening. Capillaries with inner diameters (ID) down to 2 µm are commercially available and enable investigations of very small sample volumes. However, the use of very small ID capillaries is challenging regarding the implementation of suitable detectors with appropriate detection performance. In this contribution several approaches for the use of small ID capillaries in conjunction with electrochemical detection (ED) and mass spectrometry (MS) are presented. In addition, more complex hyphenated systems integrating electrochemistry – capillary electrophoresis – mass spectrometry are discussed. Ewing and co-workers have first managed to perform CE-ED measurements using narrow bore capillaries of less than 10 µm [1]. The detection concept was based on the use of an etched carbon fibre electrode matching an etched capillary. Recently we have shown that a simple end-column detector arrangement incorporating a microdisk sensing electrode can be used in conjunction with 2 µm ID capillaries and enables limits of detection in the sub-µM range [2]. In terms of versatility of microfluidic concepts it is attractive that short pieces of fused silica capillaries can be used to achieve very fast CE separations under conditions of high electrical field strengths. In the context of CE-MS measurements field strengths up to 1.25 kV/cm could be applied resulting in separations of catecholamine model compounds within 20 s [3]. In addition, it will be shown, that capillaries with small ID of 15 µm allow for the use of nonconventional electrolytes and can lead to very efficient CE-MS separations. The concept of electrochemically assisted injection (EAI) enables the CE separation of charged product species formed electrochemically from neutral analytes. This approach is successfully coupled with MS [4]. Selected examples will illustrate that EAI-CE-MS can be used as a novel means for the CE separation of neutral analytes with full compatibility with electrospray ionization (ESI) MS. In addition, the performance of ESI-MS can be considerably improved in case of hydrophobic compounds. A semi-automated EAI system was constructed and optimized with the help of scanning electrochemical microscopy [5].

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NEW ELECTRODE MATERIALS IN ENVIRONMENTAL ELECTROANALYSIS

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The development of new electrode materials resistant to passivation, with low noise and broad potential window, mechanically robust for field measurements and/or measurements in flowing systems and with easy renewable surface (mechanically or electrochemically) is one of the priorities of modern electroanalytical chemistry. In this contribution, several non-traditional electrode materials and their application in environmental analysis will be discussed.

For electrochemically reducible organic substances of environmental importance (chemical carcinogens, pesticides, etc.) various types of amalgam electrodes (solid amalgam electrodes, amalgam paste electrodes, single crystal amalgam electrodes etc.) can be successfully used in micro and submicromolar concentration range. Their possibilities and limitation together with some practical application will be discussed together with their application for amperometric detection in flowing systems (HPLC ED and FIA ED). Attention will be paid to their pretreatment ad surface renewal (mechanical and electrochemical).

Boron doped diamond film electrodes can be used for detection of both electrochemically reducible and electrochemically oxidisable compounds because of extremely broad potential window, low noise and negligible tendency for passivation. Again, their application in flowing systems and their combination with preliminary separation and preconcentration techniques in the filed of environmental electroanalysis will be discussed.

At the end, carbon paste electrodes, carbon film electrodes and screen printed electrodes, their advantages and disadvantages, and their application in environmental electroanalysis will be discussed.

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S2: Electroanalytical Chemistry I

ION-SELECTIVE ELECTRODE BASED OF MOLECULARLY IMPRINTED CORRIN FILM

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Molecular imprinting has drawn considerable attention because of its ability to create polymers with pore architectures that are complementary to the template used in the polymerization process [1]. The advantages of molecular imprinting in creating polymers capable of chemical recognition have been demonstrated in the field of chiral separations and in the generation of synthetic receptors [2]. The basis of this polymerization is the electrodeposition of a polymeric film on the surface of an electrode immersed in a solution of an appropriate monomer. Various anions can coordinate selectively to metallomacrocyclic complexes, and this interaction is dependent on the structure of the metallomacrocycle. Vitamin B_{12}, also known as cyanocobalamin, consists of a corrin ring system coordinated with cobalt. Different derivatives of Vitamin B_{12} have shown good selectivity when they were used as ionophores to prepare anion-selective electrodes [3, 4, 5]. For this purpose, a new pyrrole substituted vitamin B_{12} derivative, functioning as a corrin monomer, was synthesized and its electrochemical polymerization by using a molecular imprinting approach was performed. Electropolymerization of the corrin monomer and its redox properties have been performed in acetonitrile in the presence of 0.1 M tetrabutylammonium perchlorate on glassy carbon electrode by cyclic voltammetry. The effect of various parameters such as the potential range, scan rate, number of scans that control the film properties and the concentration of the monomer were investigated. The optimum conditions for potentiometric measurements were tested and it was found that the electropolymerized corrin films exhibited an anion selectivity pattern that deviates from the Hofmeister series. The obtained electrode based on a novel electropolymerized corrin film was found to recognize selectively thiocyanate ions and showed a Nernstian slope of -56.04±1.02 mV/pSCN for a period of at least 6 months.

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ANTIMONY ELECTRODES IN MODERN ELECTROANALYSIS

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Among advanced analytical techniques, electrochemical stripping analysis certainly represents a field of great importance, particularly due to its unique characteristic of pre-concentrating the analytes of interest in/on the working electrode. There were many attempts to replace mercury electrode, which has been used for this purpose in the last six decades, with some other appropriate electrode materials, e.g. Au, Pt, Ag, Ir, different modifications of carbon, etc., but none of them approached the excellent electroanalytical performance of mercury. In recent years, electrochemical stripping analysis has faced some successful suggestions addressing the aforementioned issue, starting with the bismuth film electrode (BiFE) [1] which has been, by now, accepted in numerous electroanalytical laboratories worldwide for measuring trace metal ions and several selected organic compounds. This electrode was followed by the introduction of antimony film electrode (SbFE) in 2007 [2] which also exhibits extremely interesting electroanalytical features, e.g. relatively wide operational potential window, favorable hydrogen evolution overvoltage and interestingly low signal for the re-oxidation of antimony itself, etc., thus paving, together with bismuth electrode, a broad avenue in modern electrochemical (stripping) analysis for performing measurements using "mercury-free" electrodes.

In this contribution a brief overview will be given about the antimony electrode in advanced (stripping) electroanalysis. The electroanalytical performance of different configurations of the antimony-based electrodes will be discussed in combination with voltammetric/potentiometric stripping protocols for measuring trace heavy metal ions. The antimony electrodes will be also critically compared to their bismuth and mercury counterparts and finally, the possibilities for their further development and applications will be outlined.

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VOLTAMMETRIC BEHAVIOUR AND DETERMINATION OF 8-HYDROXYQUINOLINE USING GLASSY CARBON PASTE ELECTRODE AND THE THEORETICAL STUDY OF ITS OXIDATION MECHANISM

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8-Hydroxyquinoline (8HQ) is often found as an environmental pollutant due to its widespread use in industry, medicine and agriculture. It is commonly used as a complexing agent in analytical chemistry, a pesticide, a preservative, a bactericide and disinfector in some cosmetics and a component of organic light-emitting diodes. The 8-Hydroxyquinoline (8HQ) oxidation process has been investigated by Cyclic Voltammetry using a Glassy Carbon Paste Electrode (GCPE) as a working electrode. The theoretical study of the mechanism of electrochemical oxidation of 8HQ has been based on the AM1 semi-empirical quantum chemical computations of the heats of formation of the reaction intermediates, taking into account the influence of pH and solvation effects. We proposed that a two-electron irreversible process, controlled by diffusion of electroactive species, is responsible for an oxidation peak of 8HQ that appears in all cyclic voltammograms recorded on a clean electrode in the solutions of pH in the range 2–12 with a supporting electrolyte of Britton-Robinson Buffer/methanol. A single-electron oxidation of 8HQ leads, depending on pH, to the formation of various free radical species that combine to make dimers which, after being oxidized once more, give quinonoid-type compounds. Recording continuous cyclic voltammograms on the GCPE, pre-peaks appear as a consequence of dimer and quinonoid compounds formation. By applying Differential Pulse Voltammetry for 8HQ determination it was calculated that the limit of detection was 5.2×10^{-8} mol/L. For more sensitive quantitative determination of the investigated substance Adsorptive Stripping Differential Pulse Voltammery can be used since it was found that after 300s-deposition time at 0.0 V, *vs.* Saturated Calomel Electrode, a 2.1 times higher peak current than without deposition was obtained.

S2: Electroanalytical Chemistry I

SQUARE-WAVE VOLTAMMETRIC METHOD for SIMULTANEOUS ASSAY of AMLODIPINE and VALSARTAN

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Amlodipine (AMD), (Fig.1), as calcium channel blocker, and valsartan (VAL), (Fig.1), as angiotension receptor blocker are used for the treatment of high blood pressure together. Simultaneous determination of AMD and VAL in various samples is of great importance. There are few analytical techniques for spectrophotometric and chromatographic determination of AMD and VAL in literature [1-4]. There is no voltammetric method for simultaneous determination for AMD and VAL although these methods are simple, sensitive, economic, easy to be operated and they require less chemicals than other methods.

Mixture of AMD and VAL gives two anodic peaks at 0.750 V and at 0.950 V *vs*. Ag/AgCl in Britton-Robinson buffer (BR) at pH 5.0 on glassy carbon electrode corresponded to oxidations of AMD and VAL, respectively. Therefore new, sensitive, simple, and reproducible square-wave voltammetric (SWV) method was developed for direct determination of binary mixture of AMD and VAL in different samples including pharmaceuticals, human serum and human urine based on these anodic peaks. The dynamic concentration ranges were found to be 1.0–35.0 μM for AMD and 1.5–32.0 μM for VAL with limit of quantification (LOQ) values as 2.5 μM for AMD and 2.2 μM for VAL. Proposed method was successfully applied to determine the AMD and VAL content of pharmaceutical preparations, spiked human serum and spiked human urine. Recovery values in such applications were found 96.2% – 99.4% for AMD and 97.8% – 100.4% for VAL. Relative standard deviations (N = 5) were found to be less than 10.0 % for all applications.

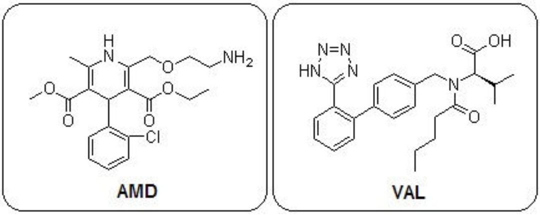

Fig. 1 Chemical structures of AMD and VAL

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S3: Education

Keynote Lecture 3

ANALYTICAL CHEMISTRY EDUCATION IN JAPAN

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The first Analytical Chemistry laboratory in Japanese University was established in 1918 in Tohoku University. Now, the education of Analytical Chemistry is recognized as a most fundamental and essential program in the undergraduate chemistry courses. In the recent twenty years, the need for the education of Analytical Chemistry is extending to the undergraduate education of Biology courses. Here, some trends in Japanese Analytical Chemistry education are reviewed.

1. Text book

Because undergraduate class and experiment are generally performed in Japanese, as in the cases of other courses, many Japanese textbooks on Analytical Chemistry more than 400 are currently available. A few English text books are translated into Japanese, but almost all text books are written by Japanese teachers of University.

2. Class and experiment of Analytical Chemistry

Education of Analytical Chemistry in undergraduate courses of Universities have been mainly carried out in six major faculties; science, engineering, agriculture, pharmaceutical science, dentistry and medicine. The subjects of Analytical Chemistry class include chemical equilibria in solution, electrochemical detection, spectroscopic analysis and analytical separation. Fundamental analytical techniques such as titration and photometry have been taught in all disciplines, but choice of advanced techniques has been dependent on the faculty. In general, analytical methods for metals and other inorganic compounds are major subjects in faculties of science and engineering. On the other hand, the analyses of biogenic substances are common in faculties of life science. However, the actual circumstances are greatly different depending on the university and the faculty. In case of Faculty of Science of Osaka University, the classes of Analytical Chemistry 1, 2 and 3 (each 90 min x 15 weeks) are prepared for the undergraduate students of Departments of Chemistry and Biology. Laboratory course in Analytical Chemistry is essential for all students of Chemistry.

ANALYTICAL CHEMISTRY EDUCATION IN THE NEW EU MEMBER STATES

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In this presentation the overview of the status of education in analytical chemistry in various European countries will be given, with a special attention to the new EU member states. The expectation of the society and the competence of those educated chemist being a results of the existing curriculum will be evaluated.

Analytical chemistry is a science allowing to understand the composition of matter and the mechanism of its transformation. It is hard to overestimate the importance of analytical chemistry to society, thus there is a big needs for the educated peoples in this area. The analytical chemistry is an interdisciplinary subject where the knowledge of basic law of chemistry, biology, physics and mathematics is required. On the top of this, analytical chemistry should be percept as a discipline which required problem solving approach, thus the analytical chemist must have a good theoretical knowledge and practical experience of various methods and instrumentation. Also the innovation and team work are consider to be a key components of the skilful analytical chemist's competence. The quality of the results in respect of its accuracy become an important issues over the last years and this include also needs to understand the specific requirements of accreditation. Therefore the higher education in analytical chemistry should posed a special concern.

Besides the analytical chemistry faculties, various specific aspects of chemical measurements are also included in the curriculum of faculties of e.g. biology, geology, medicine, pharmacy, environmental protection and engineering. All those disciplines which uses the information on the matter needs the contribution from analytical chemists. When concerning analytical chemistry, it is relevant to stress the importance of the upgrading the knowledge and competence, and for that purpose a number of long life learning coursed organised by universities have their place in educational package.

INCLUSION OF BIOANALYTICS INTO THE ANALYTICAL CHEMISTRY CURRICULUM

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In an analytical chemistry curriculum for chemists and chemical engineers one cannot expect the students to have the sufficient knowledge in biology and biochemistry to be deeply interested in studying biological systems. Nevertheless they have heard about genetic engineering, some aspects of medical technology and clinical chemistry, environmental pollution, etc. These are good connecting points for talking about bioanalysis to them.

In our department we have in recent years gradually included more and more bioanalytical topics in the analytical chemistry curriculum of chemical engineers. At the BSc level the course in analytical chemistry (4 week-hours of lectures and 4 week-hours of lab) we cover enzymatic methods of analysis and immunoassys in detail. In other, instrumental chapters, like electroanalysis or fluorescence we use many bio-related examples.

At the MSc level the students of chemical engineering have 2 week-hours lecture and 2 week-hours of lab in AC, where they get an overview of some of the main instrumental methods of analysis which were not covered at BSc level. Ca. 25% of the lectures is devoted to protein analysis by HPLC, MS and by some spectroscopies. These lectures are given by experts from industry.

About 20% of the students specialize in AC. They have ample opportunities to learn about bioanalysis both in their regular and special courses. Our specialty is a thorough compulsory course in biosensors.

At our faculty of Chemical Engineering and Biotechnology half of the student body is enrolled in bioengineering. They obtain a thorough knowledge in biology and biotechnology. Early in their BSc curriculum they take the AC course described above. Later they may specialize in food analysis or clinical chemistry.

TEACHING QUALITY MANAGEMENT AND METROLOGY IN ANALYTICAL CHEMISTRY – FIT FOR PROFESSION?

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Education in Analytical Chemistry (AC) aims at a target audience that is about to join the labor market. A great number of laboratory assistants, technicians and chemists finish their vocational training each year and enter into professional life. But are they well prepared for the demands of the market?

Generally spoken, lab assistants and technicians have received a sounder background in quality Management (QM) and metrology during their apprenticeship than academically trained chemists, who later on might be their bosses. A difficult situation occurs whenever a QM-system needs to be implemented or a lab strives for accreditation or certification. Training-on the-job is common practice to close this gap. Lifelong learning is a wonderful thing and a requirement for career planning and competition. But isn't it also our duty to offer young people an education that allows them a good start according to the needs of today?

A solution may be seen in making the subjects QM and metrology an essential (mandatory) part in teaching AC. This is not easy as universities usually do not run their research labs with a QM-system or are experienced in holding an accreditation against e.g. ISO 17025. So what part can universities contribute?

Teaching the teachers may be an option and thus enabling them to teach the students. Inviting professionals in and have them share their experiences another. Let's face it: teaching quality management and metrology at a theoretical level is quite a dry matter. How can the students' interest be drawn and maintained?

The presentation is intended to offer some answers to the questions raised.

GERMAN SPRING SCHOOL „INDUSTRIAL ANALYTICAL CHEMISTRY“

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At the Westfälische Wilhelms-Universität Münster, the Fachgruppe „Analytische Chemie“ of “Gesellschaft Deutscher Chemiker GDCh” has started a new form of education, the so called “Spring School Industrial Analytical Chemistry“, teaching master and diploma students of chemistry praxis relevant subjects of industrial analytical chemistry. The initiative of this project is based upon the results of an inquiry conducted amongst representatives from chemical and pharmaceutical companies and producers of analytical equipment during the exhibition “analytica 2008” in Munich. The main result was that more than 50% of the representatives criticized the insufficient experience and knowledge of master and diploma chemists in industrial analytical chemistry. Therefore the “Fachgruppe” and its working group “Industrieforum Analytik” carried out the first Spring School in 2011 from February 14th till 25th. The aim of this type of workshop is it to impart essential methods and typical questions of day-to-day business in an analytical laboratory.

Highly qualified speakers from analytical labs of leading German chemical and pharmaceutical companies and representatives from analytical equipment producing companies educated more than 30 students of different German universities in methods and typical problems in the praxis of an industrial analytical laboratory. Beside the analytical know-how the students received an introduction to the important aspects of entrepreneurship and social competencies like leadership, teamwork or communication. Furthermore, they all got assistance in applying for jobs in the industry. Beside the presentations all participants had the opportunity to attend the excursions to analytical laboratories of a chemical company as well as to a physical lab of an analytical equipment producer. At the end of the Spring School each student got a certificate based on a test and their universities are willing to give credit points to all students who successfully passed the test. The 20 best participants have the chance to further increase their knowledge by attending a six week course at leading German chemical and pharmaceutical companies.

The participation in a Spring School is free of charge. But the number of participants is limited. Participants may be students of master/diploma courses of chemical studies and must be recommended by their professor in analytical chemistry. Basic knowledge in analytical chemistry is absolutely necessary.

The Spring School is financed by the Fachgruppe “Analytische Chemie” and by the participating companies. Furthermore the Fachgruppe assigns scholarship funds in a limited number to their members.

FROM ANALYTICAL SCIENCE TO PROCESS ANALYTICAL TECHNOLOGY

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Since 2004, the University of Lyon offers a master degree with a training program entirely dedicated to analytical science. Every year, 48 students register to this master degree for a first year with a core curriculum in analytical tools (separation, elemental analysis, NMR, mass spec, UV-IR, signal processing, structural analysis, validation, quality assurance and quality control...). During the second year, an active pedagogy based on project based learning has been organized with challenging task assigned to students as a part of their training program. Since 2006, our students are hired during their final year by companies in order to be ¼ year time attending course at the university and ¾ year time in the company to perform research or quality control.

This training structure has been initially developed form the Knowledge, Skills and Abilities required by the French Chemical industries for laboratory analysis, and the master degree was in good adequacy with these requirements. However, chemical industry requires now specialists in industrial analysis and is facing recruitment difficulties to address challenges in real-time analysis and process optimization.

A new interdisciplinary program at the interface of chemistry, chemical engineering and analytical chemistry is now offered to students in order to address topics such as quality by design, process analytical technology, bio-processing, green chemistry, nanotechnology, micro-instrumentation, process control and data handling.

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S4: Food Analysis

Keynote Lecture 4

NATURAL CONTENT OF VITAMINS IN FOODS – AN ANALYTICAL CHALLENGE

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Modern analytical chemical methods with the use of GC and HPLC coupled to UV, DAD, MS and MS/MS detectors are very specific and show low detection limits for vitamins. Question arise whether the analytical method encompass all biological active vitamers and/or whether the quantified vitamers are really biological active in humans and with which biological activity.

The basic purpose was to measure the nutritional effect which is the ultimate measure of value for any nutrient. The original biological and microbiological methods are time-consuming, and costly, and furthermore they have a low precision.

Today the chemist has to quantify the known vitamers which contribute to vitamin activity. Ever since the discovery of vitamins this list has been enlarged and continuously the analytical methods have been improved to quantify the specific chemical vitamin active compounds.

This is a challenge, as the contents of the vitamins are present in food in very low amounts at ppb and even ppt level. E.g for sources rich in vitamin D such as cod liver oil, there is only 2-3 ppb, and in milk even at ppt level. Due to high consumption these levels are of high interest.

Like in any other analyses of natural components, the extraction process is essential. The extraction should optimally release all active vitamin compounds to be quantified.

The challenge is firstly to identify the individual compounds which posses the vitamin activity, secondly to achieve a standard material, and thirdly to develop and validate the necessary correct and precise analytical method. And finally assess the bioactivity of each of the quantified vitamers.

The talk will give example of these challenges for selected fat- and water soluble vitamins.

LABEL-FREE IMMUNOSENSORS WITH OWLS DETECTION FOR FOOD SAFETY

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Food safety has become a central concern within the European Union as it is all over the world. Consumers worry about food borne pathogens, chemicals, including mycotoxins, pesticides and veterinary residues. Safe food is a result of stringent quality control maintained throughout the food chain. In the lecture we would like to present some results obtained by the Optical Waveguide Lightmode Spectroscopy(1) (OWLS) based label-free immmunosensors used for the determination of different chemical contaminants in food responsible for the risk of food poisoning. Measuring methods were investigated for the determination of mycotoxins (zearalenone, aflatoxin, ochratoxin, and deoxynivalenol), toxin (ocadaic acid), allergenic compound (histamine), trifluralin pesticide and vitellogenin marker protein for monitoring the pollution with endocrine disrupting chemicals. The OWLS technique as a label-free immunosensor has been successfully applied for the detection of a number of different compounds in both competitive and in direct assays. In order that the sensitised surfaces to be regenerable, i.e. the sensor can be applied several times, the antigen or the antibody is immobilized on the surface by covalent attachment to silanized surfaces. When measuring in direct manner the appropriate antibody was immobilized on the sensor surface and the linear measuring range determined, while during the competitive measurement the antigen-conjugate was immobilized on the waveguide surface. Standard solutions containing different amount of toxins were mixed with antibodies of appropriate concentration, the mixture was incubated for one minute and injected into the OWLS system. Binding of the antibodies in the sample to the coated surface is competed for with free antigen in the sample and only antibodies remained in free form in the mixture bound to immobilized antigen-conjugates. The amount of antibodies bound to the surface of the chip was inversely proportional to the toxin content in the samples. The linear measuring range and the relative substrate specificity of the antibody were also studied. Different type of food samples (*e.g.* grains, fish, mussel, fruit and vegetable juice, *etc.*) were spiked with the proper chemicals and were analysed using the newly developed methods and a high degree of correlation was observed between the spike level and the detected value using the immunosensors and reference methods.

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Special thanks to MicroVacuum Ltd. (www.microvacuum.com)
by supplying measuring time on OWLS 100 instrument.

S4: Food Analysis

ULTRA FAST SEPARATION AND HIGH RESOLUTION CAPABILITIES (LCxLC-IT-TOF) FOR NATURAL PRODUCTS APPLICATIONS

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The need for more powerful and discriminating analytical techniques has grown exponentially over the last decade, along with the increasing complexity of the samples to be analyzed, and the specificity of information required. It has become ever clearer that, in order to achieve the desired level of accuracy and reliability of analytical data, the analysis of complex mixtures requires the combination of both powerful separation techniques and sensitive detection.

As far as the separation is concerned, the implementation of multidimensional (MD LC) and comprehensive (LC x LC) liquid chromatography systems has provided enhanced resolving power for highly complex samples with hundreds of closely-eluting components. The increase in separation power arises from the combination of multiple stationary phases, with different degree of orthogonality (selectivity). Whatever the separation techniques, the use of mass spectrometry (MS) adds an additional dimension to the analytical LC x LC platform, allowing more definitive identification and the quantitative determination of compounds that are not fully resolved chromatographically.

This presentation will focus on the use of comprehensive LC x LC techniques and ultra high pressure technology (UHPLC) for challenging separations in the food area (proteins, lipids, carotenoids).

High resolution is attained through the use of partially porous particles (2.7 μm), consisting of either HILIC, or Cyano, or C18 stationary phase, while the on-line coupling to an ion trap-time of flight (IT-TOF) mass spectrometer will provide the specificity of detection desired.

KINETIC APPROACH TO ASSESS END-POINT ANTIOXIDANT CAPACITY OF RED WINES USING MICROPLATE BASED METHODS

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The antioxidant capacity of red wines is commonly assessed by chemical methods based on electron transfer reactions, such as Folin-Ciocalteu (F-C), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{•}$) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{•+}$) assays. The major limitation of these assays relies on the necessity of attaining end-point measurements since the antioxidant values depend on analysis time [1]. This situation takes place because the antioxidant capacity of red wines is estimated with standard compounds (Trolox or ascorbic acid), which show a kinetic behaviour different from those obtained for red wines. In fact, using traditional standard compounds, which present a rapid kinetic profile, the antioxidant values are time-dependent and the measurements for red wines should be performed after 120, 60, 120 and 300 min using the F-C, CUPRAC, DPPH$^{•}$ and ABTS$^{•+}$ assays, respectively. This issue implies that these procedures are time-consuming and less suitable for routine/screening purposes.

The aim of the present work was to determine the kinetic profile of several antioxidant compounds, including the standard mixture proposed as reference material for antioxidant assessment [2], in order to develop analytical methods that provide results independent of the reaction time. To implement this kinetic approach for each assay, a microplate spectrophotometer was used to monitor the redox reaction of red wines and standards with each oxidizing species. The antioxidant values of red wines, measured every minute up to the end-point, were related to each studied standard and the results demonstrated that when the standard compound had reduction kinetics similar to the sample, the values attained in the first minutes of the reaction were similar to those obtained at end-point conditions. Using this strategy, the reaction time of F-C, CUPRAC, DPPH$^{•}$ and ABTS$^{•+}$ assays may be reduced to 3, 10, 30 and 5 min using the appropriate standard compound.

Acknowledgements

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IDENTIFICATION OF DEGRADATION PRODUCTS OBTAINED UNDER EXPOSURE OF QUERCETIN SOLUTION TO UV-C LIGHT

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Quercetin is the most prevalent flavonoid which belongs to the subclass of flavonols. There is enormous number of publications which deals with transformations of quercetin and identification of obtained products, regardless of mean which was use for initiation of its degradation. The most frequently studied is quercetin oxidation/degradation, initiated by electrochemical methods, radicals, presence of metal ions, enzymes, heat or different methabolic pathways. Because of its strong UV absorption quercetin is highly labile in biological systems. There are many investigations of photostability of flavonoids which has simplier structures, such as 3-hydroxy flavones, in the presence or absence of metal ions, while the publications dealing with investigation of quercetin photostability and identification of its photodegradation products are rarity.

In recent years many approaches, such as the illumination of friuts, vegetables and their products by ultraviolet light, have been investigated in order to find out the most efficient technique against food pathogens which cause their spoiling. Numerous investigations of possible using of UV-B or UV-C light for prevention of friut and vegetable browning approved that level of antimicrobial agents in plants, such as flavonoids, during short UV illumination increase, which amend their antimicrobial activity and result in prolonged resistance of fruits and vegetables against putrefaction provoken by various microbes. Thus, it is of great importance to find out which products are obtained during UV irradiation and to investigate their biological activity.

The photochemical degradation of quercetin methanolic solution was investigated in an annular (double skin) photoreactor. After 40 min irradiation by Philips TUV 15W lamp, which emits UV-C light, the decrease comparing to inital concentration of quercetin solution (4×10^{-4} M) was 30% and more than 20 products were detected in complex reaction mixture. The identification of degradation products was performed by ^{1}H NMR and by liquid chromatograph with PDA and MS detector.

PROFILING OF FLAVONOIDS IN HUNGARIAN APRICOTS (*PRUNUS ARMENIACA* L.) USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Nowadays, the scientific community increasingly recognizes that plant derived foods such as fruits and vegetables show some beneficial effects on human health which are in relation with bioactive micronutrients such as flavonoids and phenolic acids. Apricot is considered as a rich source of phenolics.

The aim of our research was to comprehensively explore the flavonoid composition of apricots grown in Hungary. Twelve apricot genotypes were collected from eleven different commercial cultivars including five traditional Hungarian cultivars (Ceglédi arany, Ceglédi óriás, Ceglédi Piroska, Korai piros, Mandulakajszi) and one hybrid from a breeding programme (hybrid 7/1). These samples cover a representative set of apricot genetic diversity. All trees were cultivated in the same experimental orchard according to normal apricot orchard management and were harvested in 2009 at the commercial maturity stage. The identification of flavonoid derivatives was carried out by an HPLC-ESI-MS/MS screening method and the quantity of flavonoid derivatives were given in their non-hydrolyzed glycoconjugate forms.

The main idea of this method that it is able to detect and identify the flavonoids in their original intact form as they are present *in planta* i.e., conjugated to various saccharides and organic acids. Having suitable information on conjugated forms is important because their bioavailability and thus biological effect of these compounds depend on the type of conjugates linked to the aglycone of flavonoids.

As a result of our study three flavonoid glycoconjugates including two quercetin derivatives (quercetin-3-*O*-ruitnoside and quercetin-3-*O*-glucoside-6'-acetate) and one kaempferol derivative (kaempferol-3-*O*-ruitnoside) were indentified and individually quantified in each cultivar; however, differences were observed in their relative abundances. Summarized flavonoid contents varied between 0.8 and 12.4 mg 100 g^{-1} of fresh fruit expressed as quercetin-rutinoside. Furthermore it must be emphasized that the flavonoid contents of cultivars with the highest values exceeded the average levels for apricot fruit.

S5: Electroanalytical Chemistry II

Keynote Lecture 5

THE ULTRA-RING ANSOR

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Ionophore containing micro and nano-particles are useful core agents to build optical sensors, potentially able to give both spatial and temporal information about a sample. However, although response time can be reduced in a nano-particle format, this puts the structure below the resolution limits for classical confocal microscopy. A capsule format can overcome this and can provide multiple additional functionalities. This capsule scaffold will be presented, based mainly on a laminated organo-silane/silica structure, with diameter from 1000nm to 2500nm.

Emerging from the sensing capabilities this sensor, we have designed a multifunctional ‘capsule’, which provides a platform technology for combining imaging contrast agents, ion-selective measurements and drug or other reagent delivery.

One philosophy in design of these capsules was that by altering the thickness and silane precursors for the shell laminate, the resultant properties of the particles could be manipulated to become ultrasound sensitive and could thus serve as both an ultrasound contrast agent and ion-selective sensor. In this context, capsules prepared with a sufficiently elastic shell can show a bright ultrasound image, thereby presenting the capsule as a contrast agent. Furthermore, their function could be selected according to applied acoustic pressure to behave as a contrast agent or as a delivery vehicle for drugs, dna etc. that might be contained within the capsule. A second contrast agent functionality could be introduced through incorporation of magnetic nanoparticles, also allowing particles to collected within a magnetic field or to be used in MRI as a contrast agent.

However, the major use of these capsules comes as fluorescent ion-selective optrodes. Assembly of ionophores in the capsule shell is shown and their response compared with particles of similar size. Since the capsule wall has nanometer dimensions, the diffusion path-length is very small and response times of <3s are typical determined by wall thickness and depending on ionophore assembly in the capsule. Reversible fast response times are demonstrated, even when a reagent such as chromoionophore I is used which typically has been reported to show photobleaching.

COMPLEX DETECTION OF DNA DAMAGE USING DNA MODIFIED SCREEN-PRINTED CARBON PASTE ELECTRODES

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Investigation of chemical interactions of the surface attached DNA is of great importance for biochemical, medical, environmental, food and other studies and applications. From the pioneer work by Palecek and Postbieglova [1] devoted to detection of small amounts of DNA, electrodes chemically modified with a layer of DNA (or generally nucleic acid) are constructed and used widely as chemical sensors and biosensors with the specific response to DNA and advantages of electrochemical analysis. Carbon based electrodes including disposable screen-printed carbon paste electrodes are of interest as non-expensive and environmentally friendly material suitable for the preparation of rather simple sensors used particularly in warning tests. Besides association interactions, structural damage to DNA is subject of such tests. Characterization of DNA damage type using the biosensors based on solid electrochemical transducers belongs to problems which should be solved.

Here we suggest an application of several detection modes at the evaluation of changes at the DNA layer of the biosensor. To ensure detection windows with necessary sensitivity, an interface formed by nanomaterials with large surface area and good electric conductivity is used at the carbon paste-based screen-printed electrode. The sensors were exploited both in batch and flow-through cells. Damage to DNA by various agents was detected using voltammetric signals of guanine moiety, redox active dsDNA intercalators, the ferricyanide anion as indicator present in solution phase and electrochemical impedance spectroscopy. Opening of the helix structure, strand-breaks and total destruction of the DNA layer have been observed.

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LABELLING OF NUCLEIC ACIDS WITH ELECTROACTIVE MOIETIES FOR THE ANALYSIS OF NUCLEOTIDE SEQUENCES AND DNA DAMAGE

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DNA is an electroactive substance yielding analytically useful redox as well as tensammetric electrochemical signals. In addition to intrinsic DNA responses, electroactive markers have been utilized to obtain reversible electrode processes at less extreme potentials than those produced by DNA itself, or catalytic signals to attain higher sensitivity. Some of the covalently (e.g., oxoosmium complexes) or non-covalently (intercalators) bound redox markers exhibit also selectivity to DNA structure. In addition to the electroactive moieties, enzymes catalyzing production of electrochemically detectable indicators have been applied in DNA labeling as well. Choice of a particular detection technique depends on the type of analysis and electrodes used. Although there have been efforts to prefer label free techniques due to their inherent simplicity, low costs and biocompatibility, not always these criteria meet requirements of the given assay. Osmium tetroxide complexes with nitrogenous ligands (Os,L) form covalent adducts with pyrimidine residues. Sensitivity of Os,bipy (bipy=2,2'-bipyridine) towards unpaired thymine residues has recently been utilized in a technique for the detection of DNA damage as well as to label signalling probes for DNA hybridization assays. Various electroactive tags can be introduced into DNA by DNA polymerases using labeled deoxynucleoside triphosphates. In recent years, preparation of electrochemically labeled nucleotides and their incorporation into DNA using primer extension (PEX) or PCR techniques have opened novel possibilities in electrochemical DNA sensing. Incorporation of the labeled nucleotides into DNA is potentially useful not only for preparation of probes for the purpose of "classical" DNA hybridization assays, but also for approaches based on sequence-specific introduction of the tags. PEX-based DNA "minisequencing" strategies involving nucleotides coded with tags producing specific signals (such as nitrophenyl, ferrocene or a Os(II) complex) results in an easy identification of the SNP type according to the peak potential and/or character of the electrode reaction.

RECOGNITION OF ANALYTES RANGING FROM NANO- TO MICRO-SIZE USING OVEROXIDIZED POLYPYRROLE BASED MOLECULARLY IMPRINTED POLYMER SENSORS

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Chemical sensors and detectors play important roles in many fields of chemical analysis. In this paper, we discuss several different types of molecularly-imprinted-polymer (MIP) based chemical sensors developed in our laboratory. We have been studying MIP sensors using overoxidized polypyrrole (oPPy) in which dopant anions are used as template molecules for recognition (Scheme 1). Recently, we have discovered that oPPy can be used for the detection of analytes ranging from nano- to micro-size. Such flexibility opens a new possibility in developing analytical sensing technology capable of detecting small to macromolecules. Such analytes include small and mid-sized molecules, such as amino acids and peptides, and in an extreme case, we have successfully detected and discriminated different kinds of bacteria using the oPPy technology.

Pyrrole is polymerized with a dopant anion, which is used as a template in this technique. The inserted anion can be removed by further oxidation (overoxidation) of the polymer film by the loss of the positive charge. Here, the hardening of the polymer film occurs concomitantly with the overoxidation to make the complementary cavity stable, and the memory of the template is precisely preserved in the film surface.

S5: Electroanalytical Chemistry II

PHOTOPATTERNING OF ULTRATHIN ELECTROCHEMILUMINESCENT HYDROGEL FILMS

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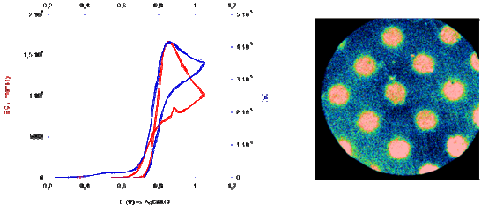
Electrogenerated chemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ is applied in clinical diagnostics, particularly in detecting affinity reactions of immunoreagents and nucleic acids.[1-2] Derivatives of $Ru(bpy)_3^{2+}$ have been immobilized in polymer and silica sol-gels films, attached to nanoparticles and covalently bound to substrates.

In the present work, photoinitiated polymerization is efficiently and rapidly carried out to immobilize ultrathin electrochemiluminescent hydrogel films. We used for our studies the [poly(4-vinylpyridine)-Ru(2,2'-bipyridine)$_2$Cl-ethylamine].[3] The redox PVP-$Ru(bpy)_2^{2+}$ polymer was photodeposited through a mask with PEGDA and a photoinitiator. Cyclic voltammetry shows reproducible signal demonstrating the stability of the film on the electrode surface. AFM experiments were performed to measure the thickness of the film which was modulated between 30 and 100 nm. In the presence of tri-*n*-propylamine (TPrA), photodeposited polymer shows intense ECL response which emission appears at the oxidation potential of Ru^{2+} centers (Fig. 1A).



A) B)

Fig. 1. A) Cyclic voltammogram (blue) and ECL emission (red) of the photodeposited film in PBS with 10mM TPrA. Scan rate: 30 mV/s. B) ECL image of obtained photopatterns.

Microscale patterns are fabricated on electrode surface by a simple photolitographic procedure and revealed by ECL imaging (Fig. 1B). The relatively fast diffusion in such hydrogel films and their spatial structuration are important characteristics for the development of new ultrathin ECL materials and for multiplexed ECL detection.

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MARIA SKLODOWSKA-CURIE, WOMAN, SCIENTIST, NOBEL PRIZE WINNER

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Maria Sklodowska-Curie is only scientist in history, who received the Nobel Prize twice once in physics (1903) for radioactivity discovery and again in chemistry (1911) for polonium and radium discovery and studies concerning the nature of these elements. Scientific works, which she started together with her husband Pierre Curie were continuing after his death, opened a new sphere of inquires in physics and chemistry and led to arise of first methods of atom interior and material researches. These studies have dominated contemporary science. Stating that radiation of radioactive substances induces chemical reactions she became the author of radiochemistry. She was able, in a very fast way, introduce her scientific results to life and check them out in practice.

Her curriculum vitae, scientific career and social activity are so fascinating and full of secrets that could be used as a topic of many studies, not only scientific. The mere fact of origin (she was born November 7, 1867 in Warsaw) and the desire liberation from the prevailing policy at that time (end of the XIX and the beginning of XX century) meant that she became a symbol of modern woman-scientist, wife, mother, and philanthropist. Her life's work (education and knowledge) performed outside of the occupied Poland. As a second homeland, she chose France. How eloquently and simply she described the story of her life: *"It is such an uneventful, simple little story. I was born in Warsaw, in professors family, I married Piotr Curie, I had two children. My scientific work I made in France.”*

S6: Challenges in Chemometrics

Keynote Lecture 6

HANDLING MISSING VALUES, OUTLIERS AND MEASUREMENT UNCERTAINTY IN THE CHEMOMETRIC ANALYSIS OF ENVIRONMENTAL DATA

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The present study addresses the problem of dealing with missing values and outlying observations simultaneously in the chemometric analysis of environmental data. The presence of samples that can be the result of analytical error and have much different values for some parameters in comparison with the majority of samples, disturbs a correct replacement of missing elements, while the absence of measurement values does not allow proper outliers' identification. The lack of measurement data in the collected experimental sets may be caused by the malfunctioning of the analytical instruments or changes in the experimental conditions due to which some measurements are not recorded for all samples. The handling of missing elements and data below the limit of detection can also be done by incorporating the measurement uncertainty information into the chemometric models.

In this study, we present and discuss approaches to principal component analysis (PCA) [1], soft independent modeling of class analogy (SIMCA) [2,3] and partial least squares regression (PLS) [4] that can process contaminated data with missing elements as well as positive matrix factorization, PMF, and the weighted variant of the multivariate curve resolution method, MCR-WALS [5], that incorporate the measurement uncertainty information.

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NEAR INFRARED SPECTROSCOPY AS A CHALLEGING ANALYTICAL TOOL

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Mid-IR-spectroscopy is widely used in analytical practice. As to NIR-spectroscopy (NIRS) it was considered as low-informative and therefore useless by many analysts. It is true that NIR spectra are much more complex than mid-IR spectra. At the same time NIR spectrum acquisition is faster compared to other analytical techniques and nondestructive. Moreover NIR carry information regarding not only chemical, but also physical phenomena.

In case the experimental data are complex but carry a lot of physicochemical information why not to extract this useful information?

Well-known drawback of NIRS is a hidden way in which the information is presented. Multivariate data analysis (MVA) is necessary to extract this information. Thus it is better not to claim NIRS as a tool, but present the entire NIR-based approach, combining NIR measurements with MVA.

Moving from Mid to NIR not only spectroscopic region is changed. The main issues, such as samples' preparation, data analysis, the results improvement etc., are changed dramatically.

The NIR-based approach and its comparison with IR spectroscopy with on the real-world examples are presented. In particular the following items are addressed.

- Sample preparation and spectra acquisitionare illustrated by the counterfeit detection of tablets with antibacterial active ingredients (API) [1].
- Influence of the employed spectral region is illustrated by the control of packed raw materials [2].
- Sensitivity of NIR in comparison with wet-chemistry analysis is illustrated by the study of the genuine and fake ampoules with API aqua solution [3].
- NIR-calibration problem and figures of merit are illustrated by determination of protein in wheat [4].

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CONTAMINANT PROFILING: A SET OF NEXT GENERATION ANALYTICAL TOOLS TO DEAL WITH CONTAMINANT COMPLEXITY

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Contaminant complexity is huge and a detailed analysis of contaminated sites can easily reveal thousands of individual compounds with different physicochemical properties and toxicity. However, current procedures for risk assessment (e.g., REACH) do not address that contaminants are present in the environment in the form of complex mixtures.

Next generation risk assessment calls for more studies on mixture toxicity, and new analytical tools to deal with contaminant complexity. These analytical tools should either be semi-targeted or untargeted to provide (semi)quantitative information on contaminants with a wide range of physicochemical properties using one or a few analytical platforms.

Analytical methods based on GC, LC, MS and spectroscopy both alone and hyphenated are the standard analytical platforms for the analysis of complex mixtures. Multi-dimensional chromatography (GC×GC and LC×LC) with MS detection are cutting edge analytical techniques for separation of complex mixtures. The enormous potential of these methods for contaminant profiling are only partly exploited today mainly due to tradition and the lack of automated methods for data processing of high-dimensional analytical data.

Chemometrics is the application of statistical and mathematical methods to chemistry and include several techniques (e.g., principal component analysis, and parallel factor analysis) that allow for a more advanced treatment of analytical data. The combination of advanced analytical chemistry with chemometrics for both automated data pre-processing and data analysis of high-dimensional analytical data (10^6 – 10^9 data points per sample) provides a powerful toolbox for contaminant profiling.

We have developed and applied numerous tools for chemical profiling of oil hydrocarbons and other contaminant mixtures as well as for environmental metabolomics since 2002. In this presentation we will give an overview of some of these tools with focus on the ‘CHEMSIC’ method. Two examples will be given on its use for oil hydrocarbon profiling and fingerprinting.

S6: Challenges in Chemometrics

A NEW FORMULATION FOR ESTIMATING THE VARIANCE OF MODEL PREDICTION

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Introduction: There are two basic ways of estimating prediction uncertainty, namely, error propagation or resampling strategies. Error propagation leads to closed-form expressions where some hypotheses are made but which provide a platform for evaluating the different sources of uncertainty. Resampling is essentially a "black box" approach which, however, is often more accurate because fewer assumptions and approximations are made. Some analytical expressions can be found in the literature for error propagation method, but all consider local linearization and other important assumptions. Particularly, the errors in the predictors are assumed to be independent and to have constant variance. This latter assumption is never fulfilled in spectroscopy. So, this paper proposes a new expression for prediction uncertainty estimation based on the error propagation strategy, using as few as possible assumptions.

Let $\hat{y} = \mathbf{x}^T\mathbf{b}$ be a model. One of the most complete existing and published expressions of prediction uncertainty is:

A: $$Var\left(\hat{y}\right) = \left(1+\frac{1}{N}\right)\left\|\mathbf{b}\right\|^2\sigma_X^2 + \mathbf{z}^T\mathbf{Var(b)z} + \frac{\sigma_y^2}{N}$$

With very few hypotheses, we calculate a new expression:

B: $$Var\left(\hat{y}\right) = \left(1+\frac{1}{N}\right)\mathbf{b}^T\mathbf{Var(X)b} + \mathbf{z}^T\mathbf{Var(b)z} + \mathbf{Var(z}^T\mathbf{b)} + \frac{\sigma_y^2}{N}$$

Where: **z** is the spectrum centred against calibration set and **Var** represents the variance covariance matrix. The main differences between the two expressions are: (i) The first term is more general in expression B, as it takes into account the complete variance / covariance of X; (ii) The third term of expression B is new; it represents a kind of covariance between the variations of **z** and those of **b**.

Material and methods: The terms of the two expressions were calculated on a dataset of N=385 x 10 repetitions of NIR spectra of feed, regressed against protein content.

Results: Table 1 reports the values of all terms of expressions A and B for several models, which differ by the preprocessing. It shows that expression A always overestimates the prediction variance, because it does not take into account systematic variance, due for example to the baselines. It is also noticeable that the third term introduced by expression B is not at all negligible.

		No prep 17LV	**2der+SNV 16LV**	**2der 17LV**	**SNV 18LV**	**Detrend 17LV**
Expression A	**Term 1**	15.49	0.51	0.78	4.08	16.02
	Term 2	0.29	0.25	0.33	0.49	0.28
	Total	**15.78**	**0.76**	**1.11**	**4.57**	**16.7**
	%Term1	98.2	67.1	70.3	89.3	98.2
	%Term2	1.8	32.9	29.7	10.7	1.8
Expression B	**Term1**	0.19	0.28	0.29	0.31	0.19
	Term2	0.29	0.25	0.33	0.49	0.28
	Term3	0.09	0.07	0.10	0.19	0.09
	Total	**0.57**	**0.60**	**0.72**	**0.99**	**0.56**
	%Term1	33.3	46.7	40.3	31.3	33.9
	%Term2	50.9	41.7	45.8	49.5	50
	%Term3	15.8	11.6	13.9	19.2	16.1

Table 1. Values of the terms according to the model

Conclusion: A new formulation for the estimation of the prediction variance, that is more suited to the mathematical specificities of the spectral data, is proposed.

TRACING THE ORIGIN OF FOODSTUFF: A CHEMOMETRIC CHALLENGE

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The increased information available to the consumers together with the competition following the institution of the Common Market (EU countries) has led safety, nutritional value, eating characteristics, ethical, environmental, economic and social aspects to be more and more important and essential topics for the food industry. In a single word, there is an increasing demand for “quality”, as a primary criterion to access the market.

Accordingly, a particular aspect of quality which has been gathering more and more attention during the recent years is that of the ty*picity* of the product. In fact, it has been widely reported in the literature that the geographic, production and species origin of a foodstuff are important variables regulating the overall quality of the product. This issue has been recognized by the European Union by the introduction of the Designations of Origin (PDO and PGI). The products which are labeled by this Denomination must be produced in a well defined geographic area and manufactured using only one or few specified botanical or animal varieties.

As a consequence, having accurate and precise analytical methods for the traceability of the origin of a foodstuff is an issue that is becoming more and more relevant. In this framework, while the possibility of identifying chemical markers of geographic, production or species origin is currently being explored using different fingerprinting techniques, it is widely recognized that, whatever the technique(s) chosen, the use of mathematical and statistical methods (*chemometrics*) to process the experimental data is essential to obtain a reliable authentication of the product.

In this communication, the successful use of different chemometric pattern recognition methods for the traceability of food products will be presented.

FEATURE RANKING WITH RANDOMFORESTS IN NUTRIMETABONOMICS

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The advent of the '-omics' during the last years, has been a challenge for a big part of the analytical chemistry community. Metabonomics in particular, has offered an interesting way to describe and provide new insights into the modulation of regulatory processes in complex biological systems. It provides a snapshot of the combined effects of genetics and environmental factors that are reflected through complex variations of the global metabolic profile.

The introduction of Nutrimetabonomics has provided a way to gain better understanding on the regulation of metabolic pathways by nutrition. However, changes experienced by the complex biological systems under nutritional interventions are often subtle.

Metabolic profiling is currently performed using state-of-the-art high throughput analytical methods such as ^{1}H Nuclear Magnetic Resonance (NMR) and several Mass Spectrometry (MS) based methodologies applied to biofluids and tissues. The use of both profiling techniques has facilitated the generation of massive datasets, making their analysis a real challenge.

RandomForests™ (Breiman 2002) is a classifier method based in an ensemble of classification and regression trees (CART). The method is a modification of the 'bagging' approach, in which each tree is constructed independently by performing bootstrapping on the dataset, and classification is achieved following a majority voting scheme.

Its properties include a low overfitting rate, few parameters to adjust, robustness to noise and its ability to provide feature ranking making Random Forests very interesting for data analysis in Metabonomics. Some of the drawbacks, such the probable decrease in performance for feature ranking on high dimensional data can be addressed with the addition of a cumulative feature ranking criteria. The possibilities of the method are demonstrated in the analysis of a set of UPLC-MS data, from a nutritional intervention study.

Plenary Lecture 5

THE EXPANDING SCOPE OF ANALYTICAL ATOMIC SPECTROMETRY: ISOTOPES, ELEMENTS, MOLECULES AND NANOSTRUCTURES VIA MASS SPECTROMETRY

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No doubt, today's analytical atomic spectroscopists are increasingly attracted back to molecular spectroscopy (e.g. to carry out chemical speciation), while recent developments in atomic Spectrometry (AS) have also brought scientists form other fields (e.g. Metallomics, Proteomics Nanotechnology, etc)to use AS tools and strategies.

Recent search for MS-based reliable quantifications not just for isotopes and atoms, but also for small and big molecules and even for nanoparticles and nanostructures will be discussed.

For small molecules, we have been able to design and construct a new MS set-up enabling three different types of information: elemental (isotopes), fingerprints of the compound (fragments) and molecular mass (molecular ion). This complete analytical speciation in a single MS instrument is achieved by resorting to an innovative GD ionization source (a µ-second pulsed-glow discharge which provides three different plasma regimes per pulse in time) coupled to a modified MS(TOF) as detector(1), most successful for environmental speciation.

After an appropriate GC separation, with on-line organic compound furnace combustion and final EI(MS) detection of formed enriched $^{13}CO_2$ molecules, the first generic approach in GC to determine every organic compound separated in the column, without the need for species-specific standards, has been demonstrated (2). Here, the molecular information is preserved in the first injection (furnace off), but structure-specific response of EI sources can be then circumvented by on-line quantitative transformation of organics to CO_2 (furnace combustion) and final isotope dilution analysis by EI (MS).

For much larger and polar molecules (e.g. proteins in biological systems), speciation is better accomplished using a LC separation coupled to ICP-MS. Of course, complementary well established "soft" ionization sources are desirable for characterization (e.g. ESI- or MALDI-), but again they are species and matrix signal dependent. A fundamental departure point from that situation may be the complementary use of ICP-MS for peptides and intact proteins absolute determinations and even for reliable enzyme activities quantitative assessment (3).

A further attractive step using ICP-MS is nanoparticles (NPs) and nanostructures characterization. Modern tools for NPs elemental/molecular/size characterization (in some aspects related to larger molecules speciation) are highly demanded and ICP-MS can be used to study, with unprecedented accuracy and precision, the synthesis, formation and stoichiometry of the nanoparticle core and shell, e.g. in quantum dots (4) as well as bioconjugations of such NPs for protein quantitative analysis.

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Pleanary Lecture 6

THE ROLE OF ACCURATE MASS MEASUREMENT IN CHEMICAL, ANALYTICAL AND MEDICAL MASS SPECTROMETRY

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High resolution mass spectrometry (MS) has become ever more accessible to the analyst with continuous improvements to MS instrumentation, processing software and analytical techniques. Recent advances in analyser technologies has seen enhanced mass measurement accuracy with rapid analysis times, that have focussed around time-of-flight and ion trapping with the Orbitrap analyser. Mass spectrometry has become a central technology for analytical measurements particularly as a superior detector for separation sciences and have become the *de facto* standard in specific areas such as Proteomics and Pharmaceuticals. The role of the mass spectrometer has never been so important and the utility of accurate mass measurement confers additional power for qualitative measurements in chemical, analytical and other branches of scientific measurement. The role of accurate mass measurement will be reviewed with examples in chemical analysis. Studies of large historical accurate mass data sets, over a range of MS technologies will be described. Examples of recent applications of accurate mass measurements for large inorganic complexes studied by matrix-assisted laser desorption/ionisation, small molecule analysis, clinical applications e.g. siRNA, and to analytical science as well as a novel application in sampling surfaces with a novel liquid (aqueous) sampling probe. The statistical significance of small and large data sets will be discussed along with recent approaches to improve the quality of data and statistical methods to assess the quality of small data sets, for example, using Bootstrapping techniques.

S7: Analytical Spectroscopy

Keynote Lecture 7

ELEMENTAL AND ISOTOPIC ANALYSIS BY ICP MASS SPECTROMETRY

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A general description of the instrumentation for and scientific value of ICP-MS will be presented, including a survey of new applications such as the search for new superheavy elements. New research on several topics from the author's lab will be described, including a) laser ablation for solid sampling, including description of the effects of large particles on atom and ion formation in the plasma, and b) origins of polyatomic ions formedduring laser ablation sample introduction.

SPECTROSCOPIC CHARACTERIZATION OF FUNCTIONALIZED SURFACES FOR LASER DESORPTION IONIZATION MASS SPECTROMETRY

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Nanomaterials play an emerging role in Laser Desorption Ionization Mass Spectrometry (LDI-MS) with the ultimate goal of overcoming some of the most important limitations intrinsically related to the use of conventional organic matrices in Matrix Assisted (MA) LDI-MS. In the last two decades, gold nanomaterials, in particular, represented the preferential choice for such application, because of a huge number of well-established synthetic routes and surface tailoring procedures able to provide fully customizable nanomaterials.

In the present study, conjugated aryl- and naphtyl- thio-derivatives, i.e. 4-mercaptonaphthalene-1,8-dicarboxylic acid and (E)-3-(4-mercaptophenyl)acrylic acid, have been synthesized and characterized by means of UV-visible, fourier transform infrared, and nuclear magnetic resonance spectroscopies.

Afterwards, the thio-derivatives have been used as covalent surface modifiers for flat and nanostructured gold surfaces, by conventional functionalization approaches based on Au-S linking chemistry. The functionalized surfaces have been thoroughly characterized by mean of x-ray photoelectron spectroscopy (XPS) which provided quantitative information about elemental composition and chemical speciation of the outermost layers.

In perspective, the modified surfaces and nanomaterials will be applied as non-conventional promoters in LDI-MS analysis of low molecular weight biomolecules, such as amino acids and peptides.

COMBINED USE OF ^{31}P NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY TO STUDY $^{18}O/^{16}O$ ISOTOPE LABELING IN METABOLIC NETWORKS AND OLIGO-PHOSPHATES

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The ^{18}O labeling technology is a very valuable tool for studying turnover rates of phosphorylated metabolites and phosphotransfer dynamics in intact tissues. This methodology is based on the incorporation of the ^{18}O isotope from $H_2{}^{18}O$ into Pi (by ATP hydrolysis) and the subsequent distribution of ^{18}O-labeled phosphoryls among phosphate-carrying molecules. The influence of the ^{18}O isotope effect on the chemical shifts is readily visible in high-resolution 1D ^{31}P NMR spectra of phosphometabolites,[1] thus enabling the quantification of major phosphometabolites and their turnover rates by ^{18}O-assisted ^{31}P NMR spectroscopy.[1,2] Recently we made major improvements to ^{18}O-assisted ^{31}P NMR spectroscopy by identifying intra-molecular correlations among ^{18}O-labels via J-decoupled 2D ^{31}P NMR chemical shift correlation spectroscopy of the ^{18}O-labeled metabolic oligo-phosphates. The observed correlations in essence discern all isotopologues and provide the means for accurate determination of ^{18}O-labeling rates of equivalent oxygen atoms within the di- or tri-phosphate moiety. Although the use of 2D correlation maps greatly enhances ^{18}O-assisted ^{31}P NMR spectroscopy, its major limitation is low sensitivity. On the other hand, mass spectrometry is very sensitive for detecting ^{18}O enrichment, but is unable to distinguish isotopomers and their associated ^{18}O distribution within the observed phosphoryl fragments. Thus, we used relatively insensitive, but isotopomer-selective, J-decoupled 2D ^{31}P NMR chemical shift correlation spectroscopy to calibrate very sensitive, but isotopomer nonselective, MS. Such a combination may be useful for comprehensive evaluation of muscle or cellular bioenergetics as well as phosphotransfer metabolomics and fluxomics.

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IN-SITU SERS SPECTROELECTROCHEMISTRY ON COPPER SUBSTRATE

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Surface-enhanced Raman scattering (SERS) spectroscopy is a tool suitable for detection of low amount of various analytes. An individual analyte has to be adsorbed from solution onto surface by either physisorption or chemisorption. To tune the adsorption of analytes and to study mixtures of analytes we developed instrumentation which enables combination of Raman spectroscopy with potentiostatic technique. An *in-situ* spectroelectrochemical cell was designed and constructed at our department. The three-electrode cell is arranged for fibre-optic Raman probe of Raman spectrometer Dimension P2 (excitation wavelength 785 nm). The SERS-active electrode is based on Pt target electrochemically coated by copper (or another SERS-active metal). In our previous studies we demonstrated that copper substrates are suitable for SERS spectroscopy exhibiting surface enhancement factor higher than 10^5. Copper is from chemical point of view more reactive material than silver or gold that means a variety of analytes can be chemisorbed.

The spectroelectrochemical cell was made from Teflon and equipped with (i) a salt bridge for any suitable reference electrode, (ii) space for auxiliary electrode and (iii) working electrode – modified Pt target with contact. Usually, the cell consisted of a saturated Ag/AgCl reference electrode, a Pt plate with large surface area and a nanostructured copper coated Pt working electrode. Different potentials were applied between working and reference electrode for each of studied analytes. Analytes were various organic derivatives with different functional groups, mainly pharmacological materials and/or biomolecules (β-carotene, acetaminophene, etc.) soluble in water and/or suitable organic solvent. Electrolytes were diluted solutions of inorganic salts (KCl, Na_2SO_4, etc.). It was observed that intensity of bands depends on applied potential. Bands of analytes were evident usually at negative potentials. We can measure reliably high-quality SERS spectra at bulk concentration down to 10^{-5} mol.l^{-1}. Lower concentration will be tested further to estimate the detection limit.

OPTIMISATION OF SAMPLE PREPARATION AND WD-XRF MEASUREMENT IN VERY SMALL-SIZED SAMPLES

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The chemical characterisation of small-sized samples is of special interest in the impurity analysis of raw materials, works of art, particle capture filters, forensic analysis, and in any circumstance where very small-sized samples are involved. These types of samples are customarily analysed by scanning electron microscopy coupled with energy-dispersive X-ray (SEM-EDX) spectrometry, micro X-ray fluorescence (µ-XRF), inductively coupled plasma-mass spectrometry (ICP-MS) itself, or in combination with laser ablation (LA-ICP-MS), in which qualitative or semi-quantitative analyses are usually obtained.

This study was undertaken to develop a sample preparation and measurement methodology that would allow wavelength-dispersive X-ray fluorescence (WD-XRF) spectrometry to be used for precise and accurate quantitative analysis of small-sized samples.

The work was conducted on samples prepared in the form of pellets and beads, using specially designed dies, 6 mm in diameter, and studying sample preparation conditions. The following variables were studied in bead preparation: minimum quantity of sample, sample/flux ratio, working temperature, and fusion time. The variables studied in pellet preparation were as follows: minimum quantity of sample, sample/binder ratio, and pressing pressure. Measurement conditions were also optimised: for example, analysis time was increased with respect to usual measurement time in order to minimise the statistical counting error.

The developed methodology was validated by analysis of reference materials and comparison with the usual working method.

The study shows that beads can be made with quantities of about 0.0150 g, compared with the usual quantity of 0.5000 g sample, thus enabling 20 times less to be used. It was further ascertained that pellets can be made with 0.1000 g, compared with the usual quantity of 9.000 g sample, thus requiring 90 times less. The developed methodology can therefore be used for quantitative chemical analysis of very small-sized samples by WD-XRF.

SURFACE-ENHANCED VIBRATIONAL SPECTROSCOPY AS A POWERFUL TOOL FOR ANALYSIS OF PLANT LEAVES

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Surface-enhanced Raman scattering (SERS) and surface-enhanced infrared absorption (SEIRA) are effects allowing vibrational spectroscopic detection and identification of molecular species on nanostructured surfaces of several metals, especially coin metals (Au, Ag and Cu). The species has to be deposited on the surface by either physisorption or chemisorption to obtain spectral signals enhanced of several orders of magnitude.

Previously, we demonstrated the potential of FT Raman and ATR spectroscopy for direct monitoring of Norway spruce needles [1 – 3]. Recently we have used FT Raman spectroscopy to analyse leaves of Salix alba L. and Populus nigra L. [4]. Bands of several components were identified, but the assignment of many other, rather weak bands was difficult.

Hence, we decided to employ the techniques of surface-enhanced vibrational spectroscopy (SEVS). We extracted the leaves with several volatile organic solvent of different polarity and the extracted mixtures were deposited on gold and silver SERS-active substrates prepared electrochemically on Pt targets. FT SERS and SEIRA spectra were accumulated and evaluated chemometrically. Considering pigments composition, the FT SERS spectra of extracts from polar solvents showed bands of xanthophylls and chlorophyll, while in samples from nonpolar solvents carotenes were evident. The components of waxy epicuticular layer were dominant in SEIRA spectra. Furthermore, we explained the way of species adsorption showing that the majority of compounds are physisorbed while only a few polar species are chemisorbed.

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S8: Art and Cultural Heritage

Keynote Lecture 8

ARCHAEOMETRY: SPECTROSCOPY MEETS ART AND ANTIQUITIES

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Archaeometry is the challenging research field on the border between natural sciences (analytical chemistry, geology, physics, etc.) and humanities (e.g. archaeology, art history, conservation sciences, etc.). Recently, spectroscopic examinations are increasingly frequently used for the study of archaeological findings and art objects. By doing so, the research can contribute to questions concerning, amongst others, provenancing, dating, authentication, conservation science, etc.

During the last years, Raman spectroscopy is becoming into vogue for this type of investigations, as several features of the technique are very favourable, such as the ability to record spectra of inorganic as well as of organic substances, the analysis at a micrometer scale and the possibility to perform in situ investigations. Apart from the molecular information that can be gathered by this approach, combining the results with data from X-Ray fluorescence analysis can be very advantageous. Raman spectroscopy is increasingly popular as analytical technique in archaeometry. Instrumental improvements make that Raman instrumentation is increasingly available for the analysis of cultural heritage objects. Micro-samples can be studied when the Raman spectrometer is coupled with a microscope; a spatial resolution of ca. 1 µm can be reached, so that easily different pigment layers or pigment grains can be distinguished. Moreover, mappings can be obtained providing a molecular image of an area. As Raman spectroscopy is a non-destructive technique and as small spectrometers with good spectral quality are becoming available, it becomes possible to investigate art objects directly.

In recent research projects, Raman spectroscopy was combined with X-Ray fluorescence. Thus, it was possible to maximise the information that was gathered, while minimising the possible damage to the art objects. Micro-Raman spectroscopy can be combined with micro-XRF measurements. Apart from these laboratory investigations, mobile Raman equipment as well as mobile XRF equipment is becoming available for archaeometrical research. These topics will be illustrated with a broad range of examples from recent research.

S8: Art and Cultural Heritage

MULTIANALYTICAL INVESTIGATION OF MEDIEVAL POTTERY FROM BRANIČEVO, SERBIA

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Selected medieval pottery sherds excavated in Braničevo, archaeological site in east Serbia were investigated. Accordign the archaeological findings this pottery is very valuable because of its clear context and precise dating in the period between beginning of the 12th and the first half of the 13th century.

The analysis of archaeological pottery is a demanding task due to the complex and inhomogeneous composition and different phases formed during thermal treatment of natural raw material. Therefore mulianalytical approach including optical and SEM microscopy, EDX, FTIR and micro-Raman spectroscopy was applied.

The ancient pottery technology (raw materials used and firing conditions of body production) was discussed based on chemical and minerological composition. The firing temperature of the non-calcareous clay past was estimated between 800 and 900° C in mainly oxidizing atmosphere. The very rich mineralogical composition (about 30 minerals) was identified by the combination of FTIR and micro-Raman spectroscopy. Using the body chemical and mineralogical composition, as starting point, the glazing techniques were examined too. Two types of glazes were identified, lead glaze and alkali glaze. Mixture of lead oxide and clay was applied over the white slip (in sgraffito type decoration) or directly over the noncalcareous body. A good correlation between the polymerization index derived from the Raman spectra and the glaze composition was obtained and the firing temperature of the glazes was estimated.

X-RAY FLUORESCENCE ANALYSIS OF 16TH CENTURY ANTWERP MAIOLICA TILES

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Maiolica is a generic term to cover all classes of lead-tin glazed soft-paste ceramic pottery manufactured in Europe from the 13th century. During our study, experiments were conducted to obtain information on the elemental composition of historically applied pigments and layered structure of painted maiolica tiles., with the main goal of 1) gaining insight to specific compositional characteristics of samples originating from different production sites and 2) better understanding of the degradation processes involved.

The measurements were performed on a maiolica collection which consists of 33 archaeological fragments originating from four different historical production centra in the city of Antwerp, Belgium. The micro-XRF spectra from these fragments were collected using a high-performance EDAX EAGLE-III laboratory X-ray microprobe equipped with a glass polycapillary lens as X-ray focusing optic. The obtained qualitative micro-XRF information results in elemental fingerprints of the pigments present on the surface of the different fragments. Due to the diverse nature and heterogeneity of the examined maiolica samples, the use of quantitative XRF data evaluation methods was also important. In this way, a database can be constructed containing major/minor and in some cases trace-level elemental information from each maiolica sample present in our collection.

In order to better understand the degradation processes occurring on maiolica fragments as well as to extract corroborative information concerning the compositional characteristics of the historically used pigments, the above described laboratory micro-XRF measurements were complemented by *in situ* experiments. The *in situ* measurements were performed on a unique maiolica tiled floor (the only known maiolica tiled floor in Belgium dating from the 16th century) of the "Rameyenhof" Castle in Gestel, Belgium. A handheld XRF spectrometer (InnovX) and a mobile Raman spectrometer (MARTA) were applied for the non-destructive analysis of this unique and extremely precious floor in order to obtain chemical compositional data during a measuring period of six days.

Results of the above described analysis will be presented.

Acknowledgements: The authors wish to thank Mr. and Mrs. De Gruyter, owners of the "Rameyenhof" Castle, and Mr. Caignie, expert of historical maiolica tiles, for granting access to the unique maiolica floor analyzed in the "Rameyenhof" Castle.

PIGMENT IDENTIFIFCATION FROM „BATTLE OF GRUNWALD“ PAINTING. POSSIBILITIES AND LIMITATIONS OF USING LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY FOR MAPPING OF MICRO-SAMPLES

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„Battle of Grunwald“ was painted by famous polish artist Jan Matejko, who devoted nearly 6 years to create this oil on canvas and complete it in 1878. The artefact has huge dimentions with the hight of 426 cm and bright equal to 987 cm. „Battle of Grunwald“ has been exhibited in the Warsaw National Museum, Poland before the conservation started in 2010.

Chemical identification of pigments was undertaken with the use of SEM-EDS, Raman spectroscopy and LA-ICP-MS. Due to the high historic value only micro-samples could be taken from the paintings therefore the collected micro-samples were devoted to successive analysis with the use of chosen instrumental techniques.

The preliminary results were obtained with SEM-EDS and Raman spectroscopy, followed by LA-ICP-MS measurements. An ICP-MS ELAN 9000 (Perkin Elmer SCIEX, Canada) equipped with the laser ablation system LSX-213 (CETAC, USA) was used during the work. Each measurement cycle consited of multi-line laser ablation and consequent ICP-MS analysis with registration of transient signals for the monitored isotopes: ^{7}Li, ^{12}C, ^{24}Na, ^{26}Mg, ^{27}Al, ^{29}Si, ^{31}P, ^{32}S, ^{35}Cl, ^{39}K, ^{42}Ca, ^{49}Ti, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{61}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{85}Rb, ^{88}Sr, ^{109}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{202}Hg, ^{208}Pb, ^{209}Bi, ^{232}Th, ^{238}U.

An open ablation cell with small intrinsic volume (9 cm^3) was used during laser ablation executed directly from micro-samples of pigment mounted on a 12 mm diameter stub. LA-ICP-MS allowed mapping of selected elements over the pre-defined area of specimen. The wash out time of the ablated aerosol from the cell was short enough to obtain the spatial resolution of the created elemental maps sufficient for insepction of pigment micro-samples with diameters of c.a. 100-200 μm.

The developed approach of consecutive use of Raman spectroscopy, SEM-EDS and LA-ICP-MS for analyzis the set of micro-samples collected from „Battle of Grunwald“ allowed reliable chemical identification of the pigments used by Jan Matejko and enabled minimalization of sampling to the micro-destructive process.

IDENTIFICATION OF PIGMENTS OF COLORED GLAZES ON SILVER LEAF FROM THE BAROQUE PERIOD IN SERBIAN ORTODOX CHURCHES WITH SEM-EDS TECHNIQUE

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The baroque influences in the decoration of Serbian orthodox churches are well documented in icon painting and woodcarving of the 18th century. However, the colored glazes on gilding and silver leaf on iconostases and icons of this period are mostly hidden under later over- paintings and therefore were not studied until recently. This paper deals with the identification of pigments in three common colors of glazes (on silver leaf): green, red and blue, using methods of optical microscopy (OM) with reflected light and UV filters (FM) and electron microscopy (SEM analysis) coupled with an EDS, energy dispersive spectroscope. The suitability of these methods for the identification of inorganic and organic pigments is discussed. The experimental results are interpreted based on the known optical properties and chemical composition of historical pigments of the 18th century.

A NEW TECHNIQUE BASED ON LED MULTISPECTRAL IMAGING AND MULTIVARIATE ANALYSIS FOR MONITORING THE CONSERVATION STATE OF DEAD SEA SCROLLS

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This research is part of "The Dead Sea Scrolls Digitizing Project" of the Israel Antiquity Authority, that involves also the Library of Congress (Washington D.C., USA) and the University of Eastern Piedmont (Italy).

The aim of this project is the development of a non-invasive technique based on LED Multi-Spectral Imaging for monitoring the conservation of the Dead Sea Scrolls collection. Imaging spectroscopy is similar to color photography, but each pixel is described by many spectral bands, instead of just the three bands of the RGB color model. LED light source allows to obtain high spectral quality and to reduce heat and stress on the objects, as only the lowest possible amount of light necessary to properly expose the objects to the spectral band of interest is generated. The aim of the approach presented in the present article is the automatic detection of a possible deterioration process, its localization and identification (which wavelengths are interested), before the human eye can detect the damage or before greater damages take place.

The technique uses multivariate analysis and statistical process control theory. All the algorithms necessary for the treatment of the data were developed using MATLAB (The MathWork, USA).

In the present case of study the monitoring of parchment was considered. Abrupt changes in humidity and temperature, which may distort the surface and degrade its appearance, are the worst enemies of parchment; the greatest problem is the physical-chemical instability of the parchment due to its sensitivity with respect to the binomial temperature-humidity.

The application of this technique to real cases was performed at the Imaging Lab of the Library of Congress in Washington D.C., United States: the monitoring of an accelerated degradation process of a sample of parchment similar to the Dead Sea Scrolls was considered.

Keynote Lecture 9

CHALLENGES FOR THE RELIABLE QUANTIFICATION OF GENETICALLY MODIFIED ORGANISMS BY PCR METHODS

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There is a strong public and regulatory demand in several parts of the world regarding the labeling of products, mainly food and feed, with respect to their content of artificially introduced genetic modifications. In the EU Regulation (EC) 1829/2003 lays down this requirement for genetically modified organisms (GMO) which are authorised in accordance with Community legislation. Consequently analytical laboratories, in particular the ones performing official food and feed control, have to be able to detect, identify and quantify an increasing number of GMOs in a variety of complex samples.

The current measurement principle of choice for GMO quantification is based on the Polymerase Chain Reaction (PCR). It offers a number of advantages such as good detection power. But the quantification of modified DNA fragments from crops such as maize or cotton with sufficient reliability for legal decisions is a considerable analytical challenge. For instance, it was assumed for many years that the necessary calibration of real time–PCR methods is a straightforward procedure like for many other measurement methods. But systematic investigations on a range of well prepared GMO crop samples as well as on synthetic DNA fragments of various kinds have shown in recent years also limitations of the approaches used so far. Among others, the correlation between the quantification parameter of PCR, namely the number of PCR cycles to pass a set fluorescence threshold, and the GM concentration in the sample is not as theoretically predictable as assumed. Moreover different biological factors such as varying DNA concentrations in different parts of the crop and its zygosity can influence the accuracy of GMO analysis. In addition different provisions about the measurement unit in various legislations complicate the situation for analytical laboratories.

This presentation will outline the main analytical challenges in the quantification of GM constituents in food and feed samples for laboratories using PCR methods. A new systematic approach for the establishment of adequate reference measurement systems, including the development of independent calibrants and quality control materials, will be explained. Consequences for the performance of PCR methods by experienced laboratories and the reliability of corresponding analytical data for decision-making will be described as well.

IDENTIFICATION OF CANDIDATE BIOMARKERS IN PROTEOMICS AND GENOMICS BY MULTIVARIATE TOOLS

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The identification of pools of biomarkers is one of the leading research areas in proteomics and genomics. The identification of biomarkers is usually carried out in proteomics from the so-called spot volume datasets, produced from 2D gel-electrophoresis, where each sample is described in terms of the volume of the spots (i.e. proteins) present on the corresponding 2D-PAGE map; in genomics, instead, this research is usually carried out on microarray datasets, where each sample is described by the fluorescent intensity of a series of oligonucleotide probes.

When the identification of candidate biomarkers has to be accomplished in such cases, problems may arise related to the large number of spots present in each map/microarray chip and the small number of samples available for each class (control/pathological or control/treated). In spite of the multivariate structure of the available data, monovariate approaches like Student t-tests are still used, notwithstanding the classical approach does not take into account the relationships existing between the variables.

In such cases the use of multivariate methods is necessary and the exploitation of variable selection procedures provides a subset of possible biomarkers; this subset has to be both reliable and exhaustive to identify all the proteins/probes responsible for the differentiation between the groups of samples. The exhaustive search is important above all in studies about the mechanism of action of a disease or a drug rather than in diagnostic applications.

Different examples of multivariate analyses on proteomics and genomics datasets are proposed here based on a Principal Component Analysis (PCA) approach (Ranking-PCA) to provide a reliable set of candidate biomarkers. Examples will be presented on proteomics datasets related to clinical (cancer) and food (meat hanging) proteomics and genomics (Parkinson disease). Another example will regard the identification of biomarkers in environmental proteomics from MALDI-TOF spectra from control and chromium-treated mytili.

DETERMINATION OF HISTAMINE IN FISH AND FISH PRODUCTS BY HPLC/UV/MS WITH PRECOLUMN DERIVATIZATION

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A high-performance liquid chromatography system coupled with UV and single-quad mass spectrometry detector was used for histamine determination in fish and fish products after precolumn derivatization with dansyl-chloride. Due to matrix effect which led to signal suppression on UV detector, mass spectrometric detection with electrospray ionization (ESI) was used to enhance sensitivity and selectivity. The stationary phase was Kromasil C18 (250x4.6mm,10µm) analytical column. The mobile phase was a mixture of acetonitrile and 5% ammonium formate in ultrapure water flowing under gradient elution. The flow rate was 1.00ml/min. and the injection volume 40µl. The UV detector was set at 254nm. In the ESI source, high purity nitrogen was employed as the nebulizer. The ESI probe temperature was set at 400°C and the needle potential at 3kV. Positive ion mode full scan data acquisition was made over the m/z 300-600 range. Calibration curve was made using standard addition method by adding known amounts of histamine reference standard (1, 3, 6, 12, 25 and 50 mg/l) to the sample.

For the determination of histamine it is necessary to homogenize the sample of fish and fish products. It was measured 5g of homogenized sample and the extraction was done with 10ml 0,2M perchloric acid. After centrifugation, 100µl of supernatant, 200µl of Na_2CO_3 and 400µl of dansyl chloride were added into the vial and left to stand in a water bath at 60°C for 5min. Derivatization was stopped adding 100µl of ammonium formate solution. In the aqueous extract was added 500µl of toluene and the organic phase was evaporated under a stream of nitrogen. The pellet was resuspended in 200µl of acetonitrile and 10µl of 5% formic acid in acetonitrile and injected for LC analysis.

Validation of method was done in according to the EC Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

For all of the samples recoveries were from 97% to 101% and RSDs were smaller than 2.2%.

The content of histamine in selected samples (frozen fish, paste and fillets,), which were obtained from the Serbian market, were under the limit of quantification of method, 10mg/kg.

ANALYTICAL SCENARIO FOR THE INVESTIGATION OF BIOTRANSFORMATIONS OF SELENIUM IN PLANTS

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Selenium is an essential element for the proper functioning of humans and animals. This is due to its incorporation in proteins important for cell metabolism, known as selenoproteins. Selenium, if present in a biologically active chemical form, affects the elimination and inhibition of the formation of oxygen free radicals, thus protecting cells from damage, aging and metabolic disorders.

Anticancer properties of selenium are of particular interest. Although the mechanisms of its activities are not fully understood, a clear correlation between the increased morbidity of cancer and the deficiencies of selenium in the diet was noted. Several investigation on population from selenium-poor areas give a statistically proved evidence that the selenium deficit increased cancer appearance, when compare with the areas rich in this element. Therefore, all researches towards development of dietary supplementation of selenium, which could compensate a natural deficiency of selenium, are considered to be important.

It is expected that the consumption of vegetables, which have the ability of biotransformation of selenium inorganic forms into its organic derivatives – selenoaminoacids (in particular Se-methylselenocysteine), which are most beneficial for humans, may play an important role in the prevention of cancer. It was found that biotransformations of selenium depend mainly on the selenium compounds used for the enrichment of plants.

The aim of this study was to evaluate the biotransformation of different selenium inorganic precursors by onions (*Allium cepa* L.) grown hydroponically. In particular, identification of selenium organic compounds in plants was of a great interest as this allows understanding of the processes of biotransformation of selenium. In order to evaluate the growth of onion roots and the mitotic activity in apical meristem of roots, Allium Test was used.

The total content of selenium in various plant organs as well as in eluent obtained with various media used for extraction, was determined after microwave-assisted digestion in closed vessels followed by inductively coupled plasma mass spectrometry (ICP-MS). High-performance liquid chromatography (HPLC), coupled to an inductively coupled plasma mass spectrometry, was used for the selenium speciation.

The developed and validated analytical scenario was used for the determination of selenium and its compounds in plants organs, all towards evaluating the possible mechanism of selenium biotransformation in onions grown under various conditions.

S9: Bioanalysis II

SENSORS APPLIED IN THE EVALUATION OF REACTIVE SPECIES EFFECTS AND ANTIOXIDANTS PREVENTION

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Electrochemical sensors use in the evaluation of reactive oxygen species (ROS) effects proved its versatility, being applicable to direct assessment of ROS (HO$^{\cdot}$, superoxide radicals and lipoperoxides radicals), to indirect evaluation of ROS effects and to antioxidant prevention against ROS damage.

The aim of this work was to provide evidences on the bio-mimetic electrochemical sensors suitability in evaluation ROS effects against suitable oxidizable substrates: lipoproteins and phosphatidylcholine (PC) and in astaxanthin effect assessment against oxidative stress. Main interest was devoted to the astaxanthin from *H. pluvialis*.

The chosen oxidizable substrates were immobilized onto conductive supports, and their ROS oxidation was monitored. The antioxidants effect was quantified with respect to prevention of ROS damage.

Superoxide evolution was monitored at 0.6 ±0.020 V. Astaxanthin superoxide quenching property was evaluated compared with other recognized antioxidants and the index of antioxidants efficacy against the radical was: astaxanthin (*H. Pluvialis)*>b-carotene> astaxanthin standard > a-tocopherol.

For lipoperoxides (LOO$^{\cdot}$) monitoring, radicals (FR) thermo-generation was used. FR induce structural modification of the lipidic layer- create lipo-peroxides- which rise an electrical measurable signal at a potential of +0.395 ±0.020 V. The generated signal correlates linearly to the peroxidation degree, FR concentration and damaging effect. The quantification was made as a difference on the signal registered in the absence and in the presence of FR. The developed systems were further applied to real samples. It was proven that astaxanthin from *H. pluvialis* has higher preservative effect against lipo- peroxidation with respect to b-carotene, a-tocopherol and coenzyme Q_{10} on a concentration range of ROS from 10^{-9} molL^{-1} to 10^{-6} molL^{-1}.

Combined use of cerium oxide nanoparticles as redox mediator enhaced the sensors sensitivity.

Acknowledgement: The work was supported by projects Sensbiosyn (FP7-232522/2009) and BIOMONOXSIS (PNII 62053/2008).

HEN OR COCK? - SEXING OF CHICKEN EGGS BY INFRARED SPECTROSCOPY

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Usually, it is not difficult to distinguish between female and male birds. Differences in size and color of the plumage are clear external sexual characteristics. However, it is very difficult or even impossible to determine the gender of a fertilized but unincubated egg. Each year, billions of day-old layer chicks are produced in the world. Male chicks are culled shortly after hatching because only female chicks are reared for egg production. This is a serious problem, both in terms of animal welfare and waste disposal.

Knowledge of egg's gender would allow to select "female" eggs for breeding and to avoid the culling of the male chicks. The gender of an egg can only be determined by an investigation of the small germinal disc. The germinal disc contains already several thousand blastoderm cells. The difference in DNA content between male and female chicks is around 2 percent. In this contribution we demonstrate that infrared spectroscopy in combination with sophisticated multivariate data analysis is capable of characterizing of blastoderm cells and to determine the gender of the egg. Just a small hole in the egg shell is required to characterize blastoderm cells. Differences in the phosphate bands of nuclei acids allow a clear assignment of the gender. The infrared spectroscopic gender determination led to the same result as the goldstandard PCR analysis. This is important for future applications because infrared spectroscopy is the only method accurate and rapid enough to be applied in hatcheries to select only female chicken eggs and to avoid the killing of male chicks.

S10: Mass Spectrometry

Keynote Lecture 10

MOLECULAR CHARACTERIZATION OF SECONDARY ORGANIC AND AMBIENT FINE AEROSOL BY LIQUID CHROMATOGRAPHY / ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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During the past decade considerable progress has been made in our understanding of the fate of biogenic volatile organic compounds (BVOCs), including isoprene, monoterpenes, and sesquiterpenes, which are emitted in large amounts from the vegetation (estimated at 1150 TgC/year globally) into the atmosphere. Secondary organic aerosol (SOA) formation from the photooxidation (hydroxyl radical-initiated reactions) of these BVOCs has been studied under atmospherically relevant conditions, i.e., in the presence of NO_x and sulfuric acid seed aerosol. Molecular characterization of organic aerosol constituents is important because it allows one to gain insights into aerosol sources and the underlying mechanisms of SOA formation and transformation (or aging). Compounds that provide this valuable information are called “tracers” or “markers”. In this lecture, emphasis will be given to the chemical characterization of several novel SOA tracers from the photooxidation of monoterpenes, including α-pinene, β-pinene, *d*-limonene, and Δ^3-carene, using liquid chromatography/negative ion electrospray ionization mass spectrometry [LC/(–)ESI-MS], high-resolution MS, and detailed interpretation of MS data, as well as, in some selected cases, through synthesis of reference compounds. The structural elucidation of the following SOA tracers, which was carried out in close international collaboration, will be addressed:

- 3-methyl-1,2,3-butanetricarboxylic acid, a higher-generation α-pinene SOA product formed in the presence of NO_x and a suitable tracer for aged biogenic SOA;
- terpenylic acid and related lactone-containing terpenoic acids, early photooxidation products; and
- di-esters formed between pinic acid and terpenylic acid, major high-MW products in ambient aerosol from forested sites.

By now the major monoterpene SOA tracers in fine ambient aerosol from sites with coniferous vegetation have been structurally characterized. This is a prerequisite to develop quantitative LC/MS methodology and perform field studies in the future where time series, day/night chemistry, and dependence on meteorological parameters can be examined in more detail.

S10: Mass Spectrometry

DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY AND ITS PECULIARITIES IN THE ANALYSIS FROM SURFACES

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Direct Analysis in Real Time mass spectrometry (DART-MS) is now rapidly emerging. Earlier, due to the horizontal supply of gas from the ionization source, the introduction of samples, e.g., HPTLC/TLC plates as cut strips, was inconvenient and resulted in low repeatability. Recently a new version of DART source was suggested, which allows adjusting the angle of the gas stream. This extends the capabilities of DART-MS due to the introduction of and access to wide surfaces.

Recently we explored the capabilities of DART-MS arising from the *desorption at an angle*: scanning analysis of surfaces, including TLC-DART-MS, and a more sensitive analysis due to the preliminary concentration of analytes on glass surfaces. In order to select the most favorable conditions, proper positioning of samples is important, therefore a simple and cheap technique for visualization of the impact region of the DART gas stream onto a substrate was developed. A filter paper or TLC plate, previously loaded with sugar, was immersed in a derivatization solution. On this substrate, owed to the hot DART gas impact, the reaction to a colored product occurred.

An improved capability of detection of DART-MS for the analysis of liquids was demonstrated by applying large volumes of model solutions of coumaphos into little glass vessels and drying these solutions prior to DART-MS analysis at ambient conditions. This allowed the introduction of, by up to two orders of magnitude or even more, increased analyte quantities as compared to the conventional DART-MS analysis of liquids. Through this improved detectability, the capabilities of DART-MS in trace analysis could be strengthened.

The results on DART-MS and HPTLC-DART-MS analysis of honey and propolis will be reported. Peculiarities of analyte identification and quantitation using DART-MS and HPTLC-DART-MS will be discussed.

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IN-SOURCE FRAGMENTATION AND A SYSTEMATIC BOTTOM-UP APPROACH FOR UNTARGETED FLAVONOID DERIVATIVES SCREENING BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Flavonoids are plant secondary metabolites, which when consumed by human they have among others protective effect against cardiovascular diseases and certain cancers. Flavonoids are mostly present in plants as glycoconjugates, i.e., conjugated to saccharides and organic acids. Having suitable information on the conjugated forms is important because their bioavailability depend on the type of moieties linked to the aglycone. In most MS screening methods the utilized identification concept is based on mass difference calculations between the parent ions and the fragments that have lost their substituents. In this study the developed protocol is also based on neutral loss calculations, however an alternative approach is introduced. The tentative identification is implemented using a bottom-up hierarchical approach, which means the the structure of the sought glycoconjugate is virtually built up from the core of the molecule based on observed diagnostic ions without the need for any preliminary knowledge on the unknown intact compounds. In this protocol in-source fragmentation plays a key role, since theoretically every ion has the possibility to be fragmented and to provide diagnostic ions simultaneously, without compromise on the analysis speed. Additionally, if in-source fragmentation is utilized to generate diagnostic ions there is no need to preliminary define the *m/z* values of the precursor ions we want to isolate and subject to forthcoming fragmentation. Illustrative examples of the proposed approach for the screening of flavonoid derivatives in real samples using ESI-QQQ-MS and ESI-TOFMS will be given in the presentation.

A FAST ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF VOLATILE BIOMARKERS IN SALIVA

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Saliva is a promising option for the diagnosing of various internal diseases and the monitoring of therapeutic levels of drugs or illicit drug use. Futhermore, analysis of saliva is useful to investigate external exposure to hazardous volatile organic compounds. In comparison to blood and urine matrices, saliva has the advantage of being non-invasively collected, being readily accessible with fewer confidentiality concerns.

In the present work, we report a methodology for the rapid determination of biomarkers of both, internal diseases and exposure to environmental pollutants.The applicability of a non separative method based on direct coupling of a headspace sampler with a mass spectrometer (HS-MS) is explored.The samples are subjected to the headspace generation process and the volatiles generated are introduced directly into the mass spectrometer, thereby obtaining a fingerprint of the sample analyzed. The mass spectrum corresponding to the mass/charge (m/z) ratios ranging beetween 45 and 160 atomic mass unit (amu) contains the information related to the composition of the headspace and is used as the analytical signal for the determination of the studied compounds.

Use a multilevel partial factorial desing permits the construction of a stable PLS 1 calibration model using 25 mixtures of the 5 studied compounds such that the concentration of all of them are uncorrelated, thus obtaining the best prediction results possible.The calibration standards were prepared in ultrapure water since no matrix effect was detected in saliva samples.

The main advantage of the proposed methodology is that no prior chromatographic separation and no sample manipulation are required. Sample preparation is reduced to placing the saliva sample in the vial which reduces the analysis time and the experimental errors associated with this step of the analytical process. The results obtained were very similar to those provided with headspace-gas chromatography-mass spectrometry (HS-GC-MS). The method is rapid, simple, and in view of the results, highly suitable for detecting volatile biomarkers in saliva.

ION MOBILITY – MASS SPECTROMETRY AS A NEW APPROACH FOR THE SCREENING OF PESTICIDES RESIDUES IN FOOD

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Pesticide residue analysis requires methods that can determine hundreds of compounds at low levels in complex food matrices. This challenge has given rise to multi residue methods, the only efficient analytical approach. This type of analytical method entails a "generic" extraction followed by a soft or no purification step to avoid any analytes looses. With over a 1000 active compounds with different physical chemical properties, gas and liquid chromatography are used as complementary separative techniques.

In the past decade, the determination has been performed on tandem mass analysers, a powerful tool to overcome co-eluting compounds with excellent sensitivity. Nevertheless, these instruments can guarantee these results per acquisition cycles for more or less 150 compounds. This represents a serious limitation when the number of pesticides to be sought for monitoring and MRL enforcement is growing each year. As multiple injections for the same sample is not viable for the laboratories, we need to have alternatives options. We propose the investigation of ion mobility (IM) coupled with mass spectrometry as a new tool for pesticide residue analysis in food.

Ion mobility spectrometry experiments were performed on a Synapt HDMS G2 spectrometer (Waters, UK), which is similar to the Q-TOF instrument, except that the hexapole collision cell is replaced by an ion mobility cell. This technique allows separating an ion packet of a given mass to charge ratio by the mean of an electric field applied to the ion mobility cell containing a neutral gas at a control pressure. Therefore, for each ion you have additional information: the time required for an ion to travel through the mobility cell.

Optimisation of IMS parameters was performed using experimental design (Plackett-Burman and Central Composite Design) on a selected number of pesticides. The optimized conditions allowed the discrimination between a couple of isobaric compounds and some isomers. Two main classes of pesticides (organophosphorus and carbamates) were also separated. Ion mobility has the potential to separate target ions from matrix background and other co-eluting compounds from liquid chromatography improving signal to noise ratio of characteristic ions. This makes easier the identification process (ions ratio, exact mass, fragmentation pathway, …).

The advantages and drawbacks of this LC-IM-MS method for the identification of 190 pesticides in different food matrices will be discussed (the selection of compounds was based on the official national and community monitoring programmes target list).

S10: Mass Spectrometry

UTILIZATION OF ULTRA PERFORMANCE MASS SPECTROMETRY COUPLED LC AND GC IN FOOD SAFETY ANALYSIS

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The promising performance parameters of ultra performance mass spectrometry - mass resolution up to 100,000 (FWHM) and accurate mass data (<1 ppm) - are enabled by utilizing a unique multi-reflecting time-of-flight mass spectrometry technology referred to as a Folded Flight Path (FFP(TM). Key to this FFP technology are the two opposing, high precision rectangular gridless ion mirrors and an array of gridless periodic focusing elements separating them. The system provides selectable high mass resolutions of between 1000 up to 100,000 by offering different effective flight paths, The FFP system permits a long flight path in a compact design (up to 64 reflections corresponding to 40 m flight path). Also, key to the system is a novel high data acquisition speed hybrid detection system providing high in-spectrum dynamic range (> 4 orders) that allows the fastest separations to be measured with speeds of 200 full spectra/sec (written to the disc) utilizing the full mass range. The gridless design permits high ion transmission through the FFP flight tube resulting in high sensitivity and minimal signal loss with increasing resolving power. The novel FFP technology enabls hyphenation not only with the high-speed HR-GC, but also with ultra high pressure LC (UHPLC) to provide separations, which meet current analytical demands – high-throughput analyses with high mass accuracy.

In this contribution, targeted and non-targeted screening of a variety of analytes in complex food and related matrices is studied by coupling FFP MS with high-speed separation techniques. The real-life samples focused on the detection of pesticides, herbicides, mycotoxins and other emerging environmental pollutants, such as polychlorinated biphenyls (PCBs) and flame retardants (BDEs). The capabilities of this technology (high resolution and mass accuracy) enabled the identification of analytes using elemental composition based accurate mass analysis and isotopic fine structure.

S11: Environmental Analytical Chemistry I

Keynote Lecture 11

TRACE ELEMENTAL SPECIATION ANALYSIS – HOW TO SAVE ORIGINAL MOLECULAR INTEGRITY

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Speciation analysis is aiming to define and quantify the distribution of an element between the different species in which it occurs. The major problem of speciation analysis is to maintain the original molecular integrity and the original distribution of species through first steps of analysis including sampling, sample storage and sample preparation. The ideal solution to this problem would be *in situ* analysis. However, very few techniques provide the necessary selectivity and sensitivity required for trace element speciation analysis. Coupled techniques combining the separation power of a chromatographic or equivalent separation technique with the detection power of the most sensitive atomic spectroscopic techniques are the most applicable. Recent developments of some these techniques will be discussed.

For water samples the best sample preparation for speciation analysis is no preparation at all or pH adjustment. The lecture will present these issues using concrete research results with arsenic speciation analysis in water.

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DETAILED MEASUREMENT MODEL FOR THE ASSESSMENT OF ATMOSPHERIC AEROSOLS WATER SOLUBLE FRACTION

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Air composition is known to have a relevant impact on public health, on the environment and on meteorology and climate. The chemical characterization of air gas and aerosol fraction is used to monitor risks from exposition to polluted air and to develop air transport and climate models. The reliability of conclusions about the systems under study depends on the quality of the measurement results.

The ionic composition of the water soluble fraction of aerosols, assessed by Ion Chromatography, IC, is particularly useful to estimate the mineral composition of aerosols of both natural and anthropogenic origins [1].

The assessment of trends in air composition must be supported on measurement results traced to a common reference and reported with measurement uncertainty.

In this work, a metrological model has been developed for the determination of ion composition of the atmospheric aerosols water soluble fraction according to an operationally defined measurement procedure. The uncertainty associated with the results is evaluated by the Differential Approach [2]. The metrological relevance of individual analytical steps and effects has been identified. The uncertainty budget is divided in the extraction, extract dilution and ionic chromatography quantification components. The evaluation of the extraction step involved the estimation of the intermediate precision from duplicate analysis of pooled urban aerosol samples prepared from real samples to ensure homogeneity. The uncertainty associated with the preparation of duplicate filters was considered.

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PROTECTING EUROPE'S WATER RESOURCES: DEVELOPMENT OF NEW WATER BASED CERTIFIED REFERENCE MATERIALS IN SUPPORT OF THE WATER FRAMEWORK DIRECTIVE

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With a growing population and high standards of living come increasing pressures on water resources. In 2000, the Water Framework Directive (WFD; [1]) was adopted and since then, the European Commission has issued a number of directives (e.g. the Marine Strategy Framework Directive, [2]) providing the legislative framework for the protection of water resources.

The WFD stipulates that all Member States have to establish monitoring programmes to assess the qualitative and quantitative status of natural water bodies within the European Union. At the heart of such programmes are measurements. Confidence in any assessment of water quality depends directly on the quality of measurement data and it is the responsibility of monitoring institutions to implement quality assurance/ quality control systems to guarantee that reported results meet assured target levels of precision and bias, also in view of compliance with legal limits. Accuracy, traceability and comparability of measurement data can be ensured if appropriate certified reference materials (CRMs) are available for the validation of analytical methods.

The role of the JRC-IRMM in supporting the WFD will be outlined and special attention will be drawn to the development of water-based CRMs in response of the needs of the laboratories charged with water quality monitoring. Of particular importance are the materials ERM-CA615 (natural groundwater; material released in 2010), ERM-CA713 (domestic wastewater) and ERM-CA403 (seawater). These three materials are targeted to comply with the requirements laid down in Directive 2008/105/EC [3] and are (to be) certified for Cd, Hg, Ni and Pb, four metals on the list of priority substances of the WFD for which the European Commission has set stringent environmental quality standards.

[1] European Commission, Directive 2000/60/EC, OJ Eur. Comm. L 327
[2] European Commission, Directive 2008/56/EC, OJ Eur. Union L 164
[3] European Commission, Directive 2008/105/EC, OJ Eur. Union L 348

PHOTODEGRADATION OF METOPROLOL USING UV, O_3 AND O_3/UV IN DIFFERENT NATURAL WATERS

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Metoprolol is a widely used selective β–blocker. It has been found in various aquatic environments. Even though adequate ecotoxicity data is not available, it is of primary importance to obtain information about its fate in water matrices. The aim of this work was to investigate the applicability of metoprolol degradation under UV radiation, in the presence of O_3 and under simultaneous influence of both O_3/UV in different waters (Danube and thermal water). It has been found that the degradation rate depends on the type of the method used, as well as on the type of the matrix. Influence of different waters is compared with degradation in deionized water. The degradation is faster in natural waters using ozonation or UV-photolysis, but no significant enhancement effect can be observed in case of their combination. To elucidate the above effect, we performed ion analysis of mentioned waters based on which we investigated the influence of different ions on the degradation of metoprolol in deionized water. The degradation kinetics was monitored by HPLC/DAD, TOC and ion chromatography.

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MONITORING AND MODELLING OF PRIORITY POLLUTANTS IN IRISH WASTEWATERS

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In 2000 the Water Framework Directive (WFD), 2000/60/EC, was introduced and a group of chemicals, including pesticides, polycyclic aromatic hydrocarbons, and metals were chosen as priority pollutants according to Annex X. The levels of these priority pollutants in the environment are regulated by set environmental quality standards (EQSs) and are affected by a number of emission factors including anthropogenic activities, population equivalents, and weather. In order for these EQSs to be enforced, regular monitoring of all water bodies must be carried out, a process which is both costly and time consuming. Waste water treatment directly affects the quality of receiving water bodies and the environment. These facilities must be operating properly to ensure removal of pollutants before outflow. It is also important that sampling and extraction methods minimize any loss of sample, employing strict controls where necessary.

We have developed a model defining emission levels relating to priority pollutants occurrence in the environment. This is based on information collected from local authorities, rainfall from Met Eireann, traffic data and road runoff and pollutant levels in waste water treatment plant (WWTP) effluents. In order to evaluate the model effluent samples have been collected from 8 WWTPs, both secondary and tertiary, over a period of 2 years, including several weeks of summer and winter high intensity sampling. These sampling periods take account of wet and dry weather flow. Solid phase extraction, (SPE), is used in the sample preparation process with subsequent analysis by gas and liquid chromatography (GC and LC) with mass spectrometric detection (MS). These results have been incorporated into the model, allowing for predictions of pollutant levels for certain emission factors. Evidence is clear that usage criteria play a significant role in pollutant occurrence and thus can help define a useful monitoring plan.

COUNTERCURRENT CHROMATOGRAPHY AND RELATED TECHNIQUES IN ENVIRONMENTAL ANALYSIS

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Rotating coiled columns (RCC) have been mainly applied to the separation of organic and bioorganic solutes by countercurrent chromatography (CCC), a support-free liquid-liquid chromatography. CCC methods for the extraction of trace rare and rare earth elements from digested geological samples have been also proposed, two-phase liquid systems with different solvents and extracting reagents being employed. Recently it has been evaluated that RCC can be used for the separation of both solutes and particles, not only in liquid–liquid systems but in liquid–liquid–solid and liquid–solid systems as well. The use of a particulate suspended matter as the stationary phase has opened a new door for unconventional applications of RCC in the analysis of heterogeneous and solid environmental samples.

A procedure has been developed for the direct recovery of toxic organic compounds (polycyclic aromatic hydrocarbons, PAHs) from a sewage sludge medium in liquid–liquid–solid systems. The recovery of PAHs from dried soil and sediment samples becomes possible if liquid–solid systems are used. It has been shown that the particulate samples can be successfully retained in the column and kept well mixed and agitated whereas organic solvents or their mixtures are pumped through the column. No clean-up step is needed before the subsequent HPLC-analysis of extracts. Besides, the dynamic multistage extraction performed in the rotating column at room temperature and normal pressure may have nearly the same efficiency as accelerated batch solvent extraction repeated three times at 150°C and 14 MPa.

A new approach to the dynamic fractionation of trace metals and metalloids in soils and sediments has been also proposed. Different eluents (aqueous solutions of acids, salts and complexing reagents) provides sequential extraction of trace elements species according to their physicochemical mobility and potential bioavailability. The method provides environmentally relevant data and is time saving since it requires only 4-5 hours instead of several days needed for routine batch sequential extraction. On-line coupling of leaching in RCC and ICP-AES measurements is possible. The extraction curves facilitate the investigation of the leaching kinetics and the study of trace and major elements associations.

RCC can be also used for the field-flow fractionation of micron and submicron particles of different nature. In general, the proposed methods and approaches look promising and can be further applied to inorganic, organic, and speciation analysis of environmental samples.

Keynote Lecture 12

NEW STRATEGIES TOWARDS NANOBIOANALYTICS

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The systematic analysis of interactions of molecules with biological entities requires the development and application of orchestrated experimental tools and analytical methods, which allow a multi-parametric quantitative determination and imaging of molecular events, molecular pathways, and molecular signals. Hence, in-situ analysis at the level of live individual cells, ensembles of small biological entities, and entire organisms with the required molecular selectivity, sensitivity, and temporal/spatial resolution is needed. Recent research is directed towards combined and modular measurement platforms for obtaining quantitative and dynamic information. Towards these efforts, combinations of individual scanning probe microscopy techniques such as combined atomic force-scanning electrochemical microscopy (AFM-SECM) have been demonstrated [1]. Still, optimized physical and chemical properties of such combined probes are a prerequisite for reliable simultaneous data acquisition in AFM-SECM measurements. Recent developments are targeted towards high-resolution electrochemical imaging, novel electrode materials such as integrated boron-doped diamond electrodes and integrated sensing schemes. Further developments include combining scanning probe techniques with *in-situ* infrared attenuated total reflection spectroscopy (IR-ATR) [3,4] for simultaneous spectroscopic access to surface processes during topographical imaging.

An overview on the state-of-the-art, current challenges, and future potential of developing such combined and modular measurement platforms will be presented. Applications relevant to biomedical and material sciences of such combined analytical techniques demonstrating the potential for nanobioanalytics will be shown.

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THERMALLY STIMULATED CURRENT SPECTROSCOPY AS A NEW TOOL IN PHARMACEUTICAL MATERIALS SCIENCE

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Recent developments in the pharmaceutical industry have led to an enormous increase in the variety of dosage forms available to the formulator. The stability of active compounds as well as the target site of the active material will govern the selection of the dosage form which is used in a given case. The design of pharmaceutical dosage forms requires not only a detailed knowledge of basic physical and chemical principles and states of aggregation, but also an understanding of the anomalies, transitions and interactions between different states of matter.

Although an extensive number of techniques provide important information about the phase transition processes and interactions between drugs and excipients, new/alternative techniques are also frequently being used to identify previously undetected transitions, interactions and reactions.

Thermally stimulated current (TSC) spectroscopy is a technique that monitors motions of molecular dipoles induced by the influence of static electrical field as sample is cooled or heated. Uniqueness of technique is in its ability to detect and characterise molecular motions at sub-ambient temperatures as well as to detect and characterise molecular mobility that cannot be detected by any other technique. Majority of materials examined using TSC reveal additional information which was not previously reported in the literature but which may be of high importance in understanding the properties of materials, as well as their interactions with other molecules in pharmaceutical formulations.

Obtained results indicate that TSC is a useful tool for the detection and characterisation of glass transition processes, polymorphic transitions and other solid-state processes in pharmaceutical materials. Compared to other thermal techniques, TSC shows greater sensitivity, especially in the case of energetically weak transitions, and better resolution compared to dielectric analysis.

Ongoing research focuses on better understanding of molecular arrangements/interactions in co-crystals, their stability as well as prediction of drug excipient compatibility.

S12: Advanced materials/Instruments development

SOLID-STATE LIGHT SOURCES: THE 21ST CENTURYLIGHT SOURCES FOR MINIATURISED AND PORTABLE ANALYSIS?

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Solid-state light sources (SSLSs) based on semiconductor technology are expected to become the light sources of the 21st century. Known as light emitting diodes (LEDs) and laser diodes (LDs), SSLSs offer advantages in many areas. In chemical analysis it is their compatibility with miniaturisation, robustness, low costs, relatively low power consumption, negligent radiative heating as ‘cold’ light sources, low optical noise, and operation in pulsed mode up to GHz frequencies.

The application range of SSLSs with focus on miniaturised analysis and separation science will be illustrated by examples, including the use of LEDs and LDs for optical detection (absorption-photometric and fluorimetric) in both research designs and commercial instrumentation in microfluidic chips and on-capillary detection in separations, with applications ranging from low-MW analytes to cells. The examples will include the use of commercially available LEDs/LDs such as a 255 nm deep-UV-LED, a high optical-power 470 nm LED, white LEDs as broad spectrum light sources for spectrophotometry, a custom made optical fibre-coupled micro-LED-array sources with integrated interference filter and simple low-cost experimental alternatives to classical fluorescence imaging instrumentation shown in capillary and microfluidic chip designs. The relevant features, strengths and limitations of current LEDs and LDs and the future potential of the SSLS technology in particular for miniaturised, portable and on-site analytical instrumentation will be discussed.

LASER INDUCED BREAKDOWN SPECTROSCOPY WITH THE USE OF FAST DIODE PUMPED SOLID STATE LASER

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Recycling of materials became increasingly important in recent years because of scarcity of resources and the associated development of raw material prices. The first step of the recycling, the sorting of scrap requires a high sensitive and ultra-fast analytical technique. For this purpose, Laser Induced Breakdown Spectroscopy (LIBS) is perfectly suited because of its high sensitivity corresponding to the limits of detection in the low ppm range, multi-element capacity, high throughput analysis and the possibility of the analysis without sample preparation. In the current work the possibilities of a novel type lasers, namely diode pumped solid state (DPSS) lasers, as ablation/excitation sources for LIBS were studied for the high throughput analysis of trace impurities in industrially relevant alloys like steel. In direct comparison with the commonly used flash lamp pumped solid state (FLPSS) lasers, DPSS lasers have lower pulse energy (typically < 1 mJ) but higher (up to several 100 kHz) and variable repetition rates. The latter allow for a faster online monitoring as well as surface mapping of heterogenic scrap material. On top of the smaller footprint of DPSS lasers, their low power consumption eliminates the necessity of water cooling making these lasers ideal candidates for portable LIBS systems.

To make optimal use of these advantages, in the present study a DPSS laser was used in combination with a novel, miniaturized Echelle spectrograph with high spectral resolution. A set of conducted experiments revealed that despite the significantly smaller, faster and more cost efficient setup the use of DPSS lasers allows a direct and unambiguous classification of the samples. Beyond that, a direct comparison with a conventional FLPSS laser setup showed that the figures of merit were even better for the new setup. Likewise the limits of detection of the DPSS-LIBS for the analytes studied were found to be in the low ppm range.

S12: Advanced materials/Instruments development

A VISCOUS FILM SAMPLE CHAMBER FOR LASER ABLATION INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

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Laser ablation - inductively coupled plasma - mass spectrometry (LA-ICP-MS) is a powerful method to determine the elemental composition of solid-state samples as it combines the high sensitivity and isotope selectivity of ICP-MS detection and the simplicity of laser ablation sampling.

This technique enables rapid multiple sampling of the analysed material, such as needed for mapping or in-depth profiling applications. However, the duration of these measurements is practically restricted by time taken for the particle to be transported from the sampling point to the ICP torch. Accordingly, several cell configurations have been devised in the last decades, trying to combine high removal rate, high efficiency (i.e. complete transport of the ablated material) and reduced memory effects. These goals may be achieved by carefully designing the geometry of the cell and its gas flow patterns. Recently, the implementation of a cell based on a cyclonic gas flow reduced the removal time to around 30 ms. The latter figure was nevertheless limited to the central part of the cell, whereas higher values (around 1 second) were registered in the other cell positions. Aim of this presentation is to introduce a new cell design which enables a homogeneous fast removal (lower than 200 ms) from a cylindrical chamber with 70 mm diameter. Such a result is achieved by combining the cyclonic flow pattern with an extraction tube coaxial with the laser beam and thus constantly positioned on the ablation spot. The cell sealing is warranted by a viscous film junction between the cell floor and cover. Optimisation and performances of the apparatus will be discussed in detail together with possible implementation of this design.

NMR SPECTROSCOPY - NOVEL TECHNOLOGIES AND APPLICATIONS

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Highly sophisticated developments in NMR technology and methodology, as well as innovative integrated software solutions, have significantly pushed the limits of NMR applications especially in the fields of natural product research, food quality control, pharmaceuticals, medicine, or material sciences.

The very latest developments are presented in the context of a choice of advanced NMR applications.

(1) Food quality control: based on a dedicated juice screening device, delivering about 40 parameters from one measurement under full automation[1], the methodology developed now can be transferred to quality/authenticity control of wine or edible oil.
(2) Clinical research: NMR can help to diagnose metabolic disorders in newborns and children[2]. With the BBIOREFCODE database of urinary metabolites, single compounds like specific biomarkers can be identified by an automated routine.
(3) Natural products screening by LC-SPE-NMR/MS
(4) Complete Molecular Confidence and Assure: automated quantification and structure verification for high throughput and automation workflows or mixture analysis, small molecule structure elucidation, and impurity identification for raw material screening can be significantly accelerated by the assistence of the innovative NMR software tools.
(5) ... and more !

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Plenary Lecture 7

THE POWER OF SELECTIVITY IN CHROMATOGRAPHY

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A key parameter in separation sciences relates to the selectivity term α_{ij}. But what drives selectivity for chromatographic processes? Phenomenologically speaking and simplifying, it is related to (minute) differences of intermolecular interactions of the analyte molecules with the (chemically modified) surface in case of adsorption chromatography but also to (minute) differences of distribution coefficients of the analytes between two liquid media in case of partition chromatography. In both cases one needs to deal with adjusted liquid mobile phase compositions which become part of a “troika” describing the (physico)chemical properties of the analytes, the surface (adsorbent or stagnant stationary phase) and of the mobile phase components.

In reality one deals very often with mixed modal selectivity principles involving in any case multiple solvation – de-solvation and re-solvation events in the course of intermolecular interactions during the chromatographic process. Apart from all the physico-chemical equilibria taking place in the chromatographic bead and in the pores of the chemically modified particles (e.g. porous silica) the selectivity causing events can be discussed on a molecular level and on molecular descriptors associated with the chemical and spatial structure of the analytes (selectands, SAs) and of the modified surface which may carry a special ligand (selector, SO). For intermolecular interactions all types of forces can be envisioned spanning from the strong to weak electrostatic, hydrogen bonding – hydrogen donating, and van der Waals types. Their activity can be tuned by the mobile phase composition. Following these straight forward concepts one can design separation systems essentially selective for (very) large biomolecules as e.g. proteins, DNAs, glycanes but seemingly for small molecules, and of particular interest are often also stereoselective systems. For very polar compounds HILIC and ion exchangers (including zwitterionic ones) have proven their exceptional suitability to solve challenges where RP systems fail.

In this lecture I will try to pinpoint on the various effects on selectivity adjustments to de-convolute interwoven effects. Representative examples highlighting inter alia enantioslective separations but also monoclonal antibody purification will be shown.

Plenary Lecture 8

ELECTRONC BIO-SENSING WITH ORGANIC THIN-FILM TRANSISTOR

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To satisfy the demand for fast and smart analytical systems a great interest has been focused on novel biosensing devices and organic field-effect transistors (OFETs) appear as a new emerging technology. OFET can electronically detect analytes in vapour systems [1] and even chiral compounds at unprecedented low concentrations [2]. Integration of membranes and proteins into electronic devices involves cross-disciplinary effort and can be exploited as highly performing recognition elements in electronic sensing devices. In this presentation an overview of the Bio-FET sensors field will be presented showing the advancements of the last years.

Recent achievements obtained with organic semiconductor FET biosensors realized through the full integration in the electronic device of the biological recognition elements will be also presented [3]. The coupling of the OTFT device and the biological recognition system is actuated by assembling supra-molecular structures in which biomolecules, such as membranes and proteins become an integral part of the OTFT active material. Specific reactions (i.e receptor/analyte binding) are then used for analyte detection. To perform the bio-sensing measurements, the solution containing the analyte is deposited on the organic semiconductor, or through a proper microfluidic system. Preliminary results show that such bio-electronic devices can be very selective reaching detection limit (LOD) in the low ppt range. In addition, such sensors allow for label-free detection.

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Keynote Lecture 13

USE OF SYNCHROTRON TECHNIQUES TO STUDY THE ENVIRONMENTAL IMPLICATIONS OF NANOPARTICLES IN THE ENVIRONMENT: THE CASE OF TERRESTRIAL PLANTS

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The impact of metal nanoparticles (NPs) on biological systems is still not well understood. The aim of this research was to determine the effects of zinc oxide (ZnO) and cerium oxide (CeO_2) NPs in velvet mesquite (*Prosopis juliflora-velutina*). Seedlings were in hydroponics with ZnO NPs (10 nm) and CeO_2 NPs (10 nm) at concentrations varying from 500 to 4000 mg L^{-1}. Cerium and Zinc concentrations in tissues were determined by ICP-OES ; while biotransformation and elemental distribution were determined by XAS and µXRF, respectively. The ICP-OES data showed that plants exposed to 500 mg ZnO NPs L^{-1} had 3837 ± 161, 1061 ± 70, and 423 ± 77 mg Zn kg^{-1} d wt in roots, stems, and leaves, respectively. While plants treated with 2000 mg CeO_2 NPs L^{-1} had 4504 ± 520, 935 ± 90, and 438 ± 121 mg kg^{-1} d wt in roots, stems, and leaves, respectively. None of the NPs reduced plant growth. Synchrotron XANES spectra demonstrated clear evidence of the presence of CeO_2 NPs within tissues but ZnO NPs were not observed. Furthermore Zn was found as Zn(II), resembling the spectra of $Zn(NO_3)_2$. The Synchrotron µXRF analysis confirmed the presence of Zn in the vascular system of roots and leaves of ZnO NP treated plants.

RECENT TRENDS IN AUTOMATIC DYNAMIC LEACHING TESTS FOR ASSESSMENT OF BIOACCESSIBLE FORMS OF TRACE ELEMENTS IN ENVIRONMENTAL SOLID SUBSTRATES

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In this presentation, dynamic column-based fractionation is presented as a new methodological approach for risk assessment of trace elements in environmental solids. Different analytical techniques capitalized on the various generations of flow analysis are overviewed and pros and cons for exploration of mobilizable metal forms within the framework of environmental availability are critically discussed.

Limitations of earlier flow-based microcolumn fractionation methods in terms of undue flow backpressure, lack of sample representativeness and impaired sample throughput have been overcome in our research group by the exploitation of liquid drivers enduring moderate pressures, the design of appropriate sample containers and multiple-extraction automatic devices along with the extraction of samples under fluidized-bed conditions [1,2]. In addition, automatic processing of leachates by sorptive microextraction techniques involving bead-injection [3] and dispersed carbon nanomaterials [4] fostered the reliable quantification of the most ecotoxicologically significant forms of hazardous elements at the sub-ng/L level.

The lecture also summarizes novel applications of dynamic extraction/fractionation as a valuable tool for investigation of the potential reuse of solid wastes (e.g., bottom ash) for civil engineering works [2,5], and for inferring whether or not biomass (biofuel) resources might be fired in combustion devices.

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DISTRIBUTION OF HEAVY METALS AND NUTRIENTS IN DIFFERENT SIZE FRACTIONS OF FLY ASH FROM BIOMASS INCINERATION

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Biomass (especially wood) counts as CO_2-neutral fuel and is therefore increasingly used for the generation of heat and electricity (transportation and pre-treatment of the biomass not taken into account). After incineration, the inorganic components remain as ashes (1-3% of the input mass).

Bottom and fly ashes contain nutrients for soils and plants (e.g. phosphorus, potassium, calcium and magnesium) but especially the fly ashes are also loaded with heavy metals. This restricts their use on agricultural used soils and in forests and they are usually disposed.

In this investigation, fly ashes from an Upper Austrian biomass incinerator plant have been separated in the lab with an air classifier into different grain size fractions to study the distribution of pollutants and nutrients. All ashes (the original ashes and the grain size fractions) were then digested in a microwave assisted digestion unit with hydrochloric acid and nitric acid at around 200°C. For the determination of metals ICP-OES (inductively coupled plasma optical emission spectroscopy) was used.

It could be found that some elements (e.g. magnesium) are distributed homogenously throughout the different grain size fractions of the fly ash whereas others (e.g. cadmium and potassium) are found in higher concentrations in the finer the ash fractions

These results are interesting in terms of recycling fly ashes from biomass incineration processes as nowadays they are mostly disposed because of their high heavy metal concentrations. By classification the fly ash can be split into a fine fraction with higheer loads of heavy metals and a coarser fraction which could be used as fertilizing agent for soils thereby reducing the necessary amount of synthetic fertilizers. Also the costs for disposing of fly ashes are minimized.

ENVIRONMENTAL STRATEGIES TO REMOVE VOLATILE AROMATIC FRACTIONS (BTEX) FROM PETROLEUM INDUSTRY WASTEWATER USING BIOMASS

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Petroleum extraction, an activity that generates considerable economic benefits, can also bring various environmental impacts, one of which is associated with the large amount of produced water discharged into aquatic environments. A particular concern is that these discharges can contain significant concentrations of toxic compounds including benzene, toluene, ethylbenzene and isomers of xylene (together termed BTEX). One option that can be employed to minimize the concentrations of these compounds entering the environment is to treat the water prior to its discharge. The present work therefore investigates the potential of peat and angico hardwood sawdust to remove BTEX from produced water. A sample of produced water was collected from the Bonsucesso treatment plant, in the city of Carmópolis, Brazil. Identification and quantification of BTEX compounds employed purge and trap gas chromatography. In batch experiments, the removal capacities using peat were 32.4, 50.0, 63.0, 67.8 and 61.8 % for benzene, toluene, ethylbenzene, m,p-xylene and o-xylene, respectively. This compared with removal capacities using angico sawdust of 20.2, 36.4, 52.8, 57.8 and 53.7 % for these compounds, respectively, demonstrating the superior performance of the peat. The optimized chromatography technique delivered accuracy exceeding 90 %, precision better than 6 %, a linear correlation coefficient (r^2) of 0.998, and limits of detection and quantification of 0.09-0.18 μg L^{-1} and 0.27-0.35 μg L^{-1}, respectively. This work has demonstrated the considerable potential of alternative materials for efficient removal of organic compounds from aqueous media.

STIR BAR SORPTIVE EXTRACTION: A SUITABLE SOLVENTLESS TECHNIQUE FOR SOIL SAMPLES?

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Stir bar sorptive extraction (SBSE) is a solventless sample technique introduced by Baltussen et al. to extract organic analytes from environmental samples by sorption onto polydimethylsiloxane (PDMS) coated stir bars (also called Twisters^{TM}). This technique has mainly been used for the analysis of contaminants in water samples. With regard to soil samples, most applications reported require a previous extraction step with techniques such as ultrasonic solvent extraction or pressurized liquid extraction. The extract, previously diluted in water, is subjected to the SBSE extraction process.

The aim of this study is to evaluate whether stir bar sorptive extraction can be applied successfully for the extraction and determination of organic compounds directly from soils without any organic solvent. Few references are available concerning the extraction of pollutants directly in soil samples. To the best of our knowledge, only Tan et al. have analyzed a range of endocrine disrupting compounds in biosolid and sludge samples, using SBSE directly in solid samples.

As model analytes for this approach we have chosen parabens (methylparaben, isopropylparaben, n-propylparaben, butylparaben and benzylparaben), triclosan and methyl triclosan. An *in situ* derivatization reaction is also proposed, in order to reduce the polarity of the target compounds. The methodoly proposed here offers important advantages over the conventional extraction techniques, such as minimizing sample handling, the complete elimination of the use of organic solvents, and simplification of the analytical procedure, with reduced time consumption. Additionally, the use of a derivatization reaction does not complicate the process, since it occurs at the same time as extraction.

TWISSELMANN EXTRACTION VS. ULTRASONICATION EXTRACTION FOR ANALYSIS OF ORGANOPHOSPHORUS FLAME RETARDANTS IN SOILS

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Great global consumption of organophosphorus flame retardants (OFR) caused increased concentration levels of these substances in nearly all environmental compartments. A new methodology is presented for analyzing of OFRs in soil samples. The extraction effectiveness of Twisselmann extraction and ultrasonication extraction was compared by analyzing spiked soil samples with toluene as a solvent. Soil extracts were evaporated to dryness, dissolved in a mixture of methanol/tap water (1:14) and ultrasonicated for 60 min. Extracts were filtered and analyzed by SPME/GC-MS.

High recoveries obtained by Twisselmann extraction ranged from 77.0% to 89.6% (TnBP, TBEP, TCPP, and TCEP). Recoveries for TDCP and TPP were only 31.5% and 42.0%, respectively (RSD 0.3-16.2%, n = 3). By ultrasonication recoveries were three to four times lower (27.4% to 30.6%) than those obtained by Twisselmann extraction, except for TDCP and TPP (RSD < 3 %, n=3). Longer extraction time with hot toluene caused better extraction efficiency and thus Twisselmann extraction was applied for analysis of soil samples collected from different sampling locations to define input sources of OFRs in soils. Two chlorinated OFRs, TCEP and TCPP, were detected in all soil samples in concentration ranges of 0.7 - 13.5 ng g^{-1} dwt and 0.59 - 8.33 ng g^{-1} dwt, respectively. The data indicated a positive correlation of the TCEP concentration in soil with population density and rain on the day before sampling. Results obtained within this study present the first data of OFRs in soil samples and further analysis should be proceeded to investigate influence of dry and wet deposition processes on the OFR concentrations in soil samples.

S14: Sensors

Keynote Lecture 14

OPTO-ELECTROCHEMICAL NANOSENSOR ARRAY FOR REMOTE DNA DETECTION

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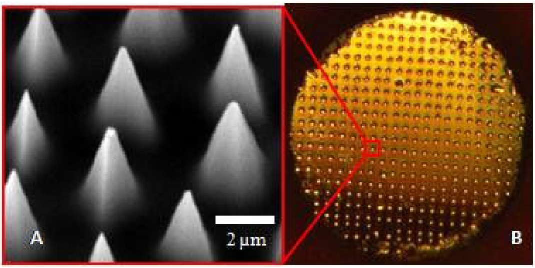
We developed a DNA nanosensor array on an original optical platform. A coherent bundle of 6,000 optical fibers is transformed in an ordered array of nanoelectrodes which are then functionalized by electrocopolymerization of a thin polypyrrole film including oligonucleotide (ODN) probes. The fabrication of the nanoelectrode array has already been detailed elsewhere.(1) To obtain a multiplexed biochip, an electrochemical cantilever has been used to deliver very small amount of solution (2). The spots are realized by electrocopolymerization of pyrrole and pyrrole-ODN monomers (3) in the limit of the picoliter drop on the conductive surface. The same process is repeated with other probe-containing solutions at determined positions of the plateform, and so different probes have been addressed onto the same nanoelectrode array (4). It allows to obtain high-density DNA chips which are able to perform remote fluorescence imaging with the advantages of ultramicroelectrode properties. Since such nanostructured optical surfaces are efficient SERS substrates (5), this approach could lead to new label-free plasmonic biosensors.



A) SEM image of the functionalized optical nanotips.
B) Image of ~400 spots deposited on nanostructured electrode.

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ADDRESSING THE ANALYTICAL CHALLENGES OF BIO-SENSING WITH NANOMATERIALS AND NANOSTRUCTURES

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The ability for direct, continuous selective and sensitive detection of large number of bio-chemical substances is of high socio-economic significance. Most of these substances, such as toxic compounds (gases, liquids), biomaterials (proteins, enzymes, virus) and nutrients (nitrates perchlorates etc) need to be detected in-vivo, or on-line and the results presented in real time. Two extreme such environments are the surfaces of the planets, and the deep see. The technology of biosensors and chemical sensors can cover these needs, assuming that they have the desired analytical characteristics.

The continuous progress of nanotechnology in material science has led to the development of nanostructure materials with unique chemical, physical, and mechanical properties. Some of these unique properties are ideal for the development of bio-chemically active nanosystems, with the desired selectivity and detection limits that can allow for the direct, on-line or in-vivo monitoring of many life sustaining or threatening substances. Even thought detection systems are usually based on electrochemical transduction, nanomaterials are promising candidates for the development of optically active systems, for a more direct and straight forward transduction.

In this presentation we will present the latest achievements in the area of bio-sensor design utilizing nanostructures. Emphasis will be placed on the development of bio-chemical sensors that can be deployed on unmanned rover units for remote sensing of substances and nutrients in the Mars planet, and for deep see research.

MINIATURIZED ELECTROCHEMICAL SENSORS BASED ON SCREEN PRINTED ELECTRODES WITH MOLECULAR IMPRINTED POLYMERS AS BIOMIMETIC RECEPTORS

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Screen printed electrodes are convenient supports for biological and synthetical receptors because they can be produced in a very reproducible and cost effective way, and can be easily derivatized and implemented in miniaturized devices. Molecular Imprinted Polymers (MIP) are synthetical receptors containing recognition sites with recognition properties similar to those of natural receptors, while being easier to prepare in a reproducible and low cost way. For these reasons they are widely employed in electrochemical sensors with amperometric, voltammetric (1) and potentiometric transduction (2).

In the present investigation two sensors for dopamine (DA) based on the same MIP membrane, but with different electrochemical transduction methods, are compared to investigate the respective merits:

- Voltammetry, based on an electrochemical platform integrating in the same strip three planar electrodes, i.e. the working electrode made of carbon ink, the counter electrode, made of carbon ink too, and the pseudo-reference electrode, made of Ag ink. The cell was completely coated with the acrylic MIP, which constitutes the actual electrolytic medium. The oxidation of DA present in the MIP membrane is the reaction taking place at the working electrode, recorded by Differential Pulse Voltammetry.
- Potentiometry, based on a planar carbon ink working electrode coated with the same MIP membrane. The potentiometric signal comes from the selective combination of the positively charged dopamine ion with MIP. The carbon ink electrode was modified with a thick layer of multiwalled carbon nanotubes (MW-CNT), similarly to what previously suggested for a conventional K-ISE (3). A non-nernstian sensitivity of 30 mV/dec at DA concentration higher than about 10 μM.

In both transduction methods the MIP membrane was obtained by in situ polymerization, as previously described (3), by dropping the polymerization mixture directly on the carbon ink screen printed electrode.

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DEVELOPING OF ELECTROCHEMICAL SENSORS FOR THE REMOTE SENSING OF HEAVY METALS: THE HYDRONET PROJECT

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We have been involved in the development of remote controlled electrochemical sensors for an autonomous marine robot in a European project called Hydronet. Our goal has bee to develop sensors for the aqueous dissolved heavy metals cadmium, mercury and chromate. The level of detection specified in the course of the Hydronet project was very low in order to enable monitoring of non-polluted aquatic systems.

The sensor for Cd(II) was based on modifying a gold electrode with a monomolecular layer of thiols and using the under potential deposition (UPD) of the metal on this self-assembled monolayer (SAM). A series of short alkanethiol SAMs bearing different end groups, i.e., sulfonate, carboxylate and ammonium, were examined. Lowest level of detection (ca. 50 ng L^{-1}) was achieved with a 3-mercaptopropionic acid (MPA) monolayer using subtractive anodic square wave voltammetry (SASV). Additional surface methods, namely, reductive desorption and X-ray photoelectron spectroscopy, were applied to determine the interfacial structure of the electrodeposited Cd on the modified electrodes. We conclude that the deposited Cd forms a monoatomic layer, which bridges between the gold surface and the alkanethiol monolayer associating with both the gold and the sulfur atoms. This modified electrode was integrated as part of a flow system to be assembled on the robot.

The second part will focus on the sensor for Hg(II). The detection of very low levels of this metal (below 1 ppb) in the field by remote sensing is not trivial. This was based on modifying a glassy carbon electrode with thiol groups using the covalent attachment of a diazonium derivative of phenyl disulfide. We showed very clearly that the sensitive detection of mercury requires thiol groups on the electrode surface. We will present the results obtained with this electrode and its characterization.

We will describe how these two sensors are about to be integrated in the robot and how the remote sensing will be carried out.

DESIGN AND INTEGRATION OF OPTICAL SENSORS IN PORTABLE INSTRUMENTS FOR GAS ANALYSIS

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The determination of gas components such as carbon dioxide and oxygen are required in different fields such as environmental monitoring, biotechnology or health. Furthermore, the ability to measure one or several analytes simultaneously using portable instruments is an interesting field of research for *in situ*, low cost and real time analysis.

We present the development and characterization of portable instrumentation with different optical sensors. Sensing membranes have been designed and characterized with conventional instrumentation and then, integrated in different portable instruments which we have developed.

Instrument 1. Hand-held optical instrument for CO_2 in gas phase based on sensing film coating optoelectronic elements.

Instrument 2. Compact optical instrument for simultaneous determination of O_2 y CO_2 in gases.

Instrument 3. A probe for soil monitoring that allows the measurement of three parameters: O_2 concentration, temperature and relative humidity of the interstitial air in soil at root depth.

A complete analytical characterization of all the instruments, a study of possible applications and a comparison between them have been carried out. The results obtained with these new instruments are very promising for gas analysis in different fields.

TWO-DIMENSIONAL SPATIOTEMPORAL VISUALIZATION SYSTEM FOR EXPIRED ETHANOL AFTER ORAL ADMINISTRATION

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A novel 2-dimensional spatiotemporal visualization system for expired ethanol after oral administration was constructed of enzyme immobilizated mesh substrate and low level light CCD camera. Distribution of gaseous ethanol was transformed to optical information with biocatalyst, and the optical information was recorded with CCD camera as a movie. This system was useful for the component in expired breath not only spatial but also temporal analysis in real-time and monitoring the blood ethanol concentration non-invasive. First, the optimiation of the substrates for visualization and the concentration of luminol solution for chemiluminescence (CL) were investigated. Then, the effect of pH condition of Tris-HCl solution was also evaluated with CL intensity, and the CL intensities detected by the system were ralated to a wide calibration range of standard gaseous ethanol (30-400 ppm). Finally, expired breath after oral administration of 5 healthy volunteers was measured. The images of expired breath were analyzed and time-concentration profiles of expired ethanol were obtained. The peaks of expired ethanol concentration appeared within 30 min after oral administration. During the 30 min, the time variation profile based on mean values showed the absorption and distribution function, and the values onward showed the ellinination fuction. The absorption and distribution of expired ethanol in 5 healthy subjects following first-order absorption process were faster than elimination process, which proves efficacious of the system for described alcohol metabolism in healthy subjects. The system showed good performance in analysis of standard gaseous ethanol and expired ethanol. Moreover, the system for expired breath can be used in assessment of other expired air analysis by varying the enzyme for clinical purpose in real-time.

S15: Chromatography

Keynote Lecture 15

SOME PEOPLE AND INSTITUTIONS IMPORTANT IN THE HISTORY OF ANALYTICAL CHEMISTRY IN SERBIA

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The lecture will review the development of chemistry in Serbia from the end of the Turkish occupation in 1830 up to near modern times. Two institutions were important, namely the State Laboratory, which emerged from the State Pharmacy, founded in 1836, in 1859, and the Lyceum. The Lyceum, founded in 1838 had in 1853 the first chemical laboratory in Serbia, it became the "Great School" in 1863 and after further reorganisation in 1905 becoming the University of Belgrade. Chemistry was also an independent curriculum subject in the Military Academy of Belgrade from 1850.

The contributions of the peoples associated with these institutions will be outlined, including those of Michael Rašković, Sima Lozanić, Marko Thomas Leko and Vukić Milovan Mićović. The circumstances of the foundation of the Serbian Chemical Society in 1897, the world's 10th oldest such society and its journals will be outlined.

NEW OBSERVATIONS ON THE ISOTHERM AND KINETICS OF MOLECULARLY IMPRINTED POLYMERS

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Molecularly imprinted polymers (MIP) are synthetic sorbents with unique selectivity patterns. This property is due to their templated preparation in the presence of the compound for which selectivity is sought. MIPs have been found useful in various analytical techniques like SPE, HPLC, sensors and binding assays.

The equilibrium isotherms of MIPs have been often found to be nonlinear in every concentration range, sometimes down to 10^{-8} M and below. This has made their chromatographic application cumbersome. In SPE they have much more success but method developers appear to ignore that even in SPE applications the nonlinearity of the isotherm may have profound effects.

We show that static (batch) binding isotherms have limited usefulness in designing dynamic methods with MIPs because the kinetics of MIPs can be quite sluggish on the scale of hours and even days. Difficulties are encountered not only in the measurement of the isotherms but also in their interpretation. The Freundlich and the bilangmuir isotherm have often been found to fit well to MIP data. We have succeeded to show that this is not accidental. To any Freundlich isotherm one can fit one or even more bilangmuir isotherms with less than 10% deviation over three orders of magnitude in equilibrium concentrations.

Due to the difficulties of obtaining reliable experimental isotherms and appropriate isotherm equations we proposed some time ago to use the distribution coefficients (D) as a useful quantity in designing analytical methods with MIPs. Obviously, D will be concentration dependent and for this reason its use has not been widely accepted. We can explain now by a simple mathematical derivation why D has a particular role in characterizing MIPs.

Several applications of MIPs are based on competition between two compounds. In HPLC, however, competition has not been observed with MIPs. Earlier we had shown experimentally the competition on MIPs by the injection on a plateau method. This time we have succeeded in finding the conditions when competition is also visible in the elution chromatography of two-component injected samples. The experimental evidence is also supported by simulation results.

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INTEGRATED APPROACH TO DETERMINATION OF HEAVY OIL RESIDUES COMPOSITION

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Complexity of heavy oil residues composition and structure makes their study a difficult task.

At present the most informative and wide spread methods of heavy oil residues analysis are FTIR spectroscopy, NMR, EPR, ICP-AES. But using the thermal decomposition at the step of sample preparation changes the original sample composition that leads to inaccurate results. That is why development of methods of determing heavy oil residues composition is of great interest.

Authors of the work suggested and tested an integrated approach to analysis of heavy oil residues composition. The procedure comprises adsorptive extraction sample preparation following by GC-MS and TLC (thin layer chromatography), ICP-AES qualitative analysis. Extraction was performed with Soxhlet apparatus (extraction by accelerated dissolution) and shaker. The universal solvent suitable for all analytical techniques listed has been selected.

To determine heavy oil residues microelement composition the optimal parameters of operation of ICP-AES with direct injection of the sample dissolved in organic solvents have been determined. Comparison of data obtained by using different sample preparation methods (liquid-liquid extraction in rotating spiral column, autoclave and microwave decomposition, dilution with organic solvents and ashing) is presented.

To determine the group composition of heavy oil residues the optimal parameters of operation of thin layer chromatograph “Iatroscan MK-6s” with flame-ionization detector were found. Extent of heavy oil residues solution in toluene at the stage of sample preparation was determined. The optimal composition of mobile phase as well as mobile phase migration distance was determined. The mobile phase is a mixtures of n-heptane - toluene and dichloremethane - methanol in the ratios depending on physical-chemical properties of a sample. This method allows to identify qualitatively and to estimate semi-quantitatively the content of asphaltenes, polar resins, saturated hydrocarbons and three groups of aromatic hydrocarbons (mono-, di-, poly-).

Data obtained on the composition of heavy oil residues from different oil refining processes of enables finding the way of their further treatment and to turn them from waste products to valuable feedstock.

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ADVANCEMENT IN OPTIMIZATION ACHIEVED BY NEWLY DEVELOPED CHROMATOGRAPHIC RESPONSE FUNCTION

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This study presents a new chromatographic response function (N_{CRF}) that is formulated here for the first time. It is developed in order to overcome the defects that mathematical design of previously developed functions appeared to have, and to provide a correct determination of the global optimal conditions in different optimization procedures. The established function has improved concept of separation and time term estimation allowing the analyst to adjust desired balance between them. It overcomes the traditional shortages of objective functions: there is no masking of poorly resolved peaks by high values of the resolution factor of the well resolved ones and both non-Gaussian and Gaussian peaks can be evaluated appropriately. Additionally, it reflects all important defects of the chromatogram such as the appearance of asymmetrical or overlapping peaks, prolonged elution time and possible peak tailings.

The obtained function was compared with previously published Morris's chromatographic exponential function (CEF) and Duarte's chromatographic response function (D_{CRF}) in the optimization strategy of raloxifene and its four impurities chromatographic separation. Thirty experimentally obtained chromatograms defined by central composite design were considered. The previously mentioned objective functions, including N_{CRF}, were calculated and compared. Analyzing the obtained results the shortcomings of CEF and D_{CRF} were reveled since they failed to rank the chromatograms appropriately. The N_{CRF}, on the other hand, made the adequate ranking and demonstrated the ability to judge the impact of the complex interactions of the selected chromatographic parameters on the mixture behavior and led to the determination of the optimal separation conditions. The newly developed chromatographic response function proved to have advanced performances and it presents the important step forward in the optimization of the chromatographic separation.

A NEW GENERATION OF CAPILLARY COLUMNS FOR SEPARATION AND SAMPLE TREATMENT: THE TITANIA (TiO_2) MONOLITH

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Recently, liquid chromatography performed on native titania (TiO_2) beds has rapidly emerged as a promising approach for selective purification and enrichment of organophosphate. Titania offers a versatile selectivity owing to combined ion- and ligand- exchange surface properties. In this context, latest challenges have concerned the on-line integration of miniaturized titania columns with hyphenated techniques. The first trend in micro-column technology was to develop capillary columns packed with micro-sized (5µm) titania particles. The second-generation has introduced the open tubular design but suffers from low loading capacity due to a reduced phase ratio compared to that of the packing design.

Nowadays, the advancements in the synthesis of titanium dioxide monoliths using the sol-gel chemistry are leading the way for the integration of this material by in situ synthesis within confined geometries with frit-free constructions. The 3D-geometry of micrometer-sized skeletal network and interconnected flow-through channels ensures the high permeability of these monoliths making them an excellent compromise between the permeability and the loading capacity needed to perform miniaturized sample enrichments for high throughput analysis.

Our study was focused on the elaboration of capillary titanium dioxide monoliths as original miniaturized devices for selective capture and extraction of phosphorylated compounds. The synthesis route of titania monoliths has been adapted to in-capillary synthesis in order to afford adapted features for chromatographic. These monoliths were used for the analysis of phosphorylated nucleotides as probe molecules to evaluate their chromatographic characteristics, and were also applied to the specific enrichment of phosphorylated molecules such as amino-acids and pesticides for clinical and environmental applications.

DISTRIBUTION AND SOURCES OF CARBON, POLYCYCLIC AROMATIC HYDROCARBONS AND POLYCHLORINATED BIPHENYLS IN SLUDGE

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Sewage sludge is widely used as organic fertilizer in various countries. However, the sludge are found to be contaminated with toxicants i.e. heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc. at elevated levels. Polychlorinated biphenyls (PCBs) are a group of chlorinated hydrocarbons widely used for industry in many different applications. Some manufacturing processes which involve hydrocarbons, chlorine and heat such as production of chlorinated solvents, paints, printing inks, agricultural chemicals, plastics and detergent bars also generate PCBs. Some data of PAHs and PCBs in the Indian ecosystem were reported. In the present work, the contamination extent, distribution pattern and sources of PAHs and PCBs congeners in steel plant, thermal power plant, municipal and agricultural sludges of the industrialized area of the country, Raipur, central India (21° 8′ 24″ N, 81° 22′ 48″ E) is described. The sludge samples were collected in the summer 2008. Twelve most toxic PAHs i.e. Phe, Ant, Fla,Pyr,Baa, Cry,Bbf, Bkf, Bap, Dba, Bgh and Ind were characterized in the present investigation. All sludge is black in color with of alkaline nature (pH = 8.1±0.2). The BC content was ranged (n = 5) from 7.5 – 9.0% with mean value of 8.4±0.7%. However, the less OC, CC and PAHs contents were seen ranging from 180 – 610, 390 – 615 and 6.3 – 11.7 mg kg^{-1} with mean value of 389±146, 466±98 and 8.4±0.5 mg kg^{-1}, respectively. Whereas PCB content was ranged from 161 – 800 ng kg^{-1} with mean value of 539±217 ng kg^{-1}. The high relative abundance (up to 84%) of monochlorobiphenyls in the sludge was marked. Significant verticle mobility of the PAHs was observed unlikely to PCBs. The speciation, stability and mobility of PCB congeners are discussed with respect to nature of the sludge.

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S16: Forensic Analytical Chemistry

Keynote Lecture 16

FLUORESCENCE CORRELATION SPECTROSCOPY - QUANTITATIVE ANALYSIS WITH SINGLE-MOLECULE SENSITIVITY

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Quantitative methods with ultimate, single-molecule sensitivity are indispensible tools in any analytical application. One such method is Fluorescence Correlation Spectroscopy (FCS). In FCS, the amplitude of spontaneous fluorescence intensity fluctuations occurring in a diffraction limited observation volume element and the time-dependent decay of the correlation of fluorescence fluctuations are analyzed to yield quantitative information about molecular numbers, diffusion properties and kinetics of chemical interactions. Since its insception in the early 70-thies [1-3], FCS instrumentation and application are continuously developing [4-6] enabling us to quantify the number of molecules, measure their transporting properties and study the kinetics of molecular interactions in the crowded and dynamic environment of a living cell [7].

The aim of this work is to present the theoretical background behind FCS and discuss its application in biomedical research and analysis.

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INTERPRETATION OF ISOTOPIC DATA: THE CHALLENGING ASPECT OF ISOTOPE RATIO MASS SPECTROMETRY?

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The flourishing number of publications on the use of isotope ratio mass spectrometry (IRMS) in forensic science denotes the enthusiasm and the attraction generated by this technology. IRMS has demonstrated its potential to distinguish samples of the same chemical nature and coming from different sources. Despite the numerous applications of IRMS to a wide range of forensic materials, the central aspect related to interpretation of isotopic data remains ambiguous. The isotopic profile may be used as information to orientate investigations (forensic intelligence) or as evidence in a prosecutorial perspective. However, in both cases, the meaning of an isotopic similarity between two specimens or their undistinguishable profile is often difficult to interpret.

The presentation demonstrates how interpretation of isotopic data is influenced by the methodological approach undertaken in the study. Through a series of applied examples, this contribution highlights the key role played by the definition of the research problem in applications of IRMS to forensic materials. In a source discrimination or source identity inference process, this stage is directly related to the definition of the source in a hierarchy of propositions and, therefore, to the level of source considered, i.e. geographical origin, synthesis pathway, manufacturers level, etc. The impact of this step on the interpretation of isotopic results and on the rest of the methodological process is more particularly exemplified for illicit drugs, arsons and explosives.

S16: Forensic Analytical Chemistry

OPTIMIZATION AND VALIDATION OF A HS-SPME/GC-MS METHOD FOR THE ANALYSIS OF RESIDUAL SOLVENT OCCLUDED IN COCAINE

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Residual solvents (RS) occluded in cocaine powder has been becoming last years an important issue in forensic drugs analysis for profiling/intelligent purposes. In spite of the works related to RS analysis in matrices i.e. pharmaceuticals and packing materials, developing a method in cocaine samples represents a complex task due to two factors: the variability of the matrix composition as well as the variability of the amount of RS occluded in depending on the manipulation previous the seizure and on the substances employed for the production.

In this work, a generic analytical method is presented for 49 residual solvents in cocaine by Headspace Solid-Phase Microextraction (HS-SPME) coupled to Gas Chromatography Mass Spectrometry (GC-MS), using a carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber. For this purpose, it was necessary to study the signal response of RS in presence of a cocaine matrix doped with adulterants and cutting agents most commonly used.

A critical step was to choice an adequate solvent capable of dissolving the target analytes and providing no interference. Solvent selection was a great challenge due to the different physical and chemical properties. Dimethylphtalate was chosen as solvent taken into account, the chromatographic response, physical and chemical properties and toxicity aspects.

HS-SPME extraction parameters (extraction time, extraction temperature and desorption time) were optimized using a statistical design of experiments procedure in order to obtain the optimal extraction conditions taking into account the interdependence of these variables. The analytical method was validated in terms of limits of detection and linearity, repeatability and reproducibility, and robustness.

COMPARISON OF LIQUID LIQUID EXTRACTION (LLE) TO SAMPLE DERIVATISATION FOR THE ANALYSIS AND QUANTIFICATION OF AMPHETAMINE TYPE STIMULANT (ATS) BY GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROSCOPY

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The fight of synthetic illicit drug (ATS) traffic is in continuous update and one of the most recent initiatives is the monitoring of the ATS precursors in order to prevent the ATS manufacturing. A contribution to this fight is furnished by the European project DIRAC (rapid screening and identification of illegal drug by IR Absorption spectroscopy and gas chromatography) which aims at developing a new advanced sensor for detecting ATS drugs and their precursors.

The compounds used in the presented research study are the three principal ATS compounds, MDMA (3,4-methylendioxymethylamphetamine), Methamphetamine and Amphetamine as well as their main precursors: ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine.

One task of the project is dedicated to the development of a simple and reliable analytical methodology to identify and quantify these substances.

GC-MS has been selected as the reference method considering it's potential to analyse this kind of compounds. However analytes that are present in a salt form need a sample treatment before to be injected.

In the presented results two sample preparation methods are investigated and compared for such analytes. The first consists in the use of a derivatising agent which transforms the salt compound into a product more suitable to be injected. Unfortunately this procedure is not ideal for quantification purposes.

The second method relies on transformation of the salt compound into its corresponding basic form following an extraction with an organic solvent (LLE). This technique provides good quantification results however is not the ideal procedure for a complete screening of street samples seizures.

The results demonstrate the complementarity and the comparability of the two techniques as well as the added value of combing the information.

The research leading to the presented results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013*) under *grant agreement* n° 242309.

SILICON DETERMINATION IN HUMAN VENTRICULAR WHOLE BLOOD

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Analytical methods for trace silicon determination in biological samples is still one of the most demanding tasks in analytical chemistry, despite its importance in medical research, toxicity studies and quality control [1]. The main limitations are silicon ubiquity and, consequently, a high risk of contamination, its low solubility in acid media and the volatility of silicon tetrafluoride that can lead to losses during sample preparation.

The main purpose of this study is to demonstrate that the difference of blood Si concentration between the left and the right ventricle can be a marker of fresh water drowning. For this purpose, it is necessary to quantify poorly soluble silicate fraction and HNO_3-soluble species. Both arise from diatoms, phyto-, geo-, zooplankton and soluble silicon naturally present in drowning medium, pass from the alveoli into the pulmonary capillaries and reach the left ventricle.

An accurate, precise and sensitive method for the determination of soluble Si in whole blood based on microwave dissolution and DRC ICP-MS analysis has been previously developed by our research group, and first significant results have been recently presented [2]. Described hereafter is an alkaline high pressure microwave assisted digestion procedure using tetramethylammonium hydroxide for total Si determination. The silicon concentration of the digested samples was measured by inductively coupled plasma (ICP-OES, ICP-MS).

Accuracy has been tested on fresh water plankton (CRM 414) with Si recovery 93(8)%, according to literature [3,4]. Possible influence of the matrix has been investigated for whole blood, before and after spikes with SiO_2, diatomaceous earth powder and CRM 414.

The procedure has been applied to blood samples from confirmed and suspected drowning cases.

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«PRACTICE OF FIRE DEBRIS ANALYSIS IN FORENSIC SCIENCE : A PLEA FOR AN HOLISTIC APPROACH»

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Fire debris analysis is a forensic discipline widely carried out, generally applying headspace extraction (with enrichment) followed GC-MS analysis. Deplorably, this discipline has not been the subject of extensive studies or fundamental researches. Most of the recent publications focus restrictively on a specific, yet important, aspect of the process and fail to consider its implication in the overall approach of fire debris analysis. There is a lack of global judgment on the mutual influence that the different steps of the process have on the final results and the decision to assess the presence of traces of an ignitable liquid in a matrix of complex and, more often than not, uncertain nature.

In this presentation we advocate for a holistic approach of fire debris analysis. This begins by the identification of the forensic problem for which data can be obtained through analytical process. This problematic is the key starting point from which the analytical strategy should be shaped. However, pressure to accreditation and de-contextualization within forensic laboratories tend to hinder this delimitation of the problem. However, on the basis of this step of specification of the problematic, accurate extraction, separation and detection methods can be selected. Results obtained after the separation-detection step (GC-MS) represent raw data that need to be carefully evaluated by the light of the different parameters that influence them. This requires the identification of these crucial parameters and the evaluation of the potential impact that each of them could have in the specific case. This evaluation process relies on a hypothetico-deductive reasoning that is seldom enlightened, if even applied.

With examples, we aim to show how a too narrow view of the practice of fire debris analysis can conduct to misleading or incomplete conclusions. We make a claim for a holistic and informed approach of the application of analytical chemistry to fire debris analysis in order to provide information useful in the perspective of the forensic problem that must be answered.

S17: Hyphenated techniques /Miniaturization

Keynote Lecture 17

PROGRESS IN MICROARRAY-BASED ANALYSIS

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Microarray technology is known from DNA analysis since 30 years. Less known is the usage of biochip and microarray. In its best configuration they represent a fully automated analysis platform.

Such read-out system for analytical flow-through microarrays based on multiplexed immunoassays has been developed. The (Munich) microarray chip reader (MCR 3) is designed as a stand-alone platform (Kloth et al., 2009). The platform is a self-contained system for the automated multiplexed immunoanalysis.The MCR 3 uses a microarray chip, which consists of two channels for parallel measurement and regeneration. One version is designed for parallel analysis of up to 15 different antibiotics in milk applying an indirect competitive immunoassay. The chip surface is regenerable for < 80 measurement cycles per chip. Assay time, incl. regeneration, is 6 min.

Multianalyte test systems are currently of high interest for the monitoring and quality control of drinking water, since microbiological methods are labor intensive and can take days. The 1st flow-through chemiluminescence microarray was developed and characterized for the rapid and simultaneous detection of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Legionella pneumophila* in water samples. The overall assay time was 13 min. In multianalyte experiments, DLs were 3×10^6, 1×10^5, and 3×10^3 cells/mL for S. typhimurium, L. pneumophila, and E. coli O157:H7, resp. Combination with oligonucleotides as receptors has been demonstrated recently by Donhauser et al. (2011).

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NANOTECHNOLOGY IN THE FLOW DOMAIN OF PROCESS ANALYSIS

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Nanoscience, Nanomaterials, Nanotechnologies and the integration of technologies for Industrial Applications forms currently a key objective of the European Union with primary concern about natural resources, food processing and distribution, health issues, climate change and clean energy as some key issues. This can be seen from the large number of research consortiums with current projects from the European Commission where Nanotechnology is one of the main areas with a budget reaching into millions of Euros. As a result micro- and nanosystems technology with PAT, MPAT and NPAT become increasingly important in various operations in real-time and new innovations linking the different PAT systems successfully to these technologies may create fruitful solutions to the problems above. The successful establishment of PATLAB in Bucharest, Romania may serve as a catalyst for PAT, MPAT and NPAT. Different scenarios regarding the innovation, development, implementation and application of nanotechnologies in the flow domain of process analysis will be outlined. This will be compared with the non-flow based domain. Careful attention will be given to the reality of nano- and microfluidics as marketable applications with flow-based PAT, MPAT and NPAT, the incorporations of micro- or/and nanosensors as detection devices and if real-time is always possible. It will be discussed if there is any improvement in the configuration and construction of downscaled chemically devices and if the movements to remote sensing with innovation of high performance real-time intelligent interactive multi-point multi-species process analytical technological nano- and microsystems are possible. The reality of the current situation with integration of these technologies from the benchtop to pilot plant to real industrial applications with possible future solutions will be highlighted and outlined.

ASPECTS OF HAZARDOUS METALS TRANSFER ACROSS BIOMEMBRANES

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The environment contamination by hazardous metals (Cd, Pb, etc.) of different origin belongs to most important problems. They have to be transported into the cells of living organisms (across the cell- and the subcellular membranes) to start their activity. Elucidation of the transport mechanisms is prerequisite for understanding distribution of pollutants in cells.

The research has been focused on investigation of transport across the phospholipid membranes (ion channels, pores, etc.) and on characterization of the forms in which the species are transported (free ions, charged and uncharged complexes with biomolecules as e.g. low-molecular weight organic acids, cysteine containing peptides or more complicated species [1-3]). The porous membranes or agar layers have been utilized as supports. The real membranes were simulated by various model supported phospholipid (lecithin, azolectin) bilayers and the real transporters by polypeptidic ionophores (e.g., calcimycin, valinomycin).

Combination of two different techniques: electrochemical methods (e.g., electrochemical impedance spectroscopy (EIS), voltammetry, ion selective electrodes (ISEs)) with electrospray-ionization mass spectrometry (ESI-MS) seems to be highly suitable for the intended purposes and yields the unique information on solution composition and on structure of the transported species on both sides of the membrane barrier. Special attention has been devoted to the transport affecting conditions (pH, electrolyte concentration, voltage, etc.).

Acknowledgements

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COMBINATION OF SPME AS NON-INVASIVE SAMPLE PREPARATION TECHNIQUE AND GCxGC-TOFMS FOR HIGH RESOLUTION METABOLITE PROFILING IN APPLES: METHOD DEVELOPMENT CONSIDERATIONS AND POTENTIAL OF NEW *INVIVO* SPME FORMATS

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Quality determination of plants and plant-derived foods relies on implementation of unbiased fingerprinting and profiling approaches in order to study overall composition of a biological system. This approach has recently been popularized as ‘metabolomics’, a sophisticated, but complicated technique due to the huge diversity of metabolites in any given species. The objective of this project is to address some of the challenges of metabolomics analysis with the use of SPME and GCxGC-TOFMS to allow comprehensive metabolomic profiling of apples. Among well-known advantages of SPME, the most important include generation of representative sample extracts, generation of clean chromatograms and its miniaturized format which causes minimum disturbance to the investigated system allowing measurements to be performed in real-time/*invivo* manner, therefore, eliminating the need for the sacrifice of biological systems. On the other hand, GCxGC-TOFMS further increases the selectivity of determinations due to three separation dimensions: i) non-polar first dimension column; ii) polar second dimension column and iii) extremely sensitive and fast TOF mass spectrometric detection. The optimized extraction method in this study involved the use of DVB/CAR/PDMS SPME fibre coating which provided the least biased volatile and semivolatile metabolite coverage and allowed the extraction of highest number of metabolites across volatility and polarity range. For extraction of metabolites, the SPME assemblies were implemented in headspace, direct and *invivo* sampling modes. The traditional *exvivo* SPME sampling formats were accompanied by appropriate metabolism quenching steps. Following sample preparation and analysis, data processing was directed toward comparing three different SPME modes of sampling in terms of metabolite coverage and precision. The results obtained are indicative of great potential of non-invasive *invivo* SPME to provide more comprehensive and more representative metabolism snapshoot, while at the same time allowing for capture of true and unstable metabolite fraction.

CHEMOMETRIC APPROACHES IN NON-TARGET GC-MS ANALYSIS

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In non-target analysis, which is often performed in environmental analysis and food research, the accurate identification and quantification of analytes represents one of the key problems. For this purpose gas chromatography (GC) coupled with mass spectrometry (MS) is the most commonly used technique. Since the number and properties of the components to be determined are usually unknown it is difficult to optimize separation conditions. At the same time qualitative and quantitative determination is hindered due to the absence of standard materials.

The identification can be improved using their retention properties in addition to their spectroscopic characteristics. A correlation between the chemical structure and the retention property of the particular compound (quantitative structure retention relationship - QSRR) was applied to approach these problems. The thermodynamic values (DH, DS) which enable calculation of retention times for any selected chromatographic condition were predicted from different types of molecular descriptors using multiple-linear regression model.

The accurate quantification of unknown compounds is an even more difficult task since there is no numerical model that enables theoretical calculations of MS response factor for particular organic compound. Theoretical procedures based on molecular similarity can be beneficial to overcome this problem. A quantitative structure-property relationship (QSPR) model was applied for the prediction of MS response factors and used for the quantification of ozone precursors present in the atmosphere. Experimental MS response factors were obtained for more than 30 different saturated and unsaturated organic compounds containing carbon, hydrogen and chlorine atoms. The prediction ability of the created model was estimated by leave-one-out cross validation procedure. The error in the prediction of response factors calculated by cross-validation procedure was around 15%.

The proposed procedure was applied for simultaneous qualitative and quantitative determination of volatile organic compounds in the atmosphere.

DETERMINATION OF BIOFLAVANONS IN FOOD SAMPLES USING LIQUID CHROMATOGRAPHY METHODS

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We have developed LC-ESI-MS/MS i LC-UV method for determination of bioflavanons (rutin, hesperidin, hesperetin) in some food samples (red onion, orange peel, honey and broccoli). Extraction of flavanons from food samples were carried out with methanol. For determination of bioflavanons the post extraction standard addition method was used, while the isolation of analytes from samples was carried out on C18 SPE cartridge.

The analytes from extracts were separated by gradient elution method on RP-C18 column using mobile phase composed of solvent A: 2 wt% of acetic acid in water and solvent B: acetonitrile. Mass detection was carried out in negative ion mode. The precursor and major product ions of the analytes were monitored in selected reaction mode (SRM) as follows rutin: (m/z) 609 → 301; 609 → 273; 609 → 257; 609 → 179, hesperidin: 609 → 343; 609 → 325; 609 → 174; 609 → 151, hesperetin: 301 → 286; 301 → 244; 301 → 179; 301 → 151, and UV detection was in spectral interval 260 – 280 nm. Peak resolution was higher than 1.8

Validation parameters obtained with solutions of standard substances, indicated linearity range 0.05 – 10 μg/mL and recoveries between 95 – 97%, thus confirming method suitability.

In the analysis of the real samples by standard additon method low detection limits (calculated as three times standard deviation of non-spiked sample) between 0.014 – 0.063 μg/mL were obtained with excellent recoveries (86 – 117 %).

Our resultes of analyses are in good agreement with the data from Food and Agricultural Import Regulations and Standard Report (FAIRS) (USA).

S18: Environmental Analytical Chemistry III

Keynote Lecture 18

FLOW INJECTION WITH CHEMILUMINESCENCE DETECTION AS A TOOL FOR INVESTIGATING THE AQUATIC ENVIRONMENT

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Instrumental analytical techniques are critical for improving our fundamental understanding of environmental processes. In addition to the challenge of environmentally relevant detection limits is the need for high temporal and spatial resolution measurements. In this context there is a growing need for rapid and reliable but relatively low cost techniques that can be remotely deployed to provide high quality environmental data. This presentation describes the evolution of flow injection (FI) based instrumentation for investigating the aquatic environment [1], with particular focus on the powerful combination with chemiluminescence detection (FI-CL). Key aspects of FI-CL systems will be discussed, including reaction chemistry, detector design, analytical performance and sample presentation.

Several examples will be used to illustrate the impact of FI-CL techniques at the interface between environmental analytical chemistry and biogeochemistry. FI-CL manifolds for the determination of macronutrients (nitrate and phosphate) will be described as sensitive alternatives to spectrophotometric detection. FI-CL manifolds incorporating solid phase preconcentration columns will also be presented for the determination of trace metal micronutrients (specifically iron and cobalt) in remote, open ocean environments. These examples will demonstrate how FI-CL can enhance our understanding of nutrient biogeochemistry and the link between chemical and biological processes in aquatic systems.

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APPLICATION OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR MONITORING METABOLITES OF NON-IONIC SURFACTANT BIODEGRDATION BY SEVERAL BACTERIA STRAINS

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Due to the massive application of detergents, surfactants are common pollutants of surface water. Non-ionic surfactants (NS) are a significant component of the surfactant stream. This group of surfactants exhibits the greatest variety of chemical composition. Several hundred individual compounds can be present in a NS mixture in surface water. Despite the fact that NS are relatively harmless to the aquatic environment, thier fate in water and soil shoud be monitored.

The general pathways of NS biodegradation in the environment are known. However, participation of different micro-organisms in this process is unknown. In this work, NS biodegrdation by two bacteria strains isolated from activated sludge: Pseudomonas fluorescens, Brevundimonas vesicularis, as well as two bacteria strains originating from soil: Sphingomonas paucimobilis, Acinetobacter baumani was investigated. The River Water Die-Away Test was used as a reference. Model oxyethylated alcohol – surfactant C12E10 was used as a NS representative.

Biodegradation testing was performed in accordance with the modyfied OECD 301E Test. Residual NS and metabolite was isolated by liquid-liquid extraction with chloroform. Liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionisation was applied for metabolites identification and quantification. Total current chromatograms, together with mass spectra were used both for identification and quantification of analytes. A multiple reaction monitoring (MRM) mass analysis was also used for quantification.

In all four instances, the ω-oxidation of the oxyethylene chain with the carboxyl end-group formation was found to be the major biodegradation pathway. Poly (ethylene glygols) (PEG) formation was found to be a parallel pathway. This is a different pathway to the predominant pathway, occuring in surface water, i.e. cetral fission with predominant PEG formation. In all instances, the residual NS and metabilte concentration changes during the 30 day biodegradation test were determined.

Acknowledgment
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LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRIC ANALYSIS OF BENZIMIDAZOLE ANTHELMINTICS AND CORRESPONDING METABOLITES IN LIQUID PIG MANURE

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A new and reliable analytical method for simultaneous determination of two benzimidazole anthelmintics and their corresponding metabolites in liquid pig manure at trace level was developed. Target compounds were fenbendazole with its metabolites fenbendazole sulfoxide and fenbendazole sulfone as well as flubendazole with six major metabolites mainly resulting from carbamate hydrolysis or keton reduction in liquid pig manure. LC/MS/MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) was applied. Identification focused on information dependent acquisition to define precursor and product ions of the target compounds. Quantitation based on multiple reaction monitoring (MRM). Different extraction procedures were tested, i.e., direct ethyl acetate extraction at different pH values, lyophilisation followed by methanol extraction and ultrasonic assisted extraction, then centrifugation followed by solid phase extraction of the aqueous phases and methanol extraction of the solids after freeze drying. Size exclusion chromatography (SEC) and solid phase extraction (SPE) were used to clean up the raw extracts. As revealed by fortification experiments, lowest quantitation limits of 2 µg kg^{-1} fresh manure with recoveries of > 86% and relative standard deviations of < 10% were achieved when manure samples were analyzed by LC/MS/MS-ESI+ after ethyl acetate extraction and two clean up steps. The detection limits ranged from 0.5 to 0.9 µg kg^{-1}. In order to compensate matrix effects during LC/MS/MS analysis, matrix-matched calibration, external and internal calibrations and standard addition techniques were studied. Finally, this method was successfully applied for the analysis of real manure samples from different sow and piglet farms.

CARDIOVASCULAR DRUGS IN ENVIRONMENTAL WATERS: METHOD OPTIMIZATION AND REAL SAMPLE ANALYSIS

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Pharmaceuticals are regarded as “emerging contaminants” in environmental waters. Up to 95% of the administered dose can be excreted unmetabolized and discharged into wastewater. Wastewater treatment plants (WWTPs) are not designed to remove pharmaceuticals and consequently the efficiency of their elimination can significantly vary. The majority of cities in Serbia do not have WWTPs, and the municipal wastewater is directly released into environment. Due to their high water solubility and often poor degradability, pharmaceuticals can pass through all natural filtrations and reach ground, and ultimately drinking water. Since cardiovascular drugs are among the most frequently used, their presence in waters is especially important as their residues can induce unwanted effects. The sensitive multiresidual method for analysis of eight most commonly used cardiovascular drugs in Serbia (enalapril, cilazapril, metoprolol, bisoprolol, atorvastatin, simvastatin, amlodipine, clopidogrel) was developed and optimized. Water samples were prepared using solid-phase extraction (SPE) and extracts were analyzed by liquid chromatography–ion trap–tandem mass spectrometry with electrospray ionization in the positive ionization mode. Parameters that affect the SPE procedure efficiency were optimized, as the elution solvents, the sample pH and the sample volume. For each pharmaceutical, MS^n analysis was performed and distinctive ions and transitions were selected for identification and quantification, as well as for confirmation purposes. Good recoveries (82–108%) were achieved with this method, except in the case of bisoprolol (28%). The analytical method was successfully applied in analysis of four river water samples collected in Serbia, as well as nine corresponding ground water samples. The residues of enalapril, atorvastatin and simvastatin were detected in river water. These drugs were not detected in corresponding ground water samples indicating that the bank filtration successfully eliminates pharmaceutical residues from water and prevents leaching from surface to ground water. In addition, in order to estimate the removal rate of cardiovascular drugs, influents and effluents of two WWTPs were analyzed.

MICROEXTRACTION BY PACKED SORBENT (MEPS) FOR THE ANALYSIS OF PHARMACEUTICAL RESIDUES IN ENVIRONMENTAL WATER SAMPLES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Microextraction by packed sorbent (MEPS) is a technique introduced in 2003 by Abdel-Rehim. It is based on the miniaturization of conventional solid phase extraction (SPE). Extraction time, sample size and solvent volumes are considerably reduced and the elution extract is directly compatible with liquid chromatography, gas chromatography or capillary electrophoresis. The sorbent can be reused with an adequate washing and reconditioning to avoid carry-over and to keep the adsoption power of the solid phase. To compensate for matrix effect, and variability in extraction and instrumental parameters an internal standard is frequently used.

MEPS has been mainly used for determination of analytes in biological fluids, but there are less applications reported in environmental samples. In this study the development of a procedure for determination of five non-steroidal anti-inflammatory drugs (NSAIDs: clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen) in waters is described. The fully automated method includes in situ aqueous derivatization followed by analytes enrichment by MEPS coupled directly to programmed temperature vaporizer-gas chromatography-mass spectrometry. The MEPS variables, such as sample volume, elution solvent, elution volume, fill and injection speed and washing steps were optimized. The MEPS applied polymer (silica-C18) could be used up to 250 times. Ibuprofen-d3 was used as internal standard. The reproducibility of the method, calculated as relative standard deviation was below 10% for all compounds. Detection limits were between 0.030 and 0.13 μg/L for ibuprofen and ketoprofen, respectively. The developed method was applied to the determination of NSAIDs in several environmental water samples, including river, sea and influent and effluent waste water.

ANALYSIS OF 11 OXY-PAHS ON ATMOSPHERIC PARTICULATE MATTER BY ADVANCED SOLVENT EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY MAGNETIC SECTOR MASS SPECTROMETRY: METHOD DEVELOPMENT, CONCENTRATION LEVELS IN FLANDERS AND HEALTH EFFECTS

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In this work, a HPLC – high resolution mass spectrometry based method was developed for the analysis of 11 oxy-PAHs [1] (including benzo[a]pyrenediones) sorbed onto airborne particulate matter (PM_{10}). A magnetic sector mass spectrometer was used, operated in multiple ion detection. The different steps of the analytical method: sample preparation, separation and detection were systematically investigated.
Pressurized liquid extraction (PLE) was evaluated as an advanced sample extraction technique and was compared to ultrasonic extraction (UE). Ethyl acetate was used as the extraction solvent and both techniques showed high recoveries from spiked quartz fiber filters (PLE: >82%; USE: >67%). The effect of the matrix on both recovery and ionization process was studied, resulting in high recoveries (>76%) and no significant disturbance of the ionization process. Through optimization of the chromatographic and mass spectrometric parameters, method detection limits between 1 and 160 pg/m³ were obtained.
Finally, the method was used for the analysis of real PM samples collected at several urban and rural locations in the Antwerp Area, Belgium, in the vicinity of homes for the elderly. Concentration levels (2 pg/m³ to 2 ng/m³) obtained were compared to other studies and were linked to changes in pulse pressure, a predictive marker of adverse cardiovascular events in elderly, living in these homes.

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Plenary Lecture 9

NEW TOOLS FOR THE ANALYTICAL LABORATORY

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In this presentation, two sources will figure prominently: a flowing atmospheric-pressure afterglow (FAPA) useful for ambient mass spectrometry, and a solution-cathode glow discharge (SCGD), applied to the determination of trace-metal concentrations in solution. “Ambient Mass Spectrometry” is a relatively new field aimed at obtaining the mass spectra of samples as they are, and in the open atmosphere. In our work, we have found that the effluent from an atmospheric-pressure helium discharge is capable not only of ionizing sample species, but also of desorbing them from solids, solutions, or liquids. The new source has applications in bioanalysis, forensics, and homeland security.

The second source is also an atmospheric-pressure glow discharge, but with a sample solution serving as the cathode. Similar to the well-known ELCAD, the SCGD offers lower detection limits and faster sample processing. Here, fundamental features of the SCGD will be highlighted and new areas of application explored.

A mass spectrometer to be discussed is based on a “Distance-of-flight” (DOF) concept. Similar in design to a time-of-flight mass spectrometer (TOFMS), the DOFMS employs not a single detector at the end of a flight tube, but rather an array of detectors positioned alongside the flight region. Before the lightest ion of interest leaves the flight tube (where in TOFMS it would encounter the detector), the ions are pushed in a direction perpendicular to their original direction of travel, where they encounter the spatially resolved detector. Unlike a sector-field spectrometer, the DOFMS design has no theoretical upper mass limit. And unlike a TOFMS, the DOFMS system offers truly simultaneous detection, high duty factor, and a much broader linear dynamic range. Its early application to isotope analysis will be featured.

Plenary Lecture 10

BIOLOGICAL TRACE ELEMENT ANALYSIS: SPECIATION AND METALLOMICS

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The analysis for trace elements and specific metal-containing contaminants in biological systems has been reaching maturity. The incoming challenge is the development of analytical methods to describe interactions of metals with biomolecules which constitute the genome, proteome, metabolome and other –omes in a cell, tissue or organism. Advances in analytical methods for trace element analysis in life sciences have brought the emergence of new research areas aiming at the description of metal interactions with biomolecules; the terms metalloproteomics, metallometabollomics or simply metallomics have been coined.

Advances in these areas focus on the development and improvement of methods for the detection, quantification, identification and characterization of complexes of metals (metalloids) at trace levels in an environment rich in biomolecules which often have similar physicochemical properties. Hyphenated techniques based on the coupling of a high resolution separation technique, such as HPLC, capillary electrophoresis of gel electrophoresis with elemental (ICP MS) and molecular (ES MS/MS) mass spectrometry offer the possibility of high-throughput acquisition of metallomics information in many biological systems. The progress in electrospray MS instrumentation being much faster than that of ICP MS, molecular mass spectrometry is invading many areas currently occupied by ICP MS. On the other hand, there are still some features of ICP MS which make it an indispensable technique in bioinorganic speciation analysis.

The lecture presents recent advances in analytical techniques for speciation of metals and metalloids interacting with the cellular metabolome and proteome. Particular attention is given to the correlations of the analytical chemistry data acquired with those obtained by molecular biology approaches or bioinformatics.

S19: Analytics in Pharmacy and Medicine

Keynote Lecture 19

LC-MS/MS IN CLINICAL ROUTINE LABORATORIES: CURRENT PERSPECTIVES AND LIMITATIONS

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The technical maturation of liquid chromatography tandem mass spectrometry (LC-MS/MS) hyphenations over the past decade brought this technology into most of the major clinical laboratories worldwide. It found its sound place amongst major basic routine technologies of laboratory medicine as enzyme based assays or immunoassays. LC-MS/MS extended the technological armamentarium of clinical laboratories significantly, both in analytical and economical terms. Especially in therapeutic drug monitoring, endocrinology, and toxicology, it became an indispensable routine tool [1-3].

Although a well designed LC-MS/MS assay generally outperforms immunoassays due to its accuracy, sensitivity, precision, and its inherent multiplexing capability, it is not free from analytical problems. Besides limitations in selectivity (i.e. “isobaric” interferences), sudden and unpredictable ion yield attenuations, often known as “ion suppression effect”, can be considered *the* Achilles heel of quantitative bio-analytical mass spectrometry [4].

Most LC-MS/MS methods used in clinical laboratories are still “home-brewn” laboratory-developed tests operating on very heterogeneous instrument configurations, although commercial IVD-CE certified LC-MS/MS assay kits have become available recently. Consequently, assay heterogeneity and lacking traceability to reference procedures or materials leads to an increased imprecision in proficiency testing as well as to inaccurate result reporting if basic rules of assay validation and “post marketing” surveillance are violated.

The position of LC-MS/MS and its advantages / disadvantages compared to immunoassays will be discussed. Technical limitations and analytical problems of LC-MS/MS instrumentation will be critically evaluated in the light of technical development.

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ENANTIORESOLUTION OF CHLORO INTERMEDIATES OF METHAMPHETAMINE BY MULTI DIMENSIONAL NUCLEAR MAGNETIC RESONANCE AND CHIRAL GAS CHROMATOGRAPHY

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Methamphetamine use exists to some extent in all European countries, and is a huge problem worldwide particularly in the US and SE Asia/Australia/NZ. This is because of methamphetamine has low production costs, easily obtained precursor chemicals, and a simple production process. There are various routes to the synthesis of methamphetamine e.g. Emde, Nagai, Birch. During these synthesis, different by-products are generated. Impurity profiling and classification of seized methamphetamine may supply valuable information with respect to the conditions and the chemicals used in the illicit manufacture and may provide information on the original source of the sample. Determination of the stereochemical makeup of forensic samples is important and provide chemical information not available from other types of chemical analysis.

The Emde synthetic route involves the chlorination ephedrine/pseudoephedrine, followed by hydrogentation of the intermediates to form methamphetamine. It is reported that methamphetamine synthesized by Emde contains following by-products: ephedrine, chloroephedrine, cis-1,2-dimethyl-3-phenylaziridine, trans-1,2-dimethyl-3-phenylaziridine, two unidentified impurities. Stereochemical relation of (+)-methamphetamine to its initial precursor (-)-ephedrine or (+)-pseudoephedrine is achieved by detection of (+)-chloropseudoephedrine or cis-1,2-dimethyl-3-phenylaziridine, and (-)-chloroephedrine or trans-1,2-dimethyl-3-phenylaziridine, respectively. Thus, knowledge about chiral profile of methamphetamine synthesized by Emde is important and may be used to determine the source of starting material and provide links to illicit laboratory.

To obtain full characterization of enantiomeric composition of methamphetamine synthesized by Emde, pure enantiomers of chloroephedrine as reference substances for analytical analysis are required. Therefore, is necessary to work out stereochemistry of the standards and thereafter the chromatographic behaviour.

The purpose of this study was to investigate the stereochemical course of part I of the Emde method involving the synthesis chloroephedrine/chloropseudoephedrine via chlorination of ephedrine/pseudoephedrine. The configuration of these chloroamines was determined by multi-dimensional NMR analysis. The separation of the enantiomers of chloroephedrines was carried out by GC on a chiral stationary phase.

THE IMPORTANCE OF CAPILLARY ELECTROPHORESIS IN CLINICAL CHIRAL ANALYSIS – METHOD DEVELOPMENT, VALIDATION AND APPLICATION

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The goal of this research is the development of electrophoretic methods for improved chiral separations of analytes that are extremely important in clinical analysis. Chiral compounds are common in several synthetic and biological systems. It has been shown that usually only one enantiomer is active, while the other might be less active, inactive or has damaging side effects. Fucose is a rare carbohydrate essential for proper cell-to-cell communication. Fucose can be found in the skin, brain cells, kidneys, retina of the eye, and breast milk. Pipecolic acid is a human metabolite that is found in biofluids. In human blood stream, the L-enantiomer is mostly present, while the D-enantiomer is primarily excreted. Even though high levels of D-pipecolic acid are not found in plasma, they are significantly increased in urine of people with chronic liver disease. Huperzine A is an important compound that is used to treat Alzheimer’s disease. However, only the (-) form of this compound is biologically active and behaves as a potential acetylcholinesterase inhibitor. Therefore, the separation of the (-) form from the (+) for the compounds mentioned above is of greatest importance. In this study, MEKC and CD-EKC were used for the chiral separation of fucose, pipecolic acid and Huperzine A. The optimum conditions were obtained by investigating different parameters, and particularly the type of the chiral selector. The most effective chiral selector was determined in regards to resolution, analysis time and reproducibility. More parameters were examined, such as the addition of modifiers, in order to optimize further the separations in regards to efficiency. The method was then validated in terms of reproducibility, linearity, LOD and LOQ. Finally, the optimum conditions were applied to a pharmaceutical formulation, in order to demonstrate the ability of the method to control the purity of the (-)-Huperzine A in pharmaceutical formulations.

IMPROVING THE ANALYSIS OF HERBAL MEDICINAL PRODUCTS BY FUSED CORE™ PARTICLE TECHNOLOGY AND EVALUATION OF DIFFERENT LIQUID CHROMATOGRAPHY STATIONARY PHASE CHEMISTRIES

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Herbal Medicinal Products (HMPs) often consist of a highly complex mixture of both active and inactive ingredients. The development of appropriate analytical separation methods can therefore be an arduous task. Increasing government regulations relating to the production and sale of HMPs have placed even more burden on the analytical chemist to develop effective and robust methods. To assure the quality of Herbal Medicinal Products the content of constituent(s) with known therapeutic activity, active markers or analytical markers have got to be determined in the starting materials, in in-process controls and batch release controls [1]. The indication of the content of constituent(s) with known therapeutic activity in the Summary of Product Characteristics (SmPC) is required for HMPs consisting of standardized or quantified herbal preparations [2].

Due to the complex composition of natural products HPLC analysis time is very often more than one hour. Fused Core™ particle technology provides the ability to reduce the analysis time without sacrificing resolution [3]. When separating the components of a complex mixture, traditional alkyl stationary phases are often inadequate. The choice of alternative phases, however, is often made arbitrarily and thus may be ineffective at solving a given issue.

In this study, the separation of active and inactive components for several popular natural products is investigated using a variety of commercially available stationary phase chemistries based on the Fused Core technology. Parameters such as resolution, retention and selectivity are compared, contrasted and related to known retention mechanisms exhibited by the phase chemistries. General trends and guidance will be provided that promises to greatly facilitate the method development in this complex environment.

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[3] Reporter (2009) Vol. 27.1, page 9.

CE-ICP-MS – as a tool to model the fragile human system transporting metallodrugs

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Metallodrugs play an increasingly important role in the arsenal of anticancer remedies. As hybrids of inorganic and organic components, they join advantages of inorganic and organic drugs: potential in treatment of a broad range of tumors and selective mode of action, respectively. However, cancer still causes death of more than 20.000 people a year, in a great extent due to many widespread and not curable tumors. Therefore, examination of potential anticancer metallodrugs needs to be intensified and, in particular, focused on a better understanding of the mode-of-action of designed non-platinum tumor-inhibiting compounds. A promising examples, indazolinium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] and tris(8-quinolinolato)gallium(III) are now appreciably progressing in clinical studies, with the outcome of few side effects and evidence of clinical activity in renal cell carcinoma. In order to make their clinical development more straightforward, the way in which the drugs are transported into cancer cell should be clarified.

Capillary electrophoresis (CE) as a high performance separation technique can be considered as the most suitable for kinetic and thermodynamic studies of protein binding of metallodrugs due to lack of organic modifiers in the separation medium, no need of the stationary phase, and small sample volume requirement. Pros and cons of CE-ICP-MS hyphenation will be discussed on the basis of our experimental results and experience gained during studying the transport mechanism of ruthenium(III) and gallium(III) developmental drugs. The approaches will also be reported to circumvent the challenging problems in exploiting the CE system that can follow the drug-processing reactions in human organism.

S19: Analytics in Pharmacy and Medicine

ANALYTICAL CHEMISTRY IN PHARMACEUTICAL RESEARCH AND DEVELOPMENT

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The objective of this presentation is to identify analytical tools commonly used at various stages of drug development pathway from discovery to market and to specify test-method related requirements. Drug development is a long process that involves target selection, biomarker identification, lead identification and optimization, non-clinical and clinical (phases 1-3) testing, formulation design and optimization, regulatory approval and post-marketing drug monitoring. Target selection, based on matabolomics and proteonomics, as well as biomarker identification, are the tasks commonly approached by MS- or NMR- based methods. Lead identification and optimization assumes physico-chemical characterization and *in vitro* and *in vivo* assessment of ADME properties, including identification of metabolic pathways mostly using LC-MS or LC-MS-MS techniques. During formulation design and optimization the major focus is development of robust and stable formulation. Critical quality attributes of active and inactive pharmaceutical ingredients, such as potency, purity, stability, compatibility, crystallinity and polymorphic stability, are determined and testing must be performed to ensure quality of the finished product. Purity testing is a complex task due to diverse nature of potentially present impurities (i.e. starting materials, synthetic intermediates, by-products, degradants, isomers, heavy metals, residual solvents, genotoxic impurities), and as such is approached by variety of analytical techniques (HPLC-DAD, HPLC-MS, chiral chromatography, head-space GC, AAS, ICPS etc). *In vitro* drug release from solid oral dosage form is a tool to assess *in vivo* release of active ingredient and drug *in vivo* bioavailability. Development of bio-indicative *in vitro* dissolution method is challenging, especially for drugs with poor solubility or low content of active ingredient in the formulation. Finally, drug, in order to be marketed, has to meet regulatory and compendial requirements . A dynamic interactive relationship between analytical development and regulatory/compendial requirements is a driving force that affects both analytical and regulatory science.

S20: Sample preparation/Separations

Keynote Lecture 20

AUTOMATED SAMPLE PREPARATION BY COUPLING SEQUENTIAL INJECTION ANALYSIS (SI) TO SEPARATION TECHNIQUES

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Sequential injection analysis (SI) is considered to be the second generation of automated flow-injection based techniques. SI offers some interesting features to analytical chemists such as: (i) precise handling of minute volumes of liquids, (ii) drastic reduction in reagents consumption and waste generation and (iii) automation through single-channeled manifolds. The potentials of SI are magnified when it is coupled to separation techniques such as liquid (SI-HPLC) and gas chromatography (SI-GC) and capillary electrophoresis (SI-CE). The resulted analytical schemes combine automated sample preparation by SI (e.g. derivatization, dilution, extraction etc) and the high selectivity and separation efficiency of these techniques.

The present lecture will present interesting published applications based on the "marriage" of SI to the above mentioned separation techniques. Additionally, the concept of Sequential Injection Chromatography (SIC) – based on the incorporation of monolithic columns to low pressure SI setups – will be discussed. Finally, new instrumental advances that might lead to the development of "High Pressure Sequential Injection - HPSI" will be highlighted.

PARTICLES IN ULTRASOUND FIELD: BEHAVIOUR, FRACTIONATION AND APPLICATION IN CHEMICAL SEPARATIONS

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Ultrasound (US) can help analytical chemists in numerous laboratory tasks, from sample preparation to detection. In sample preparation US is applied to treat liquid, solid, and heterogeneous sample. In the latter case, US can facilitate the formation, stabilization, fractionation and filtration of suspensions containing micron and submicron particles. In the last few years, some new methods have been developed for continuous fractionation and characterization of samples containing particles due to different retention of different particles in an external acoustic field. Such an approach and corresponding devices were used for the fractionation of various micro particles.

The possibility to retain particles in a US field has allowed us to arrive to the idea that beaded sorbents, most often used in solid-phase extraction (SPE), can be completely retained in a flow cell or column in the form of suspension and applied as fluidized beds instead of conventional packed beds. This novel approach has been shown to give serious advantages. First, US field makes it possible to work with SPE units filled with sorbent beads as small as a few microns which are hold in a sample solution flow. Such small beads are known to be more efficient sorbents than the grains of 40-100 μ traditionally used in SPE to avoid applying high pressures needed to overcome a pressure drop across the SPE cartridge or column. Therefore second, low-pressure suspension units can be used. Third, acoustic field may simultaneously play another role: to accelerate sorbent/sorbate interactions. It has been shown experimentally that a combination of an acoustic force field with an appropriate flow system enables the efficient recovery of various analytes from liquid samples. This has been demonstrated by separation and enrichment of metal ions from aqueous solutions using complexing sorbents and by preparation of some biological samples by means of immunosorbents.

S20: Sample preparation/Separations

NOVEL APPROACHES TO THE SYNTHESIS OF COVALENTLY MODIFIED ANION EXCHANGERS FOR ION CHROMATOGRAPHY

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Ion chromatography is the best method for determining substances of ionic nature in different environmental samples. Stationary phase usually plays the key role in chromatographic analysis and modern ion exchangers should possess such characteristics as low ion-exchange capacity, high stability, absence of swelling in a mobile phase and guarantee of high efficiency and selectivity of separation.

Polystyrene-divinylbenzene (PS-DVB) ion exchange resins meet all these requirements but there are some problems associated with using PS-DVB-based sorbents as the stationary phases for the determination of polarizable inorganic anions such as nitrate and bromide. These ions have strong affinity to the matrix of anion exchanger which is caused by their specific interactions with aromatic rings of a polymer. It results in abnormal retention, low efficiency and unsymmetrical peaks for these anions.

Negative influence of a matrix can be eliminated by increasing the hydrophilicity of functional groups or moving them away from aromatic rings with long alkyl or carbonyl spacers. In this study two novel methods of synthesis including one or both of these approaches are presented:

I	II
1. Acetylation with acetic anhydride	1. Chloroacylation
2. Reductive amination	2. Amination
3. Alkylation	3. Alkylation

The main advantage of both methods is that they allow one to vary the structure of the functional group, its hydrophilicity and spacer length easily on different stages. Traditionally halogeno- and dihalogenoalkens are used for alkylation but in order to increase the hydrophilicity of functional groups oxiranes were proposed as a novel promising class of alkylating agents.

Chromatographic properties of the obtained anion exchangers were studied by means of suppressed ion chromatography with conductometric detection. The decrease of the retention of the polarizable ions of nitrate and bromide, increase of their efficiency and improvement of the peak shape were observed as the hydrophilicity of the functional group and spacer length increased. Anion exchangers with heightened hydrophilicity provide good efficiency and selectivity for separating the mixture of seven inorganic anions such as fluoride, chloride, nitrite, nitrate, bromide, phosphate and sulfate in less than 30 min in isocratic mode with using sodium bicarbonate as a mobile phase. The highest efficiencies of the columns with the obtained anion exchangers for phosphate-ion were 50000 (using carbonate buffer solution as a mobile phase) and 35000 (using sodium bicarbonate) theoretical plates per meter.

ENRICHMENT OF PALLADIUM FROM AQUEOUS SAMPLE SOLUTIONS USING FUNCTIONALIZED NANO- PARTICLES

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Pd is widely used in today's automotive catalytic converters. Thermal and mechanical stress cause surface abrasion of the catalytic material, releasing Pd in the environment [1]. Due to its potentially harmful properties, it has become necessary to perform analysis of very low concentrations of Pd in environmental samples. The methods at hand for this task are ICP-MS, ICP-AES or ET-AAS [1]. Unfortunately, when using these methods, Pd is either strongly interfered by other elements (ICP-MS) or sensitivity does not allow measurements in the desired concentration range (ICP-AES, ET-AAS). To improve detection limits and to eliminate interferences usually sample pre-treatment techniques like solid phase extraction (SPE) with ion-exchangers are applied. Although being well established, SPE suffers from several shortcomings such as time consuming conditioning steps, incomplete recovery of the adsorbed ions and memory effects. One of the major drawbacks of SPE is the necessity to elute the analyte after adsorption using appropriate solvents.

In this work a novel sample pre-treatment procedure for enriching Pd from liquid samples is presented, featuring the idea of renewable surfaces and eliminating any elution process. Porous nano- spherical silica was functionalized with quarternary ammonium groups [2], yielding a strong anion exchanging material with a mean particle size of 700nm. Due to the high specific surface of the silica, very low amounts of this compound are sufficient to obtain nearly quantitative recovery for Pd from aqueous solutions. After separating the particles from the sample volume by means of a centrifuge and removing excess liquid, an aliquot of the remaining suspension is analyzed using ET-AAS. This straight- forward slurry approach helps to overcome the problems associated with the elution process. On that account it is possible to enrich Pd from aqueous solutions or sample digests, propelling the limit of detection in the 100ng/L range.

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THERMO-RESPONSIVE POLYMER-MEDIATED EXTRACTION OF ANTIBACTERIAL AGENTS FROM WATER

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A novel extraction method using thermo-responsive polymers was developed and applied to the removal of antibacterial agents in water. A thermo-responsive polymer, poly (*N*-isopropylacrylamide), was water-soluble at room temperature, but deposited above the lower critical solution temperature (ca. 32°C). By shaking the solution, the polymer deposits were agglutinated to form a small gum-like coagulate (polymer phase). Accompanying with the heat-induced polymer deposition, hydrophobic antibacterial agents such as triclocarban and triclosan in water were quantitatively collected and highly concentrated to the polymer phase. It was dissolved with a small amount of acetonitrile for the introduction into the HPLC system having a UV or MS detector. The method was useful to preconcentrate traces of antibacterial agents in environmental waters and effluents. For the collection of rather polar phenolic compounds, chitosan- and polyallylamine-conjugated thermo-responsive polymers were prepared by the water-soluble carbodiimide-mediated condensation method. In the presence of an appropriate oxidation enzyme, phenols in water were converted to dark brown compounds and spontaneously bound to the amino moieties of polymer molecules. Tyrosinase (from mushroom) was useful for the collection of phenol and its *p*-substituted derivatives. However, it was not effective for the oxidation of *o*- and *m*-substituted phenols. On the other hand, peroxidase (from horseradish) was a useful enzyme for the oxidization of varieties of phenolic compounds in the presence of hydrogen peroxide. The method allowed the rapid removal of *o*-, *m*-, *p*-isomers of cresols, methoxyphenols, and chlorophenols. Phenolic drugs, metabolites, and hormones including isopropylmethylphenol, acetaminophen, aminophenol, L-DOPA, and estrogens were also collected completely from water. The applicability of the proposed method to the treatment of municipal and hospital wastewaters was investigated.

RENEWABLE AUTOMATED SOLID PHASE EXTRACTION BASED ON BEAD INJECTION CONCEPT COUPLED TO LIQUID CHROMATOGRAPHY FOR ON-LINE SAMPLE TREATMENT

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The integration of sample treatment to liquid chromatography (LC) is generally implemented using robotic stations and commercial, disposable cartridges filled with suitable solid phase sorbents, requiring expensive and sophisticated equipment. Continuous flow based solutions for on-line automated sample treatment have been proposed as well, but they are not feasible for complex samples, because the accuracy and precision of LC determination are often jeopardized by carry-over effects from consecutive samples, or by permanent contamination of the flow-through extraction columns.

Recently, Lab-on-Valve (LOV) systems were proposed as an alternative that fosters the automatic renewal of the solid phase sorbent in a simple, less expensive apparatus that can be directly coupled to separative techniques [1]. In this case, sorbent renewal is achieved through the bead injection (BI) concept, where the solid sorbent is manipulated within the micro-channels of the LOV piece in an automated fashion. The present communication will address recent developments in this area, concerning novel strategies for mesofluidic handling of solid phases with non-spherical shape, namely molecularly imprinted polymers for molecular recognition of target analytes and for selective separation from complex, real samples such as milk and soil extracts. Other relevant features, including coupling strategies between LOV and LC to circumvent peak broadening effects, will be critically discussed. Finally, successful examples of LOV-BI coupling to LC will be given, including the determination of UV filters in coastal waters and phenolic compounds in food samples.

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Keynote Lecture 21

DEVELOPMENTS IN ACCREDIATION AND INTERNATIONAL MEASUREMENT INFRASTRUCTURE: RELEVANCE FOR LABORATORIES

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About 20 years after the introduction of accreditation in Europe the thus induced advantages for analytical quality assurance are well established and taken for granted. Except in small niches it is no longer possible to offer analytical services without being accredited. For this reason it is essential to constantly monitor the modern developments in quality matters for the chemical laboratory in order both to increase its efficiency and ensure high standards in reliability of new analytical procedures.

The most significant development of the last years is – besides the acceptance of quality management on the basis of ISO 17025 in the version of 2005 – the recent responsibility of national governments for matters of accreditation. This provision serves mainly the purpose to keep accreditation itself free of influences of the market in order to have a better basis for the assessment of conformity for accredited laboratories and notified bodies.

With the development of new nomenclature and definitions published in VIM 3 (also known as ISO 99) a remarkable attempt has been made to make all of metrology independent of the special field of expertise. This was accompanied for each and every branch – therefore also for chemical measurement – novelties and compromises, but it has undoubtedly helped to strengthen metrology on a worldwide basis. This has led the EURACHEM Working Group on Education and Training to issue separate interpretations and examples for some of the most important terms.

With respect to technical advances for enhancing worldwide comparability of chemical measurement CCQM has intensified its efforts in the organisation of Key Comparisons. It is particularly important that the experiences gained through these exercises are made available to all interested laboratories. For analytical chemistry advances in the estimation of measurement uncertainty through Monte Carlo approaches are of great scientific value as this leads to much more plausible results particularly for greater uncertainties and for measurements near the detection limit. Examples of this approach will be provided.

THE PRINCIPLE OF POOLED CALIBRATIONS AND OUTLIER RETAINMENT ELUCIDATES OPTIMUM PERFORMANCE OF ION CHROMATOGRAPHY

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A new principle of statistical data treatment is presented. Since the majority of scientists and costumers are interested in determination of the true amount of analyte in real samples, the focus of attention should be directed towards the concept of accuracy rather than precision. By exploiting the principle of pooled calibrations retaining all outliers it was possible to obtain full correspondence between uncertainty of calibration and uncertainty of repetition, which for the first time evidences statistical control in experiments of ion chromatography. Chloride, bromide and oxalate were determined at a high number of repetitions in standards containing different anions. The method was capable of baseline separation of 17 different anions. It was found that the limit of quantification (LOQ) was significantly underestimated by up to an order of magnitude with respect to determination of concentration of unknowns. The concept of lower-limit of analysis (LLA) and upper-limit of analysis (ULA) were found to provide more acceptable limits with respect to reliable analysis at a limited number of repetitions. These findings comply with earlier investigations of method validations where it was found that the principle of pooled calibrations provides a more realistic picture of the analytical performance with the drawback however, that generally higher levels of uncertainties should be accepted, as compared to contemporary literature values. The implications to the science of analytical chemistry in general and to method validations in particular are discussed.

OUT-OF-SPECIFICATION RESULTS OF CETIRIZINE DIHYDROCHLORIDE ASSAY

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Cetirizine dihydrochloride is used to treat allergic rhinitis and chronic idiopathic urticaria, and as a treatment adjunct in asthma. European Pharmacopoeia sets the lower specification limit $c_{\mathrm{l.s.l}} = 99.0\%$ and the upper specification limit $c_{\mathrm{u.s.l}} = 100.5\%$ of cetirizine dihydrochloride content in a bulk material (dried substance). The test/assay method is the acid-base potentiometric titration of acetone-water solution of the analyte with sodium hydroxide to the second point of inflexion. A test result c_{test} obtained by this method should be in the specification limits, i.e., $c_{\mathrm{l.s.l}} \leq c_{\mathrm{test}} \leq c_{\mathrm{u.s.l}}$.

Results which fall outside the specification limits are named out-of-specification (OOS) test results. They may be obtained when the true content c_{true} of cetirizine dihydrochloride is really less than the lower specification limit ($c_{\mathrm{true}} < c_{\mathrm{l.s.l}}$) because of a high content of known impurities. However, OOS test results may be caused also by measurement problems and, for example, exceed the upper specification limit ($c_{\mathrm{test}} > c_{\mathrm{u.s.l}}$) while $c_{\mathrm{true}} < c_{\mathrm{u.s.l}}$. When an OOS test result differs from the specification limit in the range of the measurement uncertainty, it is considered as metrologically-related. Such a consideration is important for understanding the root causes of OOS test results and preventing, where possible, their occurrence.

Probabilities of OOS test results, discussed in application to an example, are calculated for both specification limits. Global producer's and consumer's risks of incorrect decisions about the product quality, caused by the measurement uncertainty, are evaluated. Intra-factory acceptance limits for c_{test}, stronger than the specification limits, are examined.

SHOULD RECOVERY INFORMATION BE USED TO CORRECT MEASUREMENTS?

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Every step of analytical procedure carries the risk of occurrence of errors which have an impact on the quality of analytical result. Recovery (RV) is one of tools of both the quality assurance and quality control that is commonly used in analytical laboratories to evaluate the trueness of analytical result [1].

The International Union of Pure and Applied Chemistry (IUPAC) have compiled the guidelines for the use of recovery information in analytical measurement which are only a conceptual framework [2,3]. Neither in these guidelines nor in the available literature any research on the influence of various effects on the estimation of recovery was found.

Theoretical considerations prove that erroneously interpreted information resulting from RV value may gain a semblance of a reliable evaluation of an analytical result. In practice it means that the obtained RV = 100% is not equivalent to achieving a result close to the real one. On the other hand, the accurate result may be obtained in the conditions which do not guarantee the complete analyte recovery [4].

The influence of the effects (preparative and interference ones, the analyte effect) on the RV value, which may appear during a whole analytical procedure, was presented in this work. On the basis of the obtained results an attempt was made to answer the essential question raised by IUPAC: whether and in what circumstances the analytical result should be corrected for recovery?

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DETERMINATION OF TRACE ELEMENTS IN DANUBE WATER: RESULTS OF SIX INTERLABORATORY STUDIES IN SOUTH-EASTERN EUROPE

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In order to improve the quality of chemical analyses of water in South-Eastern Europe the long term project was initiated and six interlaboratory studies were organised in the period 2003-2011. The International Scientific Committee with professor dr. Anastasios Voulgaropoulos as the Chairperson organised and evaluated regional interlaboratory studies. More than 70 laboratories from Greece, Montenegro, Republic of Srpska - Bosnia and Herzegovina, Romania and Serbia took part in them. The samples for regional interlaboratory studies were prepared from filtered water of the river Danube near Belgrade by addition of corresponding substances. In each regional interlaboratory studies one of the the task for participants was to determine trace elements (Al, As, Cd, Cu, Mn, Fe, Pb and Zn) in two water samples. Results were analysed using the same methods as in interlaboratory studies organised by IRMM-JRC (Institute for Reference Materials and Measurements: EU-Joint Research Centre, Geel, Belgium) within IMEP (International Measurements Evaluation Programme). In addition to graphic presentation and analysis of results by Youden method, Cochran and Grubbs tests were also used. Reproducibility (R) and repeatability (r) were calculated according to the British standard BS 5497: Part I: 1987. All regional interlaboratory studies were generally successful and useful for participants. Systematic errors were observed in some cases and these laboratories needed to improve their performance. The quality of results for some elements improved gradually in the period 2003-2011.

DEVELOPMENT OF NEW CERTIFIED REFERENCE MATERIALS FOR CHROMATOGRAPHY BY CONTENT ASSIGNMENT WITH HIGH PERFORMANCE QUANTITATIVE NMR (HP-qNMR)

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Quantitative NMR spectroscopy (qNMR) has become an invaluable instrument for exact content assignment and quantitative determination of impurities. [1]
^{1}H-qNMR is a relative primary method because the intensity of a signal is direct proportional to the number of protons contributing to the signal of interest. [2] Therefore the signals of a sample compound and a reference substance with known content can directly be compared and the content of the sample compound can be assigned. Based on this approach an unequivocal traceability to international acknowledged reference standards, e. g. from NIST, is achieved. [3] Chromatographic techniques such as HPLC or GC usually do not provide this traceability, because they require a highly pure standard of the substance to be investigated which is often not available.
This work presents the approach of content assignment by High-Performance Quantitative NMR (HP-qNMR®) and its application on organic compounds from different classes such as amino acids, polycyclic aromatic hydrocarbons, pesticides, antibiotics, fatty acids and fatty acid esters, resulting in new Certified Reference Materials for chromatography. The high performance qNMR measurements were performed at maximum accuracy with a 600 MHz NMR instrument under ISO17025 and ISO34 double accreditation resulting in expanded uncertainties between 0.1 % and 0.5%.

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Poster presentations
Session A

Bioanalytical Chemistry	BC03-BC32
Chemometrics	CM01-CM26
Electroanalytical Chemistry	EC01-EC56
Teaching Analytical Chemistry	ED01-ED06
Food Analysis – Part I	FA01-FA40
Bio- and Chemosensors	SE01-SE30

POSSIBILITY OF KOMPLEX ELECTROCHEMICAL DETECTION OF STRUCTURAL DNA DAMAGE IN THE PRESENCE OF INTERCALATOR THIORIDAZINE

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Intercalator represents a group of chemicals that can, according to their appropriate size and chemical structure, specifically interact with base pairs of DNA. Commonly they are characterized by the possession of an extended planar heteroaromatic ring system (chromophore) that can be inserted in between adjacent base pairs. Intercalation results in an extension of the double helix, together with changes in helical twist (unwinding) for the base pairs at adjacent to each binding site. These changes in double helix might be significantly detected by electrochemical methods, especially electrochemical impedance spectroscopy. Electrochemical detection technique represents relatively cheap, nondestructive and effective method for the characterization of surface attached NA layers at the NA biosensors. With the use of DNA intercalative chemicals we should be able to obtain also more specific EIS signals of individual NA layers at the electrodes. Using this approach we could confirm match or mismatch hybridization without additional tags or mediators. Intercalators can not only serve as a stain for detection of DNA hybridization but also contribute to the detection of structural DNA damage.

In this work we study the electrochemical intercalation properties of the drug thioridazine. We perform the measurements of detection of the DNA damage by reactive oxygen species and UV radiation in the presence and absence of thioridazine. We studied the DNA damage on a sample of flavored mineral water. Information acquired from the measurements with thioridazine showed different behavior between drug and DNA interaction on DNA damage by ROS and DNA damage by UV radiation. Model DNA damage by ROS was additionally confirmed by the measurement on a real sample.

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SPANISH SALVIA EXTRACTS: AROMATIC PROFILE AND ANTIOXIDANT CAPACITY

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Spanish salvia (*Salvia lavandulifolia*) from Murcia country, was milled and extracted with water, ethanol and n-hexane. The extracts were analysed on an Agilent SB C18 5 cm x 4.6 mm x 1.8 microm column, with 0.1% formic acid water/acetonitrile gradient as mobile phase, using an Agilent 1200 HPLC-RR liquid chromatograph, DAD-SL detector and ChemStation software. Water extract showed high proportions of chlorogenic and caffeic acids and other phenolic acids. Hexane extract contains broad and high concentrations of terpenoids and some flavonoids such as quercetin and luteolin. Ethanol extract consist of phenolic acids, flavonoids and some terpenoids. Hexane extract: The main aromatic terpenoids were camphor (57.68%), eucalyptol (22.69%), bornyl acetate (9.61%), borneol (2.36%) and limonene (1.64%). Ethanol extract: camphor (60.87%), eucalyptol (20.80%), bornyl acetate (8.26%) limonene (2.34%), borneol (2.17%) and camphene (1.71%). These values were determined from calibration straights on a Supelco SLB 5ms 15 m x 0.1 mm x 0.1 microm column, with hydrogen gas carrier, using an Agilent 7890 gas chromatograph, the 5975 EI-SQ mass spectrometer detector, the ChemStation software and the NIST and Wiley mass spectrometry libraries. The antioxidant capacities of the extracts have been evaluated with the ABTS-TEAC and ORAC-TEAC assays. The disappearance of the ABTS radical has been monitored at 734 nm with a Perkin Elmer dual beam Lambda 35 spectrophotometer and UV-WinLab software. The fluorescein disappearance was recorded at 485 nm and 530 nm as excitation and emission wavelengths, respectively, after 3D optimization with a Perkin Elmer LS55 luminescence spectrometer and FL-WinLab software. This work has been partially supported by grants from several Spanish organizations. Projects BIO2009-12956 (MICINN, Madrid) and 08856/PI/08 (Fundacion Seneca, CARM, Murcia). Predoctoral fellowships JMR BES-2007-16208 (FPI, MICINN, Madrid), MP 09378/FPI/08 (Fundacion Seneca, CARM, Murcia), AC (Esencias Martínez Lozano, Murcia), VO (Alissi Brontë, Murcia).

NEW ASSAY OF THE ACETYLCHOLINESTERASE ACTIVITY BY POTENTIOMETRIC MONITORING OF ACETYLCHOLINE

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Qualitative and quantitative activity analysis of the enzyme acetylcholinesterase (AChE) have been implementing because of it applicability to a very broad range of fields. For that purpose, an acetylcholine (ACh) selective electrode, that allowed us to monitore the AChE-catalyzed hydrolysis of ACh, is here proposed.

Firstly, we proceeded to develop an ACh-selective electrode that satisfies the requirements for kinetic monitoring: sufficient sensitivity and stability, low background noise, low drift, rapid response time and adequate selectivity. To achieve this, the influence of the plasticizer and the amount of the ionic additive present in the electrode membrane was studied. A membrane containing NPOE as plasticizer and 0.5% of ionic additive was selected because it provided the best sensitivity and the best selectivity with respect to choline and some common inorganic cations.

Secondly, the selected ISE was applied for monitoring the AChE-catalyzed hydrolysis of ACh in a pH 7.5 phosphate buffer at a constant temperature of 28ºC. Variables that affect the reaction kinetics, substrate and enzyme concentrations, were studied. The application of the Michaelis-Menten equation to the results obtained in the study of the substrate influence provided values for V_{max} and K_m of $7.6x10^{-8} \pm 0.1x10^{-8}$ Ms^{-1} and $4.7x10^{-5} \pm 0.1x10^{-5}$ M, respectively.

The effect of the AChE activity was studied and a linear relationship between enzymatic activity and AChE concentration was obtained, which might be used for the determination of microquantities of AChE in problem samples.

BACTERICIDAL ACTIVITY OF SOME CALIX ARENES BASED POLYMERIC MEMBRANES

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New type of polyimide based selective membranes were obtained. As electroactive compound there were used calix[6] arene, calix[8]arene and sulphonated calix[8]arene. Due to the polymeric matrix used, namely polyimide, the selective membranes presented a very good adherence to the silicon nitride surface of a microdevice. In consequence, such membranes were suitable for clinical microdevices.

Therefore, prelimary tests to evaluate the selective membranes behaviour in biological environment were performed. The laboratory tests were conducted on ATCC 15445 *Pseudomonas aeroginosa* bacteria strains and fungi strain - *Aspergillus niger*. The latest used microorganism belongs to the monomorphic fungi type with septated hyphae hyaline. Also, microbioological tests on a yeast strain of *Candida robusta* type were carried out on the selective polyimde membranes.

There were realized antibiogram discs with 10 mg concentration on a disk for the used electroactive compounds as: calix[6]arne, calix[8]arene and sulphonated calix[8]arene. From the polymeric selective membranes obtained with each of the previously mentioned calix arene there were cut discs. It was applied the method of antifungigrame diffusion on a standardized subculture. Inoculum used should correspond approximately to 0.5 McFarland standard, so to obtain an inoculum density of 1.8 x 10^7 CFU/mL for bacteria and yeast and from 0.4 x 10^4 CFU/mL to 5 x10^4 CFU/mL for fungi, where CFU stands for colony-forming unit.

Active substances from the calix arene tested compounds: calix[6]arne, calix[8]arene and sulphonated calix[8]arene and the corrsponding selective polymeric membranes, had no inhibitory effect (bactericidal) over the strains of *Pseudomonas aeroginosa*, *Candida robusta* and *Aspergillus niger* at concentrations of 10 mg/mL for the studied compounds.

Calixarene-based active substances showed no toxicity to the cells of microorganisms used in these experiments which may allow their inclusion into products, selective microdevices, respectively, for human use.

BC10 **Session A**

HIGH-RESOLUTION ACCURATE MASS MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETRY UTILIZED TO FACILITATE METABOLITE IDENTIFICATION

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The structural elucidation of metabolites by LCMS remains challenging in many cases. Some metabolic pathways yield modifications that are labile in the ion source. This can reduce the protonated molecular ion’s intensity so that confidence in its assignment diminishes. Some analytes yield a small number of intense fragments so their spectra lack the richness required to propose a structure with certainty. To meet these challenges, the number or complexity of experiments is often increased. Performing successive product ion, precursor ion, or neutral loss experiments under different conditions adds to the scientist’s workload. Elaborate data-dependent MSn protocols can tax the duty cycle of an instrument, and are difficult to design so that all of the appropriate data are captured. The preferable solution is to gain more information from fewer, simpler experiments. Herein, we describe how multi-reflecting time-of-flight mass spectrometry (MR-TOFMS) enriches the structurally relevant information of a mass spectrum. MR-TOFMS technology can exhibit massindependent and acquisition rate-independent resolving power greater than 50,000 and sub-ppm mass accuracy. The instrument’s novel design, including gridless mirrors and periodic ion beam refocusing affords this resolution at no cost to sensitivity. These factors, together with high dynamic range detection, allow lowabundance fragment ions to be extracted from complex backgrounds, and assigned with certainty. This is demonstrated by identifying metabolites of a complex multi-component pharmaceutical product in urine, without any sample treatment other than dilution, using a fast ultra high pressure liquid chromatographic method that resulted in significant coelution.

POTENTIOMETRIC DETERMINATION OF LITHIUM IN URINE WITH AN ION-SELECTIVE ELECTRODE

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Lithium salts, normally administered as lithium carbonate, have widely been employed in the control of bipolar disorder symptom. Unfortunately, some cases of severe intoxication and even death have been reported due to its narrow therapeutic window. Therefore, the monitoring of lithium levels in drugs and biological fluids is important to ensure adequate and safe treatment.

Therapeutic levels control of lithium in patients currently involves blood analysis which leads to some discomfort in patients until the drug dosis is adjusted. For this reason, it is interesting to look for an alternative based on urine analysis. The aim of this work is to propose a new method to determine lithium in urine samples at concentration levels at which it is excreted in treated patients. In urine analysis it is neccesary to correct the analytes concentration values found because the volume of excreted urine per time is variable. The ratio analyte/creatinine is used because the creatinine amount excreted is fairly constant with time.

For that purpose, a lithium-selective electrode with a PVC membrane containing N,N,N',N',N'',N''-hexacyclohexyl-4,4',4''-propylidynetris(3-oxabutyramide) as ionophore and DOS as plasticizer, was used. The response of the electrode is affected by the sodium concentration due to the high molar concentration ratio Na/Li present in the urine and the selectivity of the electrode. Therefore, it was necessary to develop a method that allows eliminating the sodium contribution.

Lithium calibrations were carried out by adding known amounts of lithium on different urine samples from untreated patients. The concentration of other species present in the samples, including sodium and creatinine, was previously obtained in order to evaluate their possible interference. Although sodium is a strong interfering, from an exact evaluation of its selectivity coefficient and its concentration, it is possible to determine the lithium concentration in urine accurately.

SURFACE PLASMON RESONANCE IMAGING BIOSENSOR FOR 20S PROTEASOME: SENSOR DEVELOPMENT AND APPLICATION FOR MEASUREMENT OF PROTEASOMES IN HUMAN BLOOD PLASMA

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The 20S proteasome is a multicatalytic enzyme complex responsible for intracellular protein degradation in all mammalian cells, and its antigen level or enzymatic activity measured in blood plasma are potentially useful markers for various malignant and nonmalignant diseases. In this study, a novel method for the highly selective determination of 20S proteasome by using a Surface Plasmon Resonance Imaging (SPRI) technique has been developed. For the sensor development, a highly selective interaction between proteasome catalytic β5 subunit and immobilized inhibitors: Z-Ile-Glu(OBut)-Ala-Leu-H (PSI) or epoxomicin was applied. Such parameters as inhibitor concentration, pH of interaction were optimized. The biosensors analytical responses, linear response ranges, accuracy, precision and interferences were investigated. Both PSI and epoxomicin based biosensors were found to be suitable for 20S proteasome quantitative determination showing a precision of 10% for each, recoveries of 102% and 113%, respectively, and excellent tolerance of albumin, trypsin, chymotrypsin, cathepsin B and papain. In order to demonstrate the biosensors and the SPRI technique applicability, 20S proteasome was determined in plasma from healthy subjects and patients suffering from acute leukemia. Both biosensors gave similar results equal to 2 860 ng/mL in average for control and 42 300 ng/mL in average for leukemia patients. Thus, the method of the present invention is highly selective and can be useful for measurements of 20S proteasome concentration in human plasma.

CAPILLARY ELECTROPHORESIS WHITH MASS SPECTROMETRIC DETECTION FOR THE DETERMINATION OF URINARY LEVELS OF MODIFIED NUCLEOSIDES

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Modified nucleosides excreted in human urine are related to RNA turnover, a process that is increased in the presence of a tumor. They have been studied to examine their biomedical significance as possible biomarkers for cancer and other diseases. Immunoassays and mainly liquid chromatography are the analytical techniques most widely employed for the determination of urinary nucleosides. Application of capillary electrophoresis has also been reported although generally in MECC mode which involves the use of surfactants. This hinders MS detection. The main goal of this work was to develop a fast and reliable method based on capillary zone electrophoresis coupled with electrospray ionization-mass spectrometry (CZE-ESI-MS) for the determination of modified nucleosides in human urine. The target compounds were guanine, 1-methyl-guanine, 7-methyl-guanine, 9-methyl-guanine; adenosine, 1-methyl-adenosine, cytidine, guanosine, 7-methyl-guanosine. As internal standards, ribose-2-13C-adenosine and 8-13C-guanine were used. The CZE separation was carried out at pH 2.5 with 100 mM formic acid as BGE. MS detection in a single quadrupole, with ESI operating in positive-ion mode, was optimized. Analyte quantification was carried out under SIM acquisition mode. Here we also developed a procedure for the isolation-preconcentration of nucleosides from urine based on SPE with a polymeric sorbent that can be applied to a broad range of nucleosides. In most methods reported, nucleosides were isolated from urine by SPE in affinity mode, using an immobilized phenylboronic acid group, which specifically binds cis-diols. However, this is not applicable to non-cis-diol nucleosides. For the analysis of urine samples, different quantification strategies were explored to study the possible existence of a matrix effect. The standard additions method and matrix-matched calibration in synthetic urine were compared with calibration in aqueous medium. It was concluded that quantification by calibration in a synthetic matrix was the most appropriate quantification mode. These observations were confirmed by LC–MS/MS detection. The results obtained showed that the CZE-ESI methodology is rapid and robust for the determination of urinary nucleosides at 72—300 μgL^{-1} levels.

DETERMINATION OF HUMAN SERUM ALBUMIN THIOL GROUP

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Oxidative stress is increased in many pathological processes. Serum albumin (HSA) is the most abundant protein in plasma and a major extracellular antioxidant. The free thiol group of Cys 34 contributes to HSA antioxidant capacity. There is no simple, fast and cheap method for determination of the free HSA thiol group content for clinical purposes. Precipitation of serum proteins (HSA isolation) by ammonium sulfate as the first step in thiol determination was tested. The different factors such as various concentrations of AS, the method of precipitation (solid or saturated AS) and centrifugation (time and relative centrifugal force) and removal of AS on the content of HSA and globulin in all precipitates and supernatants were examined. In order to study the effects of various treatments on the composition of these precipitates and supernatants all of them were analyzed by determination of HSA, total protein and free thiol group concentrations (with bromcresol green, Biuret method and 5,5'-dithiobis-(2-nitrobenzoic acid), as well as by electrophoresis and immunoblotting (with antibodies against HSA). Also, the purity of HSA obtained by AS precipitation was compared to HSA isolated by affinity chromatography on Cibacron Blue F3G Sepharose. The 50% to 65% saturated AS fraction obtained after centrifugation 10 minutes at 3000 g has almost the same HSA purity as HSA isolated by affinity chromatography. The method is simple, fast, and accurate (recovery about 100% of total serum thiol goups). It was applied to isolation of serum HSA from 15 healthy persons and 15 patients with diabetes. The content of thiol groups in HSA of diabetics was significantly lower ($p < 0.01$) in comparison with the healthy persons. Therefore, the method developed is suitable for monitoring of HSA thiol groups in clinical practice.

THE SPECTROPHOTOMETRIC METHOD FOR MONITORING OF PROTEIN GUANIDINE GROUP CHANGES DURING CARBONYLATION

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The accumulation of methylglyoxal (MG) and other α-oxoaldehydes from both glycoxidation and lipoxidation sources lead to carbonyl stress. They are produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation. Carbonylation of the protein amino, guanidino and thiol groups is one of the important causes of vascular complications in diabetes. As the guanidine groups are very abundant on protein surface and the most reactive toward carbonyls, the changes in their levels could be the measure of carbonyl stress. Therefore, the aim of this study was to develop a simple spectrophotometric method for monitoring of the protein guanidine group changes during carbonylation. It was based on the formation of coloured adduct between thymol and product of reaction of guanidine group with hypobromite under alkaline conditions. The curve slopes of absorption vs. concentration plot of substances containing guanidine groups [arginine, human (HSA) and bovine serum albumin (BSA)] were substance dependent. The results obtained were in agreement with the Beer's law for the guanidine group concentrations of 1–36 mM. The method is simple, fast, precise (RSD in the range of 0.9-2%), accurate (recovery about 100%) It was applied for *in vitro* experiments of incubation of HSA with MG, as well as for monitoring of the changes of HSA molecule isolated by affinity chromatography from serum of patients with diabetes type 2. It was found that quantification of guanidine groups during protein carbonylation *in vitro* enables examination of the kinetics of these reactions, competitiveness of guanidine against thiol and amino groups. On the other hand it is suitable for monitoring of the HSA modification in carbonyl stress in clinical practice.

AFFINITY STUDIES OF NOVEL ANTIVIRAL DRUGS BY ISOTHERMAL MICROCALORIMETRY

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Influenza is one of the most infectious diseases with the potential to cause a pandemic with thousands of deaths[1]. The World Health Organisation (WHO) considers it as a „serious puplic health problem“, and sees urgent need in research about optimizing patient treatment[2]. However, only few antiviral drugs exist which allow for an effective treatment. Recent outbreaks of the bird flu just highlighted the urgent need for medication as a potent counteragent.

The M2 Protein is already known as a possible target for influenza drugs. The M2 proton channel is responsible for acidification of the virus protein[3]. The acidification is required for virus uptake into the cells. Furthermore acidification is required for uncoating of virus RNA from matrix proteins. Thus, inhibition of this protein inhibits critical steps in the virus reproduction cycle. Amantadine and Rimatadine were the first developed anti-influenza drugs targeted for blocking this proton channel[4]. Unfortunately, many influenza types became resistant against Amantadine and Rimantadine. This raises the need for the development of novel M2 Protein blocking agents.

Based on Adamantane, different structural derivatives were synthesized. The affinitiy of 20 different derivatives towards two different mutants of the transmembrane domain of the M2 Protein were investigated using isothermal microcalorimetry. This method monitors the heat of binding and allows for an accurate, label free determination of affinity constants.

Some of these novel substances showed even higher affinities towards the M2 protein channel than the commercially available drugs Rimandtadine and Amantadine. Thus, based on the tested substances, novel antiviral drugs could be developed.

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QUANTIFICATION OF TOTAL HESPERETIN AND HESPERETIN ENANTIOMERS IN BIOLOGICAL MATRICES VIA UPLC-MS/MS

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Hesperidin (hesperetin-7-*O*-rutinoside), a flavonoid affecting vascular function, is abundant in citrus fruits and derived products such as juices . After oral administration, hesperidin is hydrolyzed by the colonic microbiota producing hesperetin-7-*O*-glucoside, the glucoside group is further cleaved and the resulting hesperetin is absorbed and metabolized. Flavanones have a chiral carbon generating (R)- and (S)-enantiomers, with potentially different biological activities. A rapid UPLC-MS/MS method for the analysis of (R)- and (S)-hesperetin enantiomers in human plasma and urine was developed and validated. Biological matrices were incubated with β-glucuronidase/sulfatase, and hesperetin was isolated by solid-phase extraction using 96-well plate mixed-mode cartridges having reversed-phase and anion-exchange functionalities. Racemic hesperetin was analysed with a UPLC HSS T3 reversed phase column and hesperetin enantiomers with a HPLC Chiralpak IA3 column using water with 0.1% formic acid as solvent A and acetonitrile with 0.1% formic acid as solvent B. The method was linear between 50 and 5000 nM for racemic hesperetin in plasma and between 25 and 2500 nM for (S)- and (R)-hesperetin in plasma. Linearity was achieved between 100 and 10000 nM for racemic hesperetin in urine and between 50 and 5000 nM for (S)- and (R)-hesperetin in urine. Values of repeatability and intermediate reproducibility for racemic hesperetin and enantiomers in plasma and urine were below 15% of deviation in general, and maximum 20% for the lowest concentrations. In addition, the method was applied for the quantification of total hesperetin and of hesperetin enantiomers in human plasma and urine samples, obtained after oral ingestion of purified hesperetin-7-*O*-glucoside. In conclusion, the developed and validated method was sensitive, accurate and precise for the quantification of enantiomers of hesperetin in biological fluids.

ISOLATION OF THE INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3/TRANSFERRIN COMPLEX FROM THE HUMAN SERUM

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Insulin-like growth factors (IGFs) are anabolic hormones whose activities are regulatetd via binding to IGF-binding proteins (IGFBPs). IGFBP-3 is the most abundant, forming complexes with IGFs and acid-labile subunit. These complexes serve as an IGF reservoir in the circulation. IGFBP-3 was also reported to form complexes with other proteins, including transferrin (Tf). Tf is the main transporting protein for iron (Fe) and it regulates Fe distribution. A complex formation between IGFBP-3 and Tf could influence Fe metabolism and it may be postulated that the anabolic action of the IGF system extends on the Fe metabolism. In order to study this relationship, an IGFBP-3/Tf complex was isolated from human serum and investigated. A wide repertoir of affinity methods was tested: metal-, lectin- and immuno-affinity methods. The most informative results were obtained with immuno-affinity techniques: immuno-affinity chromatography, immunoelectrophoresis and immunoprecipitation with anti-Tf or anti-IGFBP-3 antibodies. The presence of IGFBP-3 and Tf during isolation was confirmed by SDS/PAGE/immunoblotting. Immuno-affinity chromatography revealed that IGFBP-3 bound to only one Tf isoform. Immunoelectrophoresis demonstrated that the IGFBP-3/Tf complex exhibited a mobility different from those of the native IGFBP-3 and Tf molecules. It could be isolated from the gel, but it was unstable and decomposed upon preparation for SDS/PAGE. Immunoprecipitation method resulted in the isolation of the stable IGFBP-3/Tf complex. A „two step“ procedure was applied. Firstly, serum sample interacted with immobilised anti-IGFBP-3 antibodies and, after specific elution, a fraction containing all IGFBP-3-immunoreactive species was allowed to interact with immobilised anti-Tf antibodies. Immunoblotting confirmed that the preparation obtained did not contain free IGFBP-3 or Tf molecules. According to the results reported here, immunoprecipitation can be recommended as a method of choice to isolate a stable IGFBP-3/Tf complex from the serum, which can be further used to study physiological relationship between IGF system and iron metabolism.

OPTIMIZATION OF ANALYTICAL METHODS FOR METALLOPROTEOMIC STUDY OF MERCURY IN FISH MUSCLE FROM AMAZON REGION – BRAZIL USING 2D ELECTROPHORESIS AND SYNCHROTRON RADIATION X-RAY FLUORESCENCE

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In the last decades studies developed have reported that about 50 tons of mercury released into the environment from gold mining activities have been deposited in water bodies and soils near the Madeira River.1,2 Thus, the elucidation of the mechanisms of mercury toxicity is critical to the socio-environment of this region. Here we presented preliminary results of metalloproteomic study of mercury in fish muscle samples from JIRAU Hydroelectric using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and synchrotron radiation X-ray fluorescence (SRXRF). The primary objective is the possible identification of biomarkers protein of mercury with samples of muscle tissue from *Mylossoma* sp. ("pacu") and *Brachyplatystoma rousseeaxii* ("dourada"). The muscle samples were macerated in deionized water and subsequently their proteins were precipitated by mixing of the extracts with ice-cold solution of acetone 80% (v/v). The protein precipitate was then centrifuged to separate the supernatant and resolubilized in specific buffer. The resulting solution was then used in the protein separations by 2D-PAGE (gel 12.5%). The protein spots obtained were cut out from the gel and analyzed by SR XRF for the determination qualitative of mercury. The preliminary analysis revealed the mercury presence in two protein spots wich presents molecular weight of 19.8 and 20.8 kDa and isoelectric point of 7.5 and 5.6, respectively.

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NOVEL ASPECTS OF THE APPLICATION OF WATER-MISCIBLE IONIC LIQUIDS IN CATALYSIS BY PLANT PEROXIDASES: FORMATION OF THE REACTION MEDIA AND SUPPORTING MATERIAL FOR THE ENZYME IMMOBILIZATION

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The area of the application of solvents of a new generation – ionic liquids (ILs) – in chemical analysis and biotechnology is constantly expanding. In this report, the attention is focused on two aspects of the application of some commercial hydrophilic ILs and plant peroxidases isolated from horseradish roots (HRP) and soybean hulls (SBP) in biochemical methods of analysis.

The first aspect involves the use of IL (1-butyl-2-methylimidazolium ([BMIm]) and N-butyl-3-methylpyridinium ([BMPy]) tetrafluoroborates)-water mixtures as the reaction media for the transformation of the model phenolic substrates of peroxidases (guaiacol and *o*-chlorophenol) by *tert*-butyl hydroperoxide. Using hydrophilic ILs instead of organic solvents (acetonitrile and DMSO) and the optimization of the reaction medium composition provided the oxidation of phenolic compounds catalyzed by plant peroxidases in the presence of 60 – 80 vol% of IL. As a result, the procedures for the determination of 3 μM – 3 mM of the indicated substrates in samples with low water content (pharmaceuticals, for example) were developed. The catalytic activity of the considered plant peroxidases controlled by spectrophotometric method, and their substrate specificity was found to depend significantly on the nature of the enzyme, IL cation, and buffer solution used as co-solvent for IL. Thus, SBP had the greatest catalytic activity and substrate specificity towards guaiacol, whereas HRP was more preferable for the transformation of *o*-chlorophenol in [BMIm][BF_4]-water mixture (70:30 vol%).

The second aspect employs ILs including [BMIm] cation and some anions (Cl^-, Br^-, CH_3COO^-) for obtaining active and stable peroxidase-cellulose composites in the form of thin and transparent films as the sensitive element of the optical biosensors. The conditions of the formation, storage and applicability of the novel composites in the reactions of aryldiamines (*o*-phenylendiamine, *o*-dianisidine, 3,3’,5,5’-tetramethylbensidine) and catecholamines (dopamine, adrenaline, α-methyldopa, dobutamine) oxidation by H_2O_2 were optimized.

The discussion of the efficiency of catalysis by plant peroxidases in the presence of ILs employed cosmotropia theory for ions of the considered ILs, the log*P* values characterizing the polarity of ILs, and the pI values of the enzymes.

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PRELIMINARY RESULTS OF THE METALLOMIC STUDY OF MUSCLE TISSUE FROM NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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The last few years in Brazil have seen a considerable increase in Nile tilapia (*Oreochromis niloticus*) production, motivating aquaculture researchers to develop studies focusing on the behavior, physiology, genetics and nutrition of this fish species [1]. There is a consensus among researchers about the fundamental importance of metal ions as structural and functional components of fishes, e.g. from the structural standpoint: the copper and zinc present in metallothioneins; and from the functional aspect: the catalyzing role of these ions on enzyme systems [2]. Considering the above, an investigation was made on Nile tilapia muscle samples obtained after protein separation. The proteins of Nile tilapia muscle was separated by 2D-PAGE and copper, iron, selenium and zinc in protein spots was qualitatively and quantitatively determined by synchrotron radiation X-ray fluorescence (SR-XRF) and graphite furnace atomic absorption spectrometry (GFAAS). Copper, iron, selenium and zinc are present in four muscle protein spots with molecular weights of 14.6, 35.1, 11.2 and 56.6 kDa, respectively and with isoelectric point (pI) ranging from 6.1 to 9.6. The concentrations of copper, iron, selenium and zinc bound to these proteins was determined by GFAAS following acid digestion of the spots presents ranges from 3.5–4.4 mg of copper, 1.05–1.5 mg of iron, 1.5–6.8 mg of selenium and 1.5 mg of zinc, respectively, per g of protein. The quantification of these elements in the protein spots enabled us to estimate that the proteins in the protein spots here studied contained proportions of one to two atoms of these elements per protein molecule.

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ENZYME-BIOSILICA NANOSTRUCTURES: A MINIATURIZED AUTOMATIC APPROACH

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The enzyme immobilization enhances the stability and hence applicability of biomolecules as reusable and robust biocatalysts. However, most methods used to synthesize mesoporous, nanostructured and/or hybridized silica materials use harsh conditions which limits their applicability to encapsulate biomolecules [1]. In contrast, the production under mild physiological conditions of nanostructured silica in natural systems by diatoms and sponges has been considered a process to mimic with great potential in the silicon technology [2].

So, the use of a biomimetic approach to synthesize biosilica nanostructures provides a rapid and simple alternative method for the enzyme immobilization that results in the physical entrapment of enzymes within silica nanospheres as they are formed. The primary advantage of the biological synthesis of silica is the benign reaction conditions (ambient temperatures, physiological pH range and aqueous solvents) which favor retention of biological activity. Thus, the catalytic nanostructures provide an ideal solution to the equilibrium that should be always regarded between minimum diffusion limitation, maximum surface area per unit mass, and high enzyme loading. Moreover, it occurs in ambient environmental conditions involving only a silica precursor and a biological catalyst, occurring the immobilization of the enzyme within seconds.

In the present communication, different precursors (templates and silica derivatives) and reaction conditions are tested, regarding not only the morphology and size of the particles obtained but also its stability and consequent activity of the enzymes. The developed nanosilica particles are used in a reactor (IMER) and incorporated in an automated flow system. This miniaturized approach could demonstrate the analytical potential of the biocatalytic nanoparticles in order to guarantee reliable, cost-effective and fast methods, which enable real-time continuous monitoring, low reagent consumption and reuse of the catalytic surface.

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INFLUENCE OF BILE ACIDS' STEROID NUCLEUS ON THEIR PARTITION COEFFICIENT BETWEEN CHLOROFORM, DIBUTYL ETHER AND WATER

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Bile acids are well known natural surfactants that modify permeability of biological membranes [1]. Partly because of that they have a wide pharmacological application. Since bile acids promote transport of drugs through cell membranes it is important to know their partition coefficients in chloroform and dibutyl ether as models for blood brain barrier. Thus, the aim of this work is to determine partition coefficients of bile acids between chloroform, dibutyl ether and water and to examine the influence of the structure of bile acids' nucleus on measured partition coefficients. Special attention is paid on bile acids' oxo derivatives since they have a lower toxicity and their physico- chemical properties are not examined yet.

Partition coefficients were determined using shake- flask method and concentration of bile acids were determined after twelve hours of shaking at room temperature in aqueous and organic layer using reversed phase HPLC with DAD detector on 210 nm.

It is found that, for both organic solvents after substitution of bile acids' hydroxyl group with oxo group in the steroid nucleus the value of *logP* falls especially if more oxo group are present. This can be explained as bile acid molecules with oxo groups are more stabilised in aqueous than in organic solvent since oxo group forms the angle of 30 ° with steroid skeleton mean plane i.e. it is in equatorial position. Thus, since spatial reasons, stabilisation is possible from the both sides of the steroid skeleton while for bile acids with axial oxo groups (cholic and deoxycholic acid) stabilisation is possible only from the α side of the steroid skeleton and for them balance is favored toward organic solvent and that is the reason why water molecules are liberated and they move back in solution while entropy rises. In dibutyl ether partition coefficients for oxo derivatives are lower since chloroform has one hydrogen as proton donor and forms hydrogen bonds with oxygen atoms while dibutyl ether as a proton acceptor doesn't have the possibility for formation of hydrogen bonds.

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ACETYLCHOLINESTERASE BASED COLORIMETRIC DIPSTICKS FOR FAST ASSAY OF NEUROTOXINS

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Acetylcholinesterase (AChE) is an enzyme playing a pivotal role in cholinergic neurotransmission. It is inhibited by a wide group of neurotoxic compounds. Owing to the practical significance, pesticides (e.g. carbaryl, dichlorvos, dimethoate) nerve agents (e.g. sarin, soman, tabun, VX) natural toxins (e.g. aflatoxins from Aspergillus flavus) and drugs (e.g. Alzheimer´s disease drugs galantamine, rivastigmine, donepezil) can be example as the AChE inhibitors. The neurotoxins can be easily assayed by devices containing AChE as a recognition element. Several colorimetric dipsticks and biosensors are typical tools containing AChE. The reported experiment is devoted to the construction of dipstick with immobilized AChE and indoxylacetate respective 2,5-dichlorophenol acetate as substrates. Original idea is to construct devices providing a contrast coloration comparing to the commercially available devices based on color change white to yellow as they use Ellman´s reagent. AChE was immobilized as a membrane stabilized by gelatin, glutaraldehyde and albumin, or cyclodextrins on cellulose, wax film, cotton gauze or alumina covered by silicagel. The immobilization procedure was optimized. After the optimization, the prepared dipsticks were tested for a long term stability and suitability for assay of neurotoxic compounds. We proved good stability with no relevant decay of the immobilized AChE activity when dipstick stored at laboratory temperature for one month. We assayed nerve agents (sarin, soman, VX), paraoxon and neostigmine as representative neurotoxins. When assessed the coloration by a naked eye, the limit of detection was approximately 100 nmol/l for the tested compounds. We infer suitability of the constructed dipsticks for fast and low cost field assay.

USE OF CAPILLARY ELECTROPHORESIS OF NUCLEOTIDES AS A RAPID METHOD FOR INSECT METABOLIC STATUS ASSESSMENT

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Capillary electrophoresis (CE) and related capillary techniques are widely exploited in the biomedical sciences not only because they have high separation power and sufficient selectivity under properly chosen conditions, but also because they can separate the most diverse types of compounds. In this study, CE of nucleotides is used as a rapid method for metabolic status assessment of diapausing larvae of the European corn borer *Ostrinia nubilalis*. Diapause is a state of arrested development that enables insects to survive predictable unfavourable environmental conditions (lack of food, high/low temperatures, hypoxia/anoxia). It is characterised as a hypometabolic state with a strong net suppression of organismal metabolic rate, targeting and coordinating both ATP-utilization as well as ATP-generation in the cell.

Whole body homogenates of the 5th instar diapausing larvae of *O. nubilalis* of different age were analyzed by capillary electrophoresis to estimate the concentration of selected nucleotides: ATP, ADP, AMP, NAD, NADP. The homogenates were deproteinated using methanol, sonicated and centrifuged. Supernatants were evaporated under nitrogen stream, and dry pellets reconstituted in ultrapure water and directly injected into capillary. CE separation was performed under following conditions: background electrolyte of 40 mM citrate titrated with γ-aminobutyric acid pH 4.4 with addition of 0.8 mM cetyltrimethylammonium bromide, U = -25 kV, detection wavelength 260 nm, injection 50 mbar/20 s, total capillary length 80.5 cm, length of capillary from injection to detection point 72 cm, internal diameter 75 μm. Between analyses of different samples, the capillary was flushed by background electrolyte for 3 min.

Results showed that the concentrations of ATP and NADP decreased during diapause, while the concentrations of ADP, AMP and NAD increased. An ATP/ADP ratio decrease during diapause confirms that diapause is a hypometabolic state with strong metabolic suppression. Our results confirmed that capillary electrophoresis can be used as a rapid and reliable method for metabolomic analysis of complex biological mixtures and metabolic status assessment.

METABOLIC STUDIES WITH BOVINE BLOOD PLASMA AFTER TREATMENT WITH IVERMECTIN EMPLOYING Hi-Res NMR

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Despite the constant advances in food science and quality control methods, which gathers Analytical Chemistry, Veterinary and Farmacology, there are several issues related to misuse of veterinarian drugs, such as Ivermectin, a powerful and effective anti-helmintic drug. The balance between avoiding diseases and respecting the recommended dosis and drug withdrawals period is the major focus of cattle producers, which is often unobtained. In this context, this job presents a novel method for detecting the consequences of misadministration of Ivermectin in blood plasma composition, in order to provide a new way to understand bovine metabonomics.

For our studies, blood plasma samples were semi-lyophilized for concentration increase and added of 20% of D_2O for the lock signal, followed by addition of 100 mM of phosphate buffer, at pH = 7.4.[1] NMR analysis were carried out in a Varian INOVA 400 spectrometer. ^{1}H data were obtained by summing 32 spectra obtained with a conventional PRESAT sequence, in order to suppress water signal, much more intense than metabolites signals. ^{13}C spectra were obtained by both traditional[2] and SSFP pulse sequences, which allows a much higher number of acquisitions in a shorter period of time. The results show that SSFP-NMR can be a valuable technique for fast ^{13}C data acquisition, which is a powerful complementary tool for metabolite identification, made via ^{1}H-NMR. After chemometric studies of these data, we are able to identify the regions in ^{1}H spectra responsible for distinguishing samples. Frequently, the large amount of peaks demands some tools for aiding this identification, so we employed both Human Metabolome Database (HMDB)[3] and Metabolomics Database[4] which allows identifying taurine, creatine, creatinine, hippurate, citrate and urea as main metabolites, and a modest variation for acetate signals, what can be related to a light ketotic condition, to be confirmed in future experiments.

Finally, NMR analysis for both ^{1}H and ^{13}C represent a fast metabolic data acquisition method and also a first step for comprehension of metabolism behavior when this is externally disturbed, as by Ivermectin administration.

A MULTICLASS METHOD FOR ENDOCRINE DISRUPTING CHEMICAL RESIDUE ANALYSIS IN HUMAN PLACENTAL TISSUE SAMPLES BY UHPLC–MS/MS

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The group of compounds commonly called endocrine disrupting chemicals covers a wide range of synthetic and natural substances able to alter the normal hormone function of wildlife and humans, consequently causing adverse health effects. Bisphenol A (BPA) and its chlorinated derivatives, benzophenones (BPs) and parabens (PBs) belong to this group of compounds. In this work, we propose a multiclass ultra high performance liquid chromatographytandem mass spectrometry (UHPLC–MS/MS) method to determine BPA, its four chlorinated derivatives (monochloro- dichloro, trichloro- and tetrachloro- bisphenol A), six BPs (benzophenone-1, benzophenone-2, benzophenone-3, benzophenone-6, benzophenone-8 and 4-hidroxybenzophenone) and four PBs (methylparaben, ethylparaben, propylparaben and butylparaben) in human placental tissue samples. The method involves an extraction step of the analytes from the samples using ethyl acetate, followed by a clean-up step by centrifugation prior to their quantification by UHPLC–MS/MS using an atmospheric pressure chemical ionization (APCI) interface in negative mode. Deuterated bisphenol A (BPA-d16) was used as surrogate. The limits of detection (LOD) found ranged from 0.03 to 0.6 ng g-1, while inter- and intra-day variability was under 13.8%. The method was validated using matrix-matched calibration standard and a spike recovery assay. Recovery rates for spiked samples ranged from 90% to 110%. This method was applied to the determination of these compounds in 50 placental tissue samples collected from women who live in the province of Granada (Spain).

SEPARATION OF NUCLEOSIDES AND NUCLEOBASES BY ZWITTERIONIC HYDROPHILIC INTERACTION CHROMATOGRAPHY: EVALUATION OF THE ELECTROSTATIC CONTRIBUTION TO THE RETENTION MECHANISM.

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Hydrophilic interaction chromatography (HILIC) is a viable alternative to RPLC for the separation of polar compounds. HILIC is based on the use of polar stationary phases combined with mobile phases with a high organic content and a small amount of water. A partition mechanism between the aqueous layer associated with the stationary phase and the organic component of the mobile phase has been proposed to explain separation in HILIC. However, later studies have shown that the mechanism of retention involves more complex equilibria. Moreover, the presence of charged sites in the stationary phase in zwitterionic hydrophilic chromatography (ZIC-HILIC) would propitiate the appearance of other retention mechanisms.

This study explores the benefits and limitations, on a quantitative basis, of using ZIC-HILIC for the separation of modified nucleosides and nucleobases (MNN). Studies have addressed the potential of MNN as markers of several diseases and, mainly, as early markers of different types of cancer. The MNN selected were 8-hydroxy-guanine, 8-hydroxy-guanosine, 8-hydroxy-2'-deoxyguanosine, 1-methyl-guanine, 7-methyl-guanine and 9-methyl-guanine.

The results obtained from the study of the different parameters affecting separation, especially salt nature and concentration, showed that separation in the ZIC-HILIC column is based on a mechanism of partition and interaction through weak electrostatic forces; however, the mechanism is complex, and it presumably involves other processes such as retention of the MNN with hydroxyl groups by hydrogen bonding.

The contribution of the electrostatic interactions to the retention of the charged analytes reached values between 25-52% when 80% acetonitrile:20% formic acid (2.6 mM, pH 3.1) with 0.25mM ammonium perchlorate was used as the mobile phase, and between 26-49% when potassium perchlorate was used instead. The electrostatic contribution decreased progressively as the salt concentration rose.

ULTRASENSITIVE CHEMILUMINESCENT ENZYME IMMUNOASSAY

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The most sensitive format of enzyme immunoassay (EIA) is the assay with chemiluminescent (CL) detection of enzyme activity of immunoreagents. Traditionally in CL-EIA horseradish peroxidase (HRP) and 4-iodophenol (PIP) are used as enzyme label and enhancer, respectively. This enhanced chemiluminescence reaction has been successfully used in development of ultra-sensitive immunochemical kits for the determination of various compounds. A drawback of HRP-C as a label in CL-EIA is the relatively quick decay of CL signal. Recently, we showed that a combination of 3-(10'-phenothiazinyl)propane-1-sulfonate (SPTZ) and 4-morpholinopyridine (MORPH) is a potent enhancement system of soybean peroxidase (SbP)-induced CL [1]. A detection limit of SbP/SPTZ/MORPH system (0.03 pM) was 40-fold lower than that of HRP/PIP system. Moreover, upon luminol oxidation the SbP/SPTZ/MORPH system produced a long-term CL signal. Therefore, a replacement of HRP/PIP with SbP/SPTZ/MORPH system allowed to solve the question connecting with the quick decay of the CL signal. The novel detection system was applied successfully in construction of ultrasensitive EIA kit for determination of thyroglobulin in human serum [2]. The study showed that a lower detection limit (LOD) of the CL-EIA with SbP/SPTZ/MORPH was 10 times lower than for the immunoassay with HRP/PIP. This opens good perspectives for use of SbP/SPTZ/MORPH system in the development of ultra-sensitive immunoassay kits.

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ENERGY TRANSFER IN MICELLAR SOLUTIONS OF EUROPIUM (TERBIUM) COMPLEXES: APPLICATION FOR DETERMINATION OF BIOLOGICALLY ACTIVE SUBSTANCES

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Luminescence spectroscopy gives many opportunities to determine the biologically active substances (BAS) for example by their own fluorescence, fluorescence of their derivatives or using sensitized fluorescence of lanthanide complexes. The last approach is more effective and popular because of its high sensitivity and better selectivity. In such complexes, the energy absorbed by organic BAS-ligand (donor) is first transferred to its triplet state through intersystem crossing and then intramolecularly transferred to a closest resonance level of the lanthanide ion (acceptor) which finally emits sensitized fluorescence displaying so-called “antenna” effect. Solubilization of the chelate in nanosized micellar assembly can be another factor strengthening the antenna effect. This solubilization promotes concentrating the reaction components, enhances stability of the chelate, stimulates additional lanthanide dehydration, and as a result increases the efficiency of excitation energy transfer and the intensity of analytical signal.

The aim of the report is to reveal major factors influencing the efficiency of electron excitation energy transfer in chelates of Eu(III) and Tb(III) ions with different BAS-ligands (tetracycline and fluoroquinolone antibiotics, amino acids, coumarine anticoagulants) in different micelles and some microemulsions and their practical application in fluorimetric analysis. It was shown that micelles of ionic and nonionic surfactants enhance the own fluorescence of some fluoroquinolones and coumarines and decrease the limit of detection to a $5 \cdot 10^{-9}$ M (lomefloxacin) or $4 \cdot 10^{-6}$ M (warfarin). The same enhancing effect observes on solubilization in micelles of lanthanide chelates with one kind of BAS-ligand or mixed-ligand complexes. Introduction of a second ligand into the binary lanthanoid-BAS complex may accompanied by two effects, namely: i) an increase in the excitation energy transfer efficiency as a result of replacement of the remaining water molecules (quenchers) from the first coordination sphere of the metal ion and additional excitation energy transfer from the second ligand to the lanthanide ion (the antenna effect), and ii) quenching of lanthanide chelate fluorescence due to the formed complexes having poorer fluorescent properties. As a result of joint action of the second ligand and micelles the analytical signal is enhanced by $n \cdot 10$ times compared to binary complex. General regularities were established at studying the influence of surfactant micelle nature on the efficiency of energy transfer in mixed-ligand complexes. Applications of the studied complexes in analysis of drugs, biological and nutrition objects, and soils are considered.

SENSITIVE FLUORESCENT DETERMINATION OF PEROXIDASE SUBSTRATES IN AQUEOUS-ORGANIC AND MICELLAR MEDIA

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Horseradish peroxidase (HRP) is one of the widely used enzymes in bioanalytical chemistry. However, efficient use of HRP in bioanalysis still has to overcome a number of limitations, in particular, limited sensitivity of earlier proposed biosensors for the determination of its substrates in clinical, nutritional and environmental samples, and low operating activity of the biocatalyst in organic media which is necessary for the determination of low water-soluble analytes in water-insoluble samples.

In the present work we propose some promising approaches for sensitive and selective fluorescent determination of a number of HRP substrates in real samples of various nature.

Catecholamines (dopamine, epinephrine, nofepinephrine, serotonine, *L*-DOPA) and flavonoids (quercetin, rutine, esculitin in particular) belong to biologically active compounds which regulate processes in human and plant systems. In order to create new highly sensitive procedures for the determination of the listed analytes in the range 1-10 nmol l^{-1}, we employed their enzymatic derivatization by benzylamine. It was shown that quantum yield of fluorescence of the substrate derivatives increased dramatically in the presence of cationic or nonionogenic surfactants (CTAB or Tween 20) and in the presence of dimethylsulfoxide (10-30 vol. %).

The approach based on the use of micellar and aqueous-organic media was successfully applied for the fluorescent determination of poorly water-soluble organic peroxides (markers of food quality). HRP-catalyzed oxidation of fluorescein by these analytes was used as the indicator reaction.

Moreover, some of the proposed recognition systems were used for the development of fluorescent solid biosensors on the basis of chitosan/peroxidase films for the determination of dopamine and epinephrine.

The analytical prospects of the developed fluorescent procedures were demonstrated in the analysis of some pharmaceuticals, herbal materials, foods, urine and human blood serum.

The study was supported by RFBR (N 09-03-00-823-a) and RF Ministry of Education and Science (contract P868 2010/25/05).

CHEMOMETRIC APROACH FOR DEVELOPMENT, OPTIMIZATION AND VALIDATION OF DIFFERENT CHROMATOGRAPHIC METHODS FOR SEPARATION OF OPIUM ALKALOIDS

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In this work, the possibilities and limitations of the systematic optimization of fast chromatographic methods for separation of compounds with similar structures, but different physico-chemical characteristics are presented. Different chromatographic methods were developed, optimized and validated for the determination of alkaloids from opium poppy (Papaver somniferum L., Papaveraceae).

The effects of different chromatographic conditions on the defined chromatographic descriptors during the optimization process were investigated by use of chemometrics. Full factorial linear design of experiments was used for the determination of the relationship between the chromatografic conditions and the retention behaviour of the analytes and the central composite circumscribed design for the final method optimization. The chemometric aproach was effectively employed for a more profound understanding of the basic processes underlying chromatographic separations and an increasement of the reliability of the proposed methods.

The separation of the opium alkaloids using gas chromatography hyphenated with flame ionasation detector and mass spectrometer (GC/FID/MSD) was achieved within ten minutes, avoiding the derivatisation step. The separation of all compounds on a reversed phase HPLC system and diode array detection (RP-HPLC/DAD) was obtained within close retention window using optimized gradient elution with high pH value of the mobile phase on a reversed phase column with bidentate C18-C18 bonding technology. The internal standards were used for both of the methods.

The methods were used for determination of opium alkaloids from poppy straw. The methods were compared for their sensitivity, efficacy, speed, simplicity, price, ecological aspects and compatibility with the extraction procedure.

MULTIVARIATE ANALYSIS OF PESTICIDE RESIDUES IN FOODSTUFFS OF PLANT ORIGIN - SELECTION OF REPRESENTATIVE ANALYTES

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The cost effective monitoring of hundreds of pesticide residues in foodstuffs of vegetable origin involves the definition of groups of analytes associated with homogeneous performance parameters of the used analytical method. The performance of the analytical method only needs to be tested on a daily base for some of these group's compounds. This work proposes a strategy for defining groups of compounds with homogeneous physical-chemical properties based on the evaluation of the similarity of the multivariate pattern of these properties using three competing multivariate analysis tools, namely, Principal Component Analysis, Hierarchical Clustering and K-Mean Clustering. This strategy was successfully used to group 100 pesticides in nine clusters represented by a compound with within group average properties. The developed groups were validated using physical-chemical data from other references or compounds and are consistent with the performance of the studied determination of pesticide residues in dried red bean. This grouping can support the reduction of the number of recovery tests performed for the validation of the analytical method or tests quality control.

INTEGRATED ANALYSIS OF HETEROGENEOUS ANALYTICAL DATA FOR THE DETECTION OF MEAT SPOILAGE

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In this study, we evaluate various approaches for the integration of data from a diverse range of analytical instruments, with the aim of detecting bacterial spoilage in meat. The chief goal is to test the hypothesis that different instruments provide complementary information which, when brought together in an integrated analysis, can provide a more reliable method for spoilage detection than any single instrument.

We have used data acquired from three main experimental techniques: spectroscopy, high performance liquid chromatography (HPLC) and electronic nose. The first step in the analysis of the data is the application of unsupervised methods for the extraction of prominent features, followed by geometric transformations. The results of these analyses are integrated into a consensus profile, which is subsequently used as input to a classification model. Classification techniques that have been investigated include partial least squares discriminant analysis (PLS-DA) and support vector machines (SVMs). Ensembles of individual classifiers have been used to maximise the accuracy and provide a confidence score for each classification.

A thorough model training, optimisation and evaluation process has been implemented throughout the analysis to ensure that the performance metrics of the classification models are representative of real world application and to provide an indication of the statistical significance of our results.

The motivation for this work is to improve the speed and accuracy with which spoilage of meat products can be identified. Preventing spoiled meat products entering the food chain is of major commercial and public health interest. This work was carried out as part of the SYMBIOSIS-EU project, funded by European Commission Framework 7. More information can be found at the project web site: http://www.symbiosis-eu.net/.

ESTIMATION OF MINIMUM RELATIVE ERRORS OF THE PARTIAL LEAST SQUARES MULTIVARIATE REGRESSION IN ANALYTICAL SPECTROMETRY

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Earlier [1], using the inverse least squares (ILS) and the classical least squares (CLS) explicitly regularized estimators of multivariate calibration [2], we developed a new method of evaluating the potentially achievable relative accuracy and precision for the determination of individual component concentrations in mixtures prior to performing analytical procedures. In the present study, we estimate the mean squared relative errors (MRSE) of the quantitative determination of mixture components using partial least squares regression (PLSR). It is assumed that there are no systematic calibration errors and the noise of the measurements is white. We use the simulated mixture spectra of Gaussian curves with different widths and the UV-VIS spectra of Excedrin tablets [3]. For a given concentration vector of the mixture, the relative weights of the pure-component spectra (RWPC) in the mixture spectrum and in the calibration matrix are changed simultaneously in the range of 0.01-0.99.

For the first time, the existence of the MRSE dependence on RWPC has been discovered. The corresponding ternary diagrams, calculated using the error propagation expressions, are in satisfactory agreement with the diagrams obtained by the method of modelling.

It is shown that there exist optimal regions of RWPC, which allow minimizing the MRSE values for a given analytical procedure. We have also studied the dependence of MRSE values on the number of PLSR factors and on the noise variances for different calibration matrices. Lower MRSE values in a wider range of RWPC and shorter computation time are the main advantages of the PLSR estimator over the Tikhonov-regularized CLS and ILS estimators [1]. The implementation of the above findings is discussed.

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HASSLE-FREE TARGET AND NON-TARGET ANALYSIS: THE APEQ METHOD

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Multi-dimensional analytical methods, such as gas chromatography with mass spectrometry (GC-MS) detection, provide great separation powers. These techniques can be used for both target and non-target analysis of compounds in complex mixtures. However, the amount of data provided by these techniques often exceeds $10^6 – 10^9$ data points per sample, which makes both target and non-target analysis difficult and laborious. New automated techniques for peak finding, curve resolution and quantification are needed to efficiently analyse the data.

Many chemometric methods are ideally suited to help analyse this kind of data. However, using these methods requires some expert knowledge, both in implementation and in analysis of the results, which is often not available to the analytical chemist. What is needed is automated chemometric methods which requires a minimum of user interaction and gives results that are easy to interpret.

The Automated Peak Extraction and Quantification (APEQ) method is a novel method for target and non-target analysis. It analyses the GC-MS data of several samples and outputs the retention times, mass spectra and relative quantity of the substances present. It is a tiered approach consisting of four tiers: (1) the chromatogram is split into regions, each containing 1-5 peaks; (2) the number of peaks in each region is estimated; (3) for each peak, the elution profile, the mass spectrum and the concentration profile in the samples is approximated. (4) For each region, the peaks and background is modelled using parallel factor analysis (PARAFAC). PARAFAC models with different number of factors are tested and the model with the optimal model complexity is selected automatically. The optimal model will in most cases be the model with lowest dimensionality (i.e., fewest factors) that has a factor describing the baseline.

The APEQ method has been used to analyse a set of 12 samples consisting of polycyclic aromatic hydrocarbons.

The total analysis time was 2 minutes on an ordinary laptop, which included finding the mass spectra of 41 compounds of which 12 was unexpected, and quantification of 492 peaks.

CHEMOMETRIC CLASIFICATION OF PLANTS ESSENTIAL OILS BY THEIR ANTIBACTERIAL ACTIVITY

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The classification of plants essential oils upon their antibacterial activity by the multivariate statistical analysis was done. The antibacterial effects of *Thymus vulgaris* L., *Lavandula angustifolia* L. and *Calamintha nepeta* (L.) Savi essential oil, expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were studied. Eleven types of bacteria were used: (1) *Pseudomonas aeruginosa* ATCC 27853; (2) *Pseudomonas aeruginosa*; (3) *Salmonella enteritidis* ATCC 13076; (4) *Salmonella enteritidis*; (5) *Staphylococcus aureus* ATCC 29213; (6) *Staphylococcus aureus*; (7) *Bacillus cereus* ATCC 10876; (8) *Bacillus cereus*; (9) *Escherichia coli* ATCC 13706; (10) *Escherichia coli* (from urine); and (11) *Escherichia coli* (from feces). For the chemometric study of the plants-bacteria system, we used softvere Mathworks MATLAB and two different methods: principal component analysis (PCA) and hierarchical clustering. For plants (as scores) and bacteria (as variable) we used full sized PC model, with cross validation method centering data at the first principal component. The score and correlation loading plots of PC model showed a similar influence of MIC and MBC values of tested bacteria for the classification of examined plants. PCA analysis revealed that essential oil of *T. vulgaris* L. and *L. angustifolia* L. in contrast to *C. nepeta* (L.) Savi are similar and have strong effect against *S. enteritidis* and *S. aureus.* The similar effect of thyme and lavender oils were also shown for *P. aeruginosa, S. enteritidis* ATCC 13076, *B. cereus* and *S. aureus* ATCC 29213. PCA model showed that other tested bacteria have the same influence at all plants essential oils and that are not relevant for their classifying. The similarity between *T. vulgaris* L. and *L. angustifolia* L. was also confirmed by the hierarchical cluster analysis used between groups-linkage as cluster method and euclidean distance as a measure.

COMBINATION OF CHROMATOGRAPHIC AND CHEMOMETRIC METHODS TO STUDY THE INTERACTIONS BETWEEN DNA STRANDS

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This work describes the combination of size exclusion chromatography and chemometric resolution methods to study the interactions between different DNA strands to build up complex structures.

DNA strands can form secondary structures of varying complexity. It is well known the double helix structure discovered by Watson and Crick in which two complementary DNA strands interact. However, besides this duplex structure, there are more complex arrangements such as triplexes (interaction of three strands) or quadruplexes (interaction of four strands). In recent years, it has been discovered that these structures could be very important "in vivo" as they could be used in antigenic therapies or, also, could be closely related to the appearance of certain types of aging cancer.

Thus, we studied the formation of these structures considering three different options: a) the formation of duplex structures from the interaction of two single-stranded DNAs, b) the formation of triplex structures from the interaction of a double stranded DNA and single-stranded DNA, and, c) the competition of duplex and quadruplex structures. In some cases, the analysis of the chromatogram at a single wavelength is enough to confirm the existence of a higher order structure. In other cases, it is necessary to acquire the entire spectrum at each point of the chromatogram and to apply chemometric resolution methods in order to detect the formation of the complex structures.

These results obtained by coupling chromatographic and chemometric techniques have been validated by using spectroscopic techniques such as molecular absorption in the ultraviolet or circular dichroism.

USEFULNESS OF PARALLEL FACTOR ANALYSIS FOR FIBER SELECTION IN A SOLID PHASE MICROEXTRATION PROCEDURE TO DETERMINE CHLOROPHENOLS BY MEANS GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETECTION

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Chlorophenols compounds are widespread in the environment; because of their toxicity official regulatory organizations have set maximum environmental concentrations. In this work for select the fiber and evaluate the performance of the developed method the use of three-way signals that provides the SPME-GC/MS system is proposed to analyse 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol.

Three kinds of SPME fibers, polyacrylate, polydimethylsiloxane and polydimethyl-siloxane/ divinylbenzene (PDMS/DVB) are compared to identify the most suitable one for the extraction process on basis of two criteria: i) To select the equilibrium time studying the kinetic of the extraction ii) To obtain the best figures of merit values, for the five analytes. For the first step, the three-way experimental data are arranged as follows: if I times of extraction are considered, the tensor of data, $\underline{\mathbf{X}}$, of dimensions $I \times J \times K$ is generated concatenating the I matrices formed by the abundances of de J ions m/z recorded in K elution times around the retention time for each chlorophenol. The second order property of trilinear data generated by SPME-GC/MS asses the unequivocally identification of each chlorophenol, as consequence the loadings in the first mode estimated by Parallel Factorial Analysis (PARAFAC) decomposition is the kinetic profile. After that, a calibration based in PARAFAC decomposition provides the best values of figures of merit for PDMS/DVB fiber. The values of decision limit, CCα, achieved are between 0.29 and 0.67 μg L^{-1}.

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PARALLEL FACTOR ANALYSIS TO OPTIMIZE THE CHROMATOGRAPHIC RESOLUTION IN THE DETERMINATION OF BISPHENOLS AND THEIR DIGLYCIDYL ETHERS BY GAS-CHROMATOGRAPHY MASS-SPECTROMETRY WITH A PROGRAMED TEMPERATURE VAPORIZER INLET

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Several food alerts caused by migration of substances from contact food materials have lead to the harmonization of legislation of EU (Commission Regulation ,EU, No. 10/2011) on plastic materials and articles intended to come into contact with In this work, the determination of bisphenol A (BPA), bisphenol F (BPF) and their respective diglycidyl ethers (BADGE and BFDGE) using a PTV-GC/MS system (BPA-d5 is used as internal standard) is shown.

For each one of the I samples that are analysed, the abundance of J m/z ratios is recorded at K times, so a tensor of dimension $I \times J \times K$ is obtained for each analyte. The decomposition of this tensor by means of Parallel Factor Analysis (PARAFAC) allows one to: i) identify unequivocally each analyte according to the maximum permitted tolerances for relative ion intensities, and ii) quantify each analyte, even in the presence of coeluents. This identification guarantees a complete specificity.

To optimize the procedure two of the heating ramps of the oven temperature program are changed according to a two levels factorial design. As results, for each analyte and each heating ramp there are five figures of merit. This data matrix has been studied by means of principal component analysis for obtaining the best figures of merit. Values of CCα between 2.10 and 4.41 ppb, with probabilities of false positive and false negative fixed at 0.05, are reached.

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BILE ACIDS STEROID SKELETON TOPOLOGICAL DESCRIPTOR

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The topological, molecular descriptors are widely used in the QSPR models. These descriptors explain relatively well the structural differences of bile acids that belong to different congeneric groups. However, with the bile acids that belong to the same linear congeneric group (e.g. the bile acids with two oxygen atoms, either from OH or oxo groups, in the steroid nucleus, glyco conjugates of bile acids with three oxygen atoms in the steroid skeleton, etc.) the majority of these topological descriptors give the same value. Because of that the aim of this work was to construct a new descriptor related to the steroid nucleus of bile acids based on the molecular graph, which also takes into account the steric environment of the oxygen atoms bonded to the steroid ring system. This should allow us to obtain the mutually different values of descriptors for the bile acids belonging to the same congeneric group. The newly constructed molecular descriptor takes into account the change of the position, steric orientation, and the number of oxygen atoms bonded to the steroid skeleton of the bile acid molecule. This steroid skeleton descriptor (*ssd*) represents the intensity of the vector obtained as the product of the matrix of orientation and of the type of oxygen atom (from OH or oxo groups) **m** and distance vector **v**, standing for the distance of the carbon atom with the bonded oxygen atom from the reference C10 atom. In the matrix **m**, the column vector is of the form $[\omega_i\ ot_i]^T$, where ω_i is the parameter of steric orientation of the *i*-th oxygen atom, whereas ot_i is the parameter for the type of the *i*-th oxygen atom. The matrix **m** is of the type [2 x *n*], where *n* is the number of C atoms of the steroid skeleton to which oxygen atoms are bonded. The final descriptor is obtained as a linear combination of *ssd* and *stericS*, where *stericS* descriptor accounts number of synclinal and synperiplanar groups in a proper Newman's projection formula of butane that contain hydroxyl and oxo groups from the steroid skeleton. Calculated novel descriptor explains very well hydrophobicity of bile acids (retention coefficient, critical micellar concentration, hemolytic potential) even in simple regression equations.

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IMPROVEMENT OF DETECTION POWER IN CHROMATOGRAPHY, VOLTAMMETRY, AND SPECTROMETRY

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Improving signal-to-noise ratios and thus limits of detection is a general challenge in analytical chemistry. Especially for environmental or clinical tasks it is important to search for new ways of analyzing smallest amounts of analytes. This may be achieved by developing new or improving existing analytical methods.

Another possibility, however, is signal treatment after measurement. Means of signal treatment like convolution with Gaussian distribution curves [1], Fourier transform (FT) [2] or Wavelet transform (WT) [3] have already been described in past. As appropriate computers had not been available at their development yet, the implementation of these methods could not be accomplished to all fields of analytical chemistry. Therefore, we present a recent comparison of these means for chromatographic, voltammetric, and spectrometric utilizations.

In one case, arsenic species (inorganic as well as organic) are separated via HPLC-ICP-MS for estimating their amount in feeding stuff. The separation has been optimized, but the inevitable noise underlying the chromatogram remains, hindering the evaluation of smaller peaks in particular. After signal treatment, the limits of detection, however, are considerably improved by the factors of 1.5-3 (Gaussian distribution curves) up to 3-4 (FT), depending on the respective arsenic species.

Wavelet transform shows the best results (factors 5-6). Particularly more symmetric wavelets like Symlet 4 result in de-noised signals without reduction of the peak heigth.

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CAPABILITY OF DETECTION, DECISION LIMIT AND OTHER PERFORMANCE CHARACTERISTICS OF AN ANALYTICAL METHOD WHEN THE CALIBRATION MODEL IS A NEURAL NETWORK

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In the present work, molecular fluorescence spectroscopy is used to determine sulfathiazole in milk. As some non-linear behaviour may occur the calibration model is computed by a MLF (multi-layer feed-forward) neural network. Despite the fact that the calibration model is non-linear, the limit of decision, CCα, and capability of detection,CCβ, of the method can be established according to Decision 657/2002/CE.

To take into account the possible quenching effect due to other fluorescent substances present in the sample, four different volumes of the whey were taken with which four series of samples were prepared with 0.5 ml, 1 ml, 1.5 ml, and 2 ml. Each sample was spiked with sulfathiazole in increasing concentration.

By considering the emission spectra (131 wavelengths) of the 28 calibration samples, a calibration model is built by using a MLF neural network trained with the Levenger-Marquardt algorithm. To improve the bad objects/variables rate, a previous PCA was made.

Then a line was fitted by OLS between the predicted concentrations (computed with the neural network) and the true concentrations, which allows concluding that the method is unbiased.

The European Union has established (Regulation 37/2010) a maximum residue limit for this sulfonamide of 100 µg kg^{-1} for this value CCα and CCβ are respectively 103.3 µl l^{-1} and 106.6 µl l^{-1} with probabilities of false non compliance and false compliance equal to 0.05.

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USEFULNESS OF PARALLEL FACTOR ANALYSIS IN THE DETERMINATION OF TRIAZINES IN PRESENCE OF NON INTENCIONALLY ADDED SUBSTANCES BY GAS CHROMATOGRAPHY WITH A PROGRAMMED TEMPERATURE VAPORIZER INLET

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The presence of non-intentionally added substances (NIASs) is becoming important. It is common to use high-resolution mass spectrometry detectors to identify these substances. However, this does not solve the fact that a NIAS contributes to the relative ion abundance at an m/z ratio corresponding to fragmentation of other molecules. This is the cause of false negatives in the identification of compounds (SANCO/10684/2009).

An alternative consists of considering the multivariate structure of the abundances of *K* ions recorded at *J* times. Concatenating *I* of such matrices, a three-way tensor $\underline{\mathbf{X}}$ is obtained, which is decomposed by means Parallel Factor Analysis (PARAFAC) into as many factors as substances that are coeluting, providing the spectral and chromatographic profiles of each of them. If amount of each analyte changes along the *I* samples the unequivocally identification is assessed. These are the basis of a three-way calibration. But, for a test sample eventually containing NIAS, it is not possible to prepare samples with different concentration before identifying the substance.

In this work, the introduction of different volumes of sample on a GC system, used in full scan mode, with a programmed temperature vaporizer (PTV) inlet, provides a tensor of data suitable for PARAFAC decomposition. This is applied to the determination of simazine, atrazine, terbuthylazine, prometryn and terbutryn in the presence of NIASs. The triazines were unequivocally identified with decision limits between 7.5 and 25.0 μg L^{-1}.

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MATHEMATICAL MODELING OF THE ANALYTICAL SIGNAL IN SINGLE INTEFACE FLOW ANALYSIS

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The efforts regarding the theoretical modeling of flow analysis techniques so far have focused exclusively the original Flow Injection Analysis strategy (FIA), with approaches from different fields being used for this purpose ranging from chemometrics (e.g. neural networks) to fluid dynamics (e.g. axial dispersion plug flow model) and chromatography (e.g. tanks-in-series).

Single Interface Flow Analysis (SIFA) differs from the more traditional flow analysis technique in the sense that it is based on the interpenetration of reagent and sample zones at the single interface reaction. Hence it does not depend on the insertion of defined volumes of sample and reagent. Also, due to its characteristics, the analytical signal presents itself not as a peak given by the sample dispersion at leading and a trailing interfaces with the reagents, but as the product of the dispersion of reagent and sample at a single reaction interface. This flow analysis strategy has not yet been comprehensively addressed in a theoretically perspective. Although the dispersion behavior of a colored sample has been focus of previous work [1], the most common situation where a reaction occurs has not previously been focused.

In this communication, we present results regarding the mathematical modeling of the analytical signal in a SIFA determination involving an enzymatic reaction with the reaction product being detected by UV-VIS spectrophotometry.

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SOFTWARE «1:1 OR 2:2» FOR JOB'S METHOD

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During provided webometric research it was found out that in spite of the fact, that job's method is known for a long time, it is still used in the chemical researches (examined time 1946-2011 years, sample=2400, system for providing research - Google.Scholars). The average statistical impact-factor of journals in which that articles were published was 3,375 (using Google.Scholars 1500 most relevant articles to the request "Job's method" were examined). Analyzing over 200 most relevant article to the requests "Job's method" and "continues variation method" (using ScienceDirect) was found out that 56,7% of studied complexes have complex ratio 1:1.

We used proposer mathematical model [1] in the creation of software "1:1 or 2:2". In that model data obtained at classical job's method research is used for determination is the complex ratio is 1:1 or 2:2. Additionally, the limitation of the proposed mathematical model was studied using opportunities of software "1:1 or 2:2".

Software was approved on the real complexes of xylenol orange, eriochrome cyanine R, chrome azurol S with Cu(II), Fe(III), Al(III), Zn(II). Examined obtained results we supposed that Cu(II)-chrome azurol S and Fe(III)- xylenol orange have complex ratio 2:2.

Software is freely available in three languages: English, Ukrainian and Russian on http://www.chem.org.ua/11-or-22/?lang=en.

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APPLICATION OF METHODS OF MULTIVARIATE ANALYSIS IN EVALUATION OF RAMAN SPECTRA OF HEALTHY HUMAN BRAIN TISSUES

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In this study we evaluated potential of multivariate analysis in assignment and classification of Raman spectra of different healthy brain tissues. The objects of this evaluation are the Raman spectra of the following brain tissues: *pons, nucleus caudatus, cortex cerebri, cortex cerebelli, nuclei basales* and *septum pellucidum.* Raman spectra of the 6 specimens of each tissue, prepared by cutting 50 μm thick slicess on cryotome, were collected by a DXR Raman microscope with 532 nm excitation. The spectral characteristics of brain tissues were determined using K-Mean Cluster Analysis (KMCA) and Principal Component Analysis (PCA). The established characteristics have helped to analyse the similarities between Raman spectra of these tissues and of *septum pellucidum*, whose microstructure has been the object of many histological disputes. The results of KMCA have shown the greatest similarity between *nuclei basales* and *septum pellucidum* tissues, which has also been confirmed by the results of PCA analysis. The results of PCA indicated that all analyzed tissues contain lipids and sterol-derivate compounds. The differentiation spectra PCA analysis have revealed that tissues, mainly consisted of grey matter, have much greater representation of components related to the presence of DNA, proteins and derivates of sterol compounds. The preliminary study results pinpoint that use of advanced statistical techniques of Raman spectra classification can give qualitatively new information compared to standard immunohistological techniques, which can help in further differentiation of nervous tissue. Further they could serve as reference in characterization of brain tissues affected by disease.

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MULTIVARIATE ANALYSIS OF DISCRIPTIVE CHEMICAL AND SENSORY DATA ON TAP AND MINERAL WATERS

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Sensory analysis tests were performed on 13 tap and 12 mineral water samples, which were blindly assessed by 14 trained panellists. Trained panellists judged these 25 samples in duplicates in two days sessions. Averaged panellists taste quality scores from 1 (worst) to 10 (best) were evaluated for the different samples in relation to their chemical content. The same samples were chemically analyzed for 13 different parameters.

Principal Component Analysis performed simultaneously on the chemical and sensory data sets resulted in a 5 PC components model explaining more than 95% of the total variance. This exploratory data analysis allowed for the discrimination of the two classes of water samples- tap and mineral water. According to these 5 principal components, preferred water sensory parameters characterizing samples were 'hardness' related to high concentrations of bicarbonate, sulphate, calcium, magnesium ions and to 'alkalinity'- higher pH values . On the contrary, panellists scored low waters with higher 'total mineralisation' and lower pH-'acidity' parameters. Salinity, due to high concentrations of sodium, potassium and chloride ions, was also low scored by the panellists during the evaluation. The panellists did not like either those waters with relative high chlorine concentrations present in tap water samples.

Partial Least Squares regression of panellist sensory evaluations on chemical parameters resulted in a two latent variables model, explaining 89% of the total variance and fitting a calibration model with 0.80 R-square. Analysing the importance of chemical parameters in the regression model using the Variable Importance of Projection (VIP) scores method, did reveal which were the more significant variables in sensory testing evaluations. Concentrations of sodium, potassium, chloride and nitrate ions, chlorine and dry content were confirmed to be the more important parameters. Moreover, these 6 variables were found to be scored with negative values in PLS weight and PLS regression vectors leading to the conclusion that they contribute negatively to the panellist preferences.

THE USE OF LINEAR REGRESSION FOR CALIBRATION IN ANALYTIC CHEMISTRY

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A vast majority of calibration work in analytical chemistry involves responses that increase linearly with the concentration of the analyte. Non-linear responses, when encountered, are often linearized electronically or through some data pre-treatment. Alternatively, linearity is ensured by dilution of samples prior to determination. For the calibration of linear responses the method of linear least squares regression is used almost exclusively. In accordance with this, or vice-versa, IUPAC has published guidelines where this practice is outlined and advocated [1]. These guidelines clearly specify the limiting conditions which this algorithm is based on and the importance of taking these into account, when using this calibration technique. One of the two limiting conditions is that there should be no errors associated with the concentration or x-variable. This will never be strictly met, but for most practical purposes this is probably not a major concern. The second condition demands homoscedasticity in the measured response or y-variable, which means that the error in the estimated response is constant over the calibrated concentration range. Should your calibration fulfil both conditions you can apply a so-called normal or ordinary regression algorithm and this is what most analysts do. The IUPAC guidelines however firmly stress that before this most simple least squares algorithm can be used one must clarify, preferably through statistical testing, that the above stated conditions are indeed met. If they are not, weighted regression is recommended. The algorithm for weighted regression is less straight-forward and it requires some assumption on the nature of the heteroscedastisity. It also requires more specialized software as compared with ordinary linear regression.

For instrumental analysis, homoscedasticity is rather an exception than a rule and, since ordinary regression is very widely used, it may be feared that this problem is often solved by ignoring or even ignorance. It is the belief of the present author that the use of linear regression in the way suggested in the IUPAC guidelines, although very widely adopted, should be avoided in routine work. An alternative approach, which has been very thoroughly tested, will be suggested and explained in this lecture.

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ASSESSING THE QUALITY OF PEARLS USING ARTIFICIAL NEURAL NETWORKS AND DIFFUSE REFLECTANCE UV-VISIBLE SPECTROSCOPY

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Assessing the quality of pearls involves various tools and methods, however the process is mainly visual and often subjective. While there is no official standard for grading pearls, they are normally classified by origin and are then graded by luster, nacre thickness, surface quality, size, color and shape. The aim of this study was to investigate the capacity of Artificial Neural Networks (ANNs) to classify and estimate the quality of 27 different pearls from their Diffuse Reflectance (DRA) UV-Visible spectra. The UV-Visible spectrum of a pearl is unique and differ-ent pearls may show significant differences in their UV-Visible absorbance due to differences in nacre composition. The iridescent appearance and quality of nacre has been attributed to light diffraction, both diffraction and interference (interaction between waves), or interference alone.

Due to the opaque nature of pearls, spectroscopy measurements were performed using the Diffuse Reflectance UV-Visible spectroscopy technique. The spectra were acquired at two different locations on each sample to assess surface homogeneity. The spectral data were smoothed to reduce the noise using the Moving Average method by counting the average of twenty spectral intensities in every fifth wavelength record. The resulting spectral data (inputs) were then fed into Artificial Neural Networks and correlated to the pearl's quality/grading criteria (outputs). For the pearl quality assessment the pearls name, species, locality, luster, luster enhancing, color, color enhancing, donor condition, recipient, surface, and shape were used with 11 different ANNs trained, tested and validated.

The developed models were successful in predicting the pearls type (South sea pearl versus Freshwater Pearl, Tahitian pearl or Akoya Pearl), mollusk growing species (Pinctada maxima, Freshwater mussel (species unknown), Pinctada fucata martensi or Pinctada margaritifera), possible lustere and colour enhancing (possible, likely and no), donor condition/type (relaxed or normal/standard seeding), recipient/host colour (yellow, white or unknown/possibly black), donour colour (silver, yellow, white or unknown/possibly black), pearl lustre (1,2,3 or reject), pearl colour (white, cream with various overtone, gold, lavender or reject, pink and various colour), origin (Bali, China; Japan or South Pacific). The results of this study shows that the UV-Vis spectroscopy method could be used to predict with accuracy pearl colour, pearl origin, colour enhancement and treatment and as a quantitative measure of pearl lustre. This technique provides a less subjective method of assessing pearl quality (grading) and may become a valuable tool for the pearl grading industry.

CHEMOMETRIC CLASIFICATION AND STRUCTURE-ACTIVITY STUDY OF CHOLINESTERASE DUAL INHIBITORS

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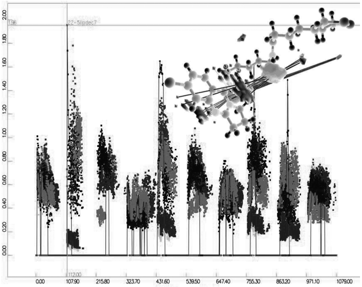
Set of eighty-eight structurally diverse, dual acetylcholineesterase (AChE) inhibitors are classified by principal component analysis (PCA) of 3D-dependent, alignment independent descriptors (GRIND)[1,2] derived from molecular interaction fields (MIF).[3] Conformations of all compounds studied are adjusted to ligand cocrystalized with the respective enzyme binding site (PDB entry 2CKM).[4] Tautomeric and protomeric states of compounds studied are ascribed in a way to fit experimental conditions under which determination of their potency were done. The first and second generation of GRIND descriptors are used and compared. First three PC's give good classification of compounds in respect to their structural properties, using both methods. Inclusion of potency data obtained under the exactly same conditions, spanning 4.2 p(IC_{50}) units, and subsequent structure-activity study by partial least square analysis (PLS) give good results. Fractional factorial design are used to obtain final models. X variable space is covered by 836 and 870 variables. Four latent variables (LV) models show r^2 0.91 and q^2 0.76 - 0.81 (validated by 5 randomly chosen subgroups of compounds). Very good external predictivity is proven using both external literature set, as well as potency values of compounds synthesized by our group. To the best of our knowledge this is the first reported PCA/PLS analysis on such number of AChE inhibitors so far. Along with this, statistical quality of models obtained significantly exceeds so far reported ones, using smaller set of compounds.



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ASSESSMENT OF RIVER WATER QUALITY BY APPLYING MULTIVARIATE ANALYSIS

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The main goal of this work is focused on water quality classification of the Sava River (Serbia) and evaluation of pollution data obtained by the monitoring of significant quality parameters measured during the period 2008–2010. Selected chemometrics techniques, such as principal component analysis (PCA), factor analysis (FA) and cluster analysis (CA), were applied to the obtained dataset composed of sixteen chemical and physical–chemical quality parameters for 36 water samples. Multivariate analysis of very complex matrix demonstrated the power of chemometrics in identification of factors as groups of correlated variables with the highest percentage of explanatory variance. Natural clusters of analyzed variables with a similar pollution character were identified for the investigated period of time. The classification of water purity of the Sava River made by the Inspection of Environmental Protection showed minor differences between the periods of monitoring. On the basis of the chemometric approach, it was also found that some locations were under the high influence of municipal contamination, and others under the influence of agriculture within the observed time period. Chemometrics tools appeared as very efficient for river water classification and rapid assessment of water quality necessary for management of water resources.

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ELECTROCHEMICAL DETERMINATION OF ATOMOXETINE IN IONIC LIQUID MODIFIED CARBON PASTE ELECTRODE

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Based on the unique electrochemical properties of ionic liquids (IL) such as high ionic conductivity, broad electrochemical potential window and good redox-robustness, in this work we have prepare and characterize carbon paste (CP) modified electrodes through incorporation of the IL ethyl-dimethyl-propylammonium bis(trifluoromethylsulfonyl) imide, and then it was used to determine the selective noradrenaline reuptake inhibitor drug atomoxetine (N-methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine).

Different percentages of carbon paste and IL were investigated (5% to 30% w/w). The electrochemical response of the IL-CPEs was evaluated by cyclic voltammetry using the redox mediators ferrocenemethanol and potassium ferrocyanide, and the results indicate that in relation of bare CPE a) the presence of IL in the carbon paste enhanced the electrochemical current, b) the background response on the IL-CPEs was greater and c) the IL-CPE enhanced the active electrode area. According to the results a 30%-LI (w/w) was selected for the application of IL-CPE to quantitative determination of atomoxetine.

Atomoxetine exhibited an oxidation signal that shifting at lowers potentials as the pH increases. The peak current was more intense than that obtained using bare CPE at all the pH studied (e.g. around 3-fold at pH 3). For analytical purposes, differential pulse voltammetry technique in Britton-Robinson buffer at pH 3 was selected, which exhibited adequate analytical parameters. CV (%) of within-day reproducibility and inter-day reproducibilities were lower than 6% at different concentration levels. The calibration curve method was employed with a concentration range of $5.0\times10^{-5} - 7.0\times10^{-4}$ M, and was described by the expression: I_p (μA) = 32.7×C (M) - 0.0012 ($r^2 = 0.998$)

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DEVELOPMENT AND CHARACTERIZATION OF IBUPROFEN SELECTIVE ELECTRODE BASED ON CYCLODEXTRIN AS IONOPHORE

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Ibuprofen (α-methyl-4-[isobutyl] phenylacetic acid), is the oldest nonsteroidal anti-inflammatory drugs (NSAIDs) massively prescribed to reduce fever and to treat pain and inflammation in rheumatic disease and other musculoskeletal disorders [1]. The analytical control of ibuprofen is important in several kinds of samples and more recently in environmental water resources, with demonstrable chronic toxicity activity. Two metabolites of ibuprofen (carboxyl-ibuprofen and hydroxyl-ibuprofen) were also found in surface waters and sewage treatment plants [2]. Cyclodextrins has been exploited as ionophores in selective electrodes regarding to several advantages like selectivity, sensibility, repeatability, stability and lower detection limits [3]. In this work an ibuprofen selective electrode is proposed aiming screening proposes as well the coupling to separation techniques. The membrane incorporates α-cyclodextrin (1.2% w/w) as ionophore, 2-nitrophenyloctyl ether (65.6% w/w) as plasticizer and tetradodecylammonium bromide (0.4% w/w) as additive. This sensing cocktail was dropped directly on the conductive surface made up with a mixture of epoxi resin (Araldite) and graphite powder. The conventional ibuprofen electrode as a slope of 57.8±0.1mV decade^{-1}, a practical detection limit of $(3.34\pm0.03)\times10^{-6}$ mol L^{-1} and a linear range between $(3.87\pm0.00)\times10^{-6}$ and $(1.00\pm0.00)\times10^{-2}$ mol L^{-1}. The electrode has a response time lower than 15 seconds and it is stable for at least 2 months. Ibuprofen electrode shows lower selectivity coefficients concerning the representative ions of the sample matrices. However this electrode presents nernstian responses for other nonsteroidal anti-inflammatory drugs such as salicilate and diclofenac. This result has been understood as an opportunity to be applied in a chromatographic system for monitoring different drugs. The separation methodology is still understudy.

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NEW FLOW-THROUGH POTENTIOMETRIC CELL FOR EXPEDITOUS PERM-SELECTIVE MEMBRANE OPTIMIZATION AND APPLICATION

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Recent insights on the mechanism of response of polymeric membrane-based ion-selective electrodes (ISEs) revealed that trace level analysis is impaired by zero-current ion fluxes which also prevent the accurate assessment of inherent membrane selectivities and the achievement of optimized detection limits [1]. To overcome the problem, several guidelines were proposed either concerning the optimization of the membrane composition and thickness as well the careful establishment of the inner reference solution composition and measurement conditions for each actual application [2,3]. From those studies becomes evident the need for a versatile configuration of the potentiometric cell namely fulfilling the requirements for selective membrane optimization and the usefulness in different analytical control applications. Accordingly a new flow-though potentiometric cell is proposed, which enables easy replacement of ion-selective membranes and in which both the appropriate inner reference solution and sample flows according to the selected application. An initial evaluation study was developed using a 400 μm thickness calcium selective membrane with the composition of 3.3%(w/w) regarding the ionophore ETH5234, 1.1% of the additive NaTFPB, 63.3% of the o-NPOE plasticizer and 32.3 of PVC. The best performance, corresponding to a dynamic range between 10^{-7} and 10^{-2} of Ca^{2+} was achieved for an inner reference solution containing $5x10^{-2}$ mol/L EDTA, 10^{-8} Ca^{2+} and 10^{-4} NaCl adjusted to pH 9.0 and using an optimum flow-rate of solutions of 1.0 mL/min. The performance results compared favourably with those reported for other ISEs with conventional rod configuration possessing inner reference solution. Simple procedures were developed for the accurate determination of calcium in biological fluids, waters and natural fruit juices.

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EC04 **Session A**

EVALUATION OF THE ANTIOXIDANT CAPACITY OF FLAVOURED WATERS USING A PURINE BIOSENSOR: OXIDATIVE DAMAGE BY HYDROXYL, SUPEROXIDE AND SULFATE RADICAL (A COMPARATIVE STUDY)

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Oxidative lesions in DNA are the primary risk factor for gene mutations, playing a key role in carcinogenesis and aging. Free radicals are continuously generated in living cells, such as, in the inner mitochondrial membrane, outer membrane, and in several metabolic pathways in mammalian cells, for instance, in the microsomal electron transport [1]. Hydroxyl radical ($OH^{\bullet}$), superoxide radical ($O_2^{\cdot-}$) and the sulfate radical ($SO_4^{\bullet-}$) are some examples of free radicals produced in the human body.

Some food and beverage compounds have an important external contribution to body defences against oxidative insult. It provides cells specific antioxidants that are able to scavenge multiple types of free radicals contributing to maintain cellular health.

In this work, a purine (adenine and guanine) biosensor was developed to assess the total antioxidant capacity of flavoured waters and flavours. This biosensor consisted in the electro-deposition of a purine base on a glassy carbon electrode.

In order to evaluate the oxidative lesions in the purine base, this biosensor was immersed in hydroxyl, superoxide and sulfate radicals. Damage produced in the DNA layer was evaluated by square wave voltammetry by measuring the decrease in the oxidative peak current. Ascorbic acid, gallic acid, caffeic acid, coumaric acid and resveratrol antioxidants were added as standards and its subsequent protective effects was achieved. A comparative study was done on the oxidative damage promoted by the three different free radicals.

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DETERMINATION OF MERCURY IN STREET DUST SAMPLES BY ULTRASONIC EXTRACTION AND STRIPPING VOLTAMMETRY ON GOLD NANOPARTICLES MODIFIED SCREEN-PRINTED ELECTRODES

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Gaseous elemental mercury, particle-bound mercury and Hg(II) in rainwater are often selected as analytes to investigate atmospheric mercury distribution and behaviour. As an alternative to the several spectroscopic techniques for Hg determination, electrochemical analysis, particularly stripping voltammetry on gold or gold-based electrodes and recently on new disposable screen-printed platforms, have attracted significant interest because of their excellent sensitivity, short analysis time, and affordable and miniaturized equipments. In the last few years, modified electrodes based on nanoparticles research have become one of the most active areas in environmental analysis. In comparison to conventional electrodes, sensors based on metal nanoparticles present a high surface area, increased mass transport, low detection limit and better signal to noise ratio. On the other hand, extraction of Hg from complex samples (food, soil, sediments) is recognized as a crucial step before its determination due to its usual low concentration. Ultrasonic bath and probe have been described as efficient and inexpensive alternatives for solid sample pre-treatment.

We explore in this work the applicability of novel commercial gold nanoparticles screen-printed electrodes for the determination of particle-bound mercury in street dust samples by square wave anodic stripping voltammetry (SWASV) after ultrasonic bath and ultrasonic probe extraction. Faced centred central composite design was developed to optimize the pre-treatment. NIST Standard Reference Material® 2583 Trace Elements in Indoor Dust and ICP-MS data were used to test the method. Applicability to particle-bound mercury extraction and determination in real street dust samples from urban areas is described.

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NEW CARBON NANOTUBES-BASED SONOGEL-CARBON MATERIAL: DEVELOPMENT, CHARACTERIZATION AND ELECTROCHEMICAL APPLICATIONS

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The present work reports the development and characterization of a new type of Sonogel (SNG)-Carbon material, where the conductive material (carbon powder) has been substituted by multi-wall carbon nanotubes (MWCNTs). The exceptional electrical and mechanical properties of CNTs together with their enormous surface-to-volume ratio are well-known. Therefore, it is expected to enhance remarkably the conductive properties of resulting material, and hence, of the electrodes built with it. As a consequence of this, sensitivity of the electrochemical devices versus different analytes is intended to be improved, as well as to decrease their detection limits.

The synthesis of the CNTs-based SNG electrodes, via acidic sonocatalysis and sol-gel processing, is described. The amount of CNTs added to the sol in order to obtain materials with adequate mechanical and conductive properties is optimized. The electrodes built with this new material have been electrochemically characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Some structural characterization techniques have been also employed, such as scanning electron microscopy (SEM), scanning transmission microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS), among others.

The decoration of CNTs with gold sononanoparticles (AuSNPs) is also reported. Electrodes based on this conductive nanocomposite material have been tested and their electrochemical performance has been compared to CNTs-based SNG electrodes and common Sonogel-Carbon electrodes [1].

Some electroanalytical applications of this new CNTs-based SNG material are reported.

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ELECTROCHEMICAL DETERMINATION OF ASCORBIC ACID IN BABY JUICES BY USING METAL OXIDE-BASED SONOGEL-CARBON ELECTRODES

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Metal oxides, and in particular, CeO_2 have demonstrated to possess anti-fouling properties, what make them very useful for the determination of analytes in real samples. The present work reports the development and characterization of a new metal oxide-Sonogel Carbon electrode for the electrochemical determination of ascorbic acid in apple juices for babies. The electrochemical devices were built using Sonogel-Carbon (SNGC) [1] as supporting material, which were modified by depositing different concentrations of CeO_2 nanoparticles on their surface.

These electrodes were electrochemically characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The CeO_2 nanoparticles were also decorated with gold sononanoparticles [2] at different proportions to enhance sensitivity.

Besides, the electrochemical devices obtained were characterized structurally by several techniques: scanning electron microscopy (SEM), scanning transmission microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD), among others.

Finally, the devices developed were applied for the determination of ascorbic acid in apple juices for babies. The recovery percentages and the relative errors are also reported.

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ENANTIOANALYSIS OF PIPECOLIC ACID WITH STOCHASTIC AND POTENTIOMETRIC MICROSENSORS

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Stochastic and potentiometric sensors based on porphyrins and polymeric surfactants (poly-SULV, poly-SUVV) were applied for enantioselective assay of pipecolic acid. The matrix used for the design of the stochastic sensors was diamond paste while the matrix used for the design of potentiometric sensors was carbon paste. Surface characterisation of the active side of the microsensors was performed using AFM. The response characteristics of the microsensors were determined for the enantiomers of pipecolic acid. The response characteristics, selectivity and enantioselectivity studies proved that the proposed microsensors can be used for clinical analysis. The enantiomers of pipecolic acid can be directly determine in biological fluids, e.g., urine and serum.

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POTENTIOMETRIC DETERMINATION OF DISSOCIATION CONSTANTS OF SOME SCHIFF BASE LIGANDS IN BINARY WATER-ORGANIC SOLVENT MIXTURES

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The Schiff bases and complexes with them are interesting because of their properties such as their ability to reversibly bind oxygen[1], catalytic activity[2], antibacterial and antifungal properties[3]. The Schiff base complexes can be greatly modified by introducing different substituents[4].

In this work acidity constants of some Schiff base ligands synthesized from diamine (etane-1,2-diamine or propane-1,2-diamine) and corresponding β-diketone (pentane-2,4-dione and/or 1-phenylbutane-1,3-dione, pentane-2,4-dione and/or 1,1,1-trifluoropentane-2,4-dione, or 1,1,1-trifluoropentane-2,4-dione and/or 1-phenylbutane-1,3-dione) were potentiometrically determined in methanol-water (1:1, *v/v*) and dimethylsulfoxide-water (1:1, *v/v*) at t = 25 ± 1°C and constant ionic strength (I = 0.1 mol/dm^3 (NaCl)). Titrations were performed with TTT-60 titrator with an ABU-12 autoburette (Radiometer) and pH measured with PHM240 pH-Meter (Radiometer) with a combined GK2401B electrode (Radiometer). Measured pH values were converted into pc_H values according to relation[5] $pc_H = -\log [H_3O^+] = pH - A$, where A is the correction factor determined by potentiometric titration of the standard HCl solution with the standard NaOH solution in the used experimental conditions. pK_w Values was calculated from the same set of titrations. The software Hyper Quad 2008[6] was used to evaluate the dissociation scheme in the studied pc_H range and to calculate the values of acidity constants from four times repeated titrations.

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OPTIMIZATION AND VALIDATION OF A NEW METHOD FOR THE DETERMINATION OF Pb (II) IN ENVIROMENTAL SAMPLES BY ANODIC STRIPPING VOLTAMMETRY ON A BISMUTH DISK ELECTRODE

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Heavy metals measurement are critically important in environmental pollution assessment. In the past, mercury (Hg)-based electrodes have been the material of choice for stripping analyses due to their attractive analytical properties. Due to the toxicity and inconvenient disposal of Hg, alternative electrode materials are being explored, including bismuth [1]. Among the bismuth-based electrodes, little work has been done using a bismuth disk electrode (BiDE). In this work, we have explored the voltammetric stripping behaviour of Pb(II), Cd(II) and Zn(II) on a homemade BiDE. Optimization of chemical and instrumental parameters was carried out to develop a reliable and convenient method for the determination of these metals by square-wave anodic stripping voltammetry (SWASV). Calibrations resulted in good linearity for all metals studied with appropriated detection limits for environmental monitoring applications. The method has been validated with surface water, wastewater and soil certified reference materials with good agreement between measured and certified values. Finally, the method has proven its applicability in the determination of Pb(II) in samples of fortified rainwater and sewage sludge from a steel industry, expanding the previously described applicability of the BiDE [2]. All results obtained have been contrasted by ICP-MS with good agreement. The BiDE has been shown a good option for Pb(II) determination in environmental samples by SWASV due to its proper detection limits, inexpensive and easy fabrication, and low toxicity.

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METALLIC OXIDE COATING ELECTRODES AND ANALYTICAL APPLICATION THEREOF

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Electrochemical characterization of palladium and platinum oxide electrodes are reported. Wire electrodes for acid-base sensing are produced by coating palladium and platinum wire with sodium hydroxide and then oxidizing in oxygen at 750 and 450 °C, respectively. After suitably insulating to define the active area and cover up bare palladium (platinum), the wires are useful for potentiometric measurement. The electrodes are stable a few years in distilled water storage with no essential change in response.

The palladium oxide electrode enable fast measurements, with good sensitivity (54 ± 0.9 mV per decade), in acidic and base solutions (linear range of response up to the concentration 1×10^{-6} M) in acetonitrile and 49 ± 1.2 mV per decade in propionitrile. The response of the platinum oxide electrode was linear in the concentration range between 5×10^{-2} and 1×10^{-6} M, and a sensitivity of 52 ± 1.6 mV per decade in acetonitrile was obtained. The response time of the electrodes was less than 15 s in the both solvents. The properties of the palladium oxide electrodes were similar to those of the platinum ones.

Some potential analytical applications of the sensors are pointed. Palladium and platinum oxide electrodes for the potentiometric titrations of acids (benzoic, anthranilic, salicylic acids, sulfadiazine) and bases (*N,N'*-diphenylguanidine, tributylamine, and collidine) in acetonitrile and propionitrile were studied. Potassium hydroxide, tetrabutylammonium hydroxide and perchloric acid proved to be very suitable titrating agents for these titrations. The standard deviation of the determination of the investigated acids and bases was less than 0.7% from those obtained with a glass electrode.

The obtained results confirme that the palladium and platinum oxide electrodes can be successfully applied as indicator electrodes for the titration of weak acids and bases in acetonitrile and propionitrile. These electrodes exhibited useful features for determination of weak acids and bases with respect to its mechanical resistance, very low cost, simple preparation, great hardiness and chemical inertness to the non-aqueous working mediums in comparison with glass electrode.

NATURAL MONOCRYSTALLINE PYRITE AS SENSOR FOR POTENTIOMETRIC TITRATIONS OF COBALT(II) WITH IRON(III) AND CERIUM(IV) IN THE PRESENCE OF 1,10-PHENANTHROLINE

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Mineral pyrite as a new indicator electrode for potentiometric redox titrations cobalt(II) in the presence of the ligand 1,10-phenanthroline (phen) was used. Iron(III) and cerium(IV) solutions were employed as titrating agents. The titrations of cobalt(II) with iron(III) and cerium(IV) were carried out in acid solutions in the presence of 0.02 M and 0.01 M phen, respectively.

Indicator pyrite electrode was prepared in the folowing manner:

A quadratic piece of natural crystalline (a = 0.5 cm) was used as the electrode material. The crystal to be in contact with the solution was polished with diamond paste. A narrow glass tube was fixed with glue to the other side of electrode and then filled with mercury. One end of a copper or silver wire was immersed in the mercury and this device was mounted into a wider glass tube (Ø = 1 cm), which was then cemented with a cold sealing mass based on methyl methacrylate. After solidification of this mass, the working surface of electrode was polished to a high gloss. The electrode was then rinsed with distilled water and alcohol and dried in air, after which it was ready for use.

When this electrode was employed as an indicator electrode, the equilibrium potential in the course of the titration and at the titration end-point was rapidly established (within a few minutes). The results obtained by using pyrite electrode was compared with those obtained by application of a platinum electrode under the same conditions, and good agreement, reproducibility and accuracy were obtained. The relative error of the determination for 0.001 M cobalt(II) was less then 3% . The advantages of the pyrite electrode are long-term stability, fast response, easy preparation and low cost. The effect of some ions on the titration of cobalt(II) with cerium(IV) in presence phen was also investigated. Accurate and reproducible results were obtained.

DETERMINATION OF *CARBENDAZIM* FUNGICIDE BY DIFFERENTIAL PULSE STRIPPING VOLTAMMETRY AT A TRICRESYL PHOSPHATE-BASED CARBON PASTE ELECTRODE

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In this study, carbon paste electrode based on tricresyl phosphate (TCP-CPE) as a binding liquid has been applied as working electrode for the voltammetric characterization and determination of the *Carbendazim* fungicide (methyl *1H*-benzimidazol-2-ylcarbamate).

The effects of the pH value of the supporting electrolyte (Britton-Robinson buffer, pH 2.0-8.0) as well as the presence of cyclodextrin on the electrochemical behaviour of *Carbendazim* were investigated. In the investigated potential range (from -0.1 V to 1.3 V) one oxidation peak was obserevd, and its potential shifted from 1.06 V to 0.69 V while the pH of the supporting electrolyte increased from 2.0 to 8.0. The oxidation peak intensity also changed significantly with the change of the pH, and the most intensive and well shaped signal was obtained at pH 4.0. This indicates that protons are involved in the oxidation mechanism.

Addition of cyclodextrin ($3.6\ 10^{-5}$ mol dm^{-3}) to the electrolyte (Britton-Robinson buffer, pH 4.0) significantly enhanced the sensitivity of the *Carbendazim* determination.

The experimental conditions of the differential pulse stripping voltammetric method for the determination of *Carbendazim* were as follows: start potential -0.1 V, end potential 1.3 V, deposition potential -0.35 V, deposition time 120 s and scan rate 100 mV s^{-1}. The developed method offered a good linearity in the concentration range of $5\ 10^{-7} – 1\ 10^{-5}$ mol dm^{-3} with the correlation coefficient 0.995 and the limit of detection of $3\ 10^{-7}$ mol dm^{-3}. Satisfactory recovery achieved with spiked water was 101.9% at the concetration range of $1\ 10^{-6} – 3\ 10^{-6}$ mol dm^{-3}, inferring that the established method can be applied to real sample analysis.

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SCREEN-PRINTED ELECTRODES MODIFIED WITH BISMUTH PRECURSOR COMPOUNDS FOR THE DETERMINATION OF TI(I) BY ANODIC STRIPPING VOLTAMMETRY

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Over the last decade, it has been demonstrated that the environmentally-friendly bismuth film electrodes (BiFEs) exhibit favourable analytical properties in stripping voltammetry, in many ways comparable to those of the toxic mercury film electrodes (MFEs) [1]. The commonest method to generate a BiFE involves electroplating in the *in-situ* or *ex-situ* modes. The main drawback of electroplating is the added experimental complexity and the requirement for Bi(III) solutions while the morphology of the bismuth deposit is strongly dependent on the electroplating conditions. An alternative approach to fabricate a BiFE is the use of bismuth precursors; this involves bulk-modification of an electrode with a bismuth precursor compound (typically a Bi(III) compound); generation of the bismuth film occurs *in-situ* by reduction of the B(III) in the precursor during the reductive deposition step in anodic stripping analysis. So far, screen-printed electrodes (SPEs) loaded with bismuth oxide have been reported [2-3]; these sensors are disposabe and do not require a separate bismuth plating step. The aim of the present work was to assess five different bismuth(III) compounds (bismuth citrate $[O_2CCH_2C(OH)(CO_2)CH_2CO_2]Bi$, bismuth titanate ($Bi_2O_3 \cdot 2TiO_2$), bismuth oxide ($Bi_2O_3$), bismuth aluminate ($Bi_2(Al_2O_4)_3 \cdot xH_2O$) and bismuth zirconate $2Bi_2O_3 \cdot 3ZrO_2$) as potential bismuth precursors for the fabrication of disposable modified SPEs for the determination of Tl(I) by anodic stripping voltammetry. The chemical and instrumental paramemetrs were investigated in detail and the analytical features of the modified SPEs were established. The results indicate that the nature of the precursor and its content in the graphite ink critically affect the sensitivity of the analysis.

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ELECTROCHEMICAL BEHAVIOR OF REBOXETINE AND ITS VOLTAMMETRIC DETERMINATION

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Electrochemical techniques have a great advantage due to their simplicity, low cost, and relatively short analysis time. Reboxetine (RBX), chemically known as (RS)-2-[(RS)-a-(2-ethoxyphenoxy)benzyl] morpholinemethanesulphonate is a novel selective noradrenaline reuptake inhibitor with proven efficiency in a wide range of patients with major depressive disorder.

Various analytical techniques for the determination of RBX include high performance liquid chromatography (HPLC) with ultra-violet (UV) [1-3], fluorescence [2] or mass spectrophotometric detection [4,5]. In this study electrochemical behavior of RBX at hanging mercury drop electrode (HMDE) and glassy carbon electrode (GCE) was studied for the first time. For this purpose cyclic and square-wave voltammetry techniques were used, probable reaction mechanisms were proposed. Besides, according to linear relation between peak currents and RBX concentration voltammetric determination methods were described. Within proposed assay methods limit of detection of 0.004 μM was achieved for trace determination of RBX with differential puls cathodic adsorptive stripping voltammetry method. Described procedures were successfully applied for the assay of RBX in tablet dosage form and without prior extraction process recovery values were calculated around 100 %.

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CARBONIZED NANOSTRUCTURED POLYANILINES: APPLICATION FOR LEAD AND CADMIUM IONS SENSING

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Recently, carbonized polyaniline nanostructures have been used as supporting material for manganese dioxide catalyst for detection of heavy metal ions. It was noticed that carbonized polyaniline nanostructures themselves are active for sensing of lead and cadmium ions. Herein the synthesis of carbonized nanostructured polyanilines and their application for determination of different analytes is explored. Polyaniline is one of the most important conductive polymers commonly produced by electrochemical or chemical oxidative polymerization of aniline from acid aqueous solutions. The properties of the final polymer are influenced by several parameters: pH at the beginning of the oxidation reaction, reaction temperature, type of oxidant and aniline/oxidant ratio as well as acid anion used for doping. Herein polyanilines were synthesized by the oxidative polymerization of aniline in the presence of 5-sulfosalicylic and 3,5-dinitrosalicylic acid as well as without added acid, and then carbonized in nitrogen atmosphere by means of gradual heating up to 800 °C. Synthesized carbonized polyanilines were characterized using X-ray diffraction analysis. Subsequently, application of carbonized polyanilines based electrodes for sensing of heavy metals was investigated. Carbonized nanostructured polyaniline whose precursor was synthesized by the polymerization of aniline in the aqueous solution of 3,5-dinitrosalicylic acid showed the highest catalytic activity for lead and cadmium ion sensing. Sensing behaviour of electrodes based on carbonized polyanilines is comparable to other electroanalytical systems, allowing low concentrations of lead and cadmium ions to be detected.

ELECTROCHEMICAL CHARACTERIZATION AND ELECTROANALYTICAL DETECTION OF A MANGANESE (II) COMPLEX

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Manganese (II) complexes with various ligands, such as phorphyrins [1], cyclic polyamines [2], polyamine-polycarboxylate [3], have been synthesized and tested as anti-inflammatory drugs. On this ground, [Mn(thiophenyl-2-carboxylate)$_2$(H$_3$tea)], where H$_3$tea= triethanolamine, could be a potential antioxidant, anti-inflammatory agent. It can be considered that the electrochemical and electroanalytical behavior of manganese (II) compounds is an important matter in order to detect them in various samples and understand theirs biological role.

In the present work we report the electrochemical behavior of [Mn(thiophenyl-2-carboxylate)$_2$(H$_3$tea)] using cyclic voltammetry. To our knowledge it is the first time that [Mn(thiophenyl-2 carboxylate)$_2$(H$_3$tea)] is electroanalytically approached. The cyclic voltammetric peaks response was evaluated with respect to pH, sweep rate, sweep potential and concentration of manganese (II) complex. Moreover, we report the electroanalytical detection of the manganese (II) complex using adsorptive stripping voltammetry at a bare as well as at a dsDNA modified carbon paste electrode.

Keywords; Mn (II) complexes; biological activity; cyclic voltammetry, adsorptive stripping voltammetry, dsDNA

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SENSITIVE ELECTROANALYTICAL DETECTION OF dsDNA AT A CARBON NANOTUBES PASTE ELECTRODES

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Due to their special chemical, electrical and mechanical properties, carbon nanotubes (CNTs) have attracted much attention as analytical tools. Especially, in the electroanalytical field because of their high surface area, the good electronic conductivity and ability to promote electron transfer, CNTs are used as electrode materials.

The aim of the present work, is the combination of the feature of MWNTs and the advantages of the carbon paste, in order to fabricate CNTs paste electrodes that allow the easy, fast and sensitive detection of dsDNA, whereas the performance of three types of multi-wall carbon nanotubes (MWNTs) is investigated. The effect of surface pretreatments and accumulation conditions on the adsorption and electrooxidation of the dsDNA at CNTPEs is also described. Experimental results indicated that the electroactivity inherent to carbon nanotubes paste electrodes provided a large enhancement of the guanine oxidation signal compared to that obtained at the conventional carbon paste electrode (CPE). Based on the signal of guanine, under the optimal conditions very low levels of the dsDNA can be detected following short accumulation times for all types of MWNTs ($MWNTs_1$, $MWNTs_2$, $MWNTs_3$), with detection limits of 2.64 mg L^{-1}, 2.02 mg L^{-1} and 1.46 mg L^{-1}, respectively.

Keywords: *Carbon nanotubes paste electrodes; Carbon paste electrode; dsDNA; Adsorptive transfer stripping voltammetry*

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ELECTROCHEMICAL BEHAVIOR of ARIPIPRAZOLE and VOLTAMMETRIC METHODS for its DETERMINATION

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Aripiprazole (ARP) is a drug active molecule in the classes of atypical antipsychotic and antidepressant used in the treatment of schizophrenia, bipolar disorder and clinical depression. Determination of ARP in pharmaceutical preparations and biological samples including human serum and human urine is of great importance. In literature, there is no study about electrochemical behavior of ARP and also there is no voltammetric study dealing with determination ARP.

In this study; electrochemical behavior of ARP on glassy carbon electrode (GCE) was investigated by using voltammetric methods. One irreversible $2e^-/2H^+$ oxidation peak at about 1.05 V (vs. Ag/AgCl/3M KCl) was obtained. Voltammetric methods including differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) with and without adsorptive stripping (AdS) mode for direct determination of ARP were developed. These methods were based on the linear calibration of oxidation peak current with ARP concentration. Calibration curves were linear over the range of 11.40-157.0 µM (5.11-70.39 ppm) in DPV and SWV. When adsorptive stripping mode was applied, linear range was lowered to 0.22-13.56 µM (0.098-6.08 ppm). Values for limits of dedection (LOD) were calculated to be 6.36 µM and 5.52 µM for DPV and SWV, respectively. In anodic adsorptive stripping voltammetry (AAdSV) LOD values were found to be 0.14 µM and 0.11 µM for DPV and SWV, respectively. Proposed methods were successfully applied to pharmaceuticals, spiked human serum and spiked human urine and such applications have recovery values between 97.0 % and 102.7 % with relative standard deviation of less than 10 %. These methods have distinct advantages over other existing chromatographic and spectrophotometric methods regarding their accuracy, precision, sensitivity, detection and quantification limits.

OPTIMIZATION OF CONDITIONS OF DESIPRAMINE DETERMINATION BY DIFFERENTIAL PULSE VOLTAMMETRIC WITH THE USE OF THE SIMPLEX METHOD

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Desipramine is a tricyclic antidepressant which is used in the treatment of depression symptoms, so there is constant need to control concentration of the drug in a human body.

The aim of this study was to optimize the conditions of Differential Pulse Voltammetry (DPV) method of desipramine determination with the use of Simplex Method (SM). Cyclic voltammetry was used to determine the oxidation potential of the compound of interest in the range of 0–1200 mV and the scan rate of 100 mV/s. These parameters were established for quantitative measurements in Differential Pulse method, however the method sensitivity was insufficient. Optimization of the working conditions was conducted with the Simplex Method for parameters such as: potential of the pulse (E_P), time of the pulse (t_P), potential difference between the pulses (E_S). Glassy carbon ($A = 7.065\ mm^2$) and graphite electrode ($A = 4.14\ mm^2$) were examined as the working sensor. Glassy carbon electrode was tested in a stationary system vs. Ag/AgCl reference electrode and platinum as the auxiliary electrode. In the second case, measurements were performed with the use of a three screen-printed electrode system. Both working and auxiliary electrodes were made of graphite but silver-silver chloride electrode was utilized as a reference electrode.

The evaluated method enabled to determine desipramine in water solution at concentrations below 0.1 μM. In comparison with the conditions established with cyclic voltammetry, the signal was at least 25 times higher for both sensors. The results obtained for glassy carbon and graphite electrode were recalculated with respect to the area of each sensor. All the presented results were obtained with precision less than 10% (RSD).

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AMPEROMETRIC DOT-SENSORS BASED ON ZINC PORPHYRINS FOR SILDENAFIL CITRATE DETERMIATION

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Three types of zinc porphyrines have been studied as modifiers of carbon paste or diamond paste in the assembly of six amperometric dot-sensors used in the detection of sildenafil citrate using differential pulse voltammetry. The linear concentration range for the proposed electrodes was 10^{-4} – 10^{-11} molL^{-1}, the lowest detection limit (2.46 pmolL^{-1}) being achived by the use of the diamond paste dot-sensor modified with zinc–5,10,15,20-tetra(4-sulfophenyl)porphyrin. Sildenafil citrate was succesfully determined from its pharmaceutical formulations, namely Viagra, by the use of the proposed method.

Acknowledgements
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USING OF A NEW CALIX[4]ARENE DERIVATIVE AS AN IONOPHORE FOR HYDROGEN ION-SELECTIVE ELECTRODE

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The pH measurements are generally carried out by use of glass electrodes. Although pH-sensitive glass electrode has distinguished response characteristics and has been in use for such a long period it has certain setbacks such as its high resistance, fragility, its instability in hydrofluoric acid or fluoride solutions and its unsuitability to serve as microelectrode for biological applications. Therefore, it is a promising area of interest to design selective receptors as ionophores for the construction of PVC membrane pH electrodes.

Various derivatives of calix[4]arenes have been used as ionophores in membrane electrodes that are selective towards different ions. Hence, we focused on the possibility of using synthesized 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(4-nitrophenyl-1-yl)-thiosemicarbazide-carbonyl-methoxy]-26,28-dihydroxy-calix[4]arene in the construction of a new hydrogen ion-selective electrode.

The proposed electrode has a membrane composition of 2% ionophore, 68.3% *o*-nitrophenyloctylether and 29.7% PVC (plasticizer/PVC mass ratio of 2.3) for a total mass of 425.5 mg. An internal filling solution of 1.0×10^{-3} M $CaCl_2$ was used. The electrode exhibited linear response with a sub- Nernstian slope in the pH range 1.9–12.4 at room temperature. It was successfully employed as an indicator electrode in acid-base titrations.

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VOLTAMMETRIC STUDY OF THE INTERACTION OF GENOTOXIC 2-NITROFLUORENE WITH DNA AT A HANGING MERCURY DROP ELECTRODE

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The interaction of genotoxic environmental pollutant 2-nitrofluorene with a double-stranded calf thymus DNA has been studied using a hanging mercury drop electrode (HMDE) as an electrochemical sensor. Two types of DNA damage were investigated and electrochemically detected (using cyclic voltammetry and differential pulse voltammetry): (i) The DNA damage caused by the direct interaction with 2-nitrofluorene and (ii) the DNA damage caused by short-lived radicals generated by the electrochemical reduction of the nitro group in 2-nitrofluorene. For the study of direct interaction, HMDE was modified by DNA and the interaction of DNA with 2-nitrofluorene was studied, after their incubation, right at the HMDE surface (adsorptive transfer stripping technique) or the DNA was preincubated with 2-nitrofluorene and, subsequently, the interaction was studied voltammetrically (DNA titration technique). Using both detection techniques, the formation of DNA–2-nitrofluorene complex was observed and the mutual interaction was interpreted as an intercalation between the DNA base pairs, although such interaction was not clearly confirmed by UV-visible absorption spectroscopy. After reductive activation of 2-nitrofluorene giving short-lived nitro anion radicals, the oxidative DNA damage induced by these reactive species was evaluated from the height of cathodic peak CA (due to reduction of cytosine and adenine). On the basis of obtained results, we suppose that expected formation of 8-oxoguanine (one of the most common DNA lesions) leads to guanine–cytosine base pair interruption and DNA double-strand breaks. These results are in good accordance with those previously obtained during our voltammetric investigations at screen-printed carbon paste electrodes [V. Vyskočil, J. Labuda, J. Barek: Anal. Bioanal. Chem. (2010) 397:233–241].

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DETERMINATION OF THE RELATIVE ACIDITY SCALE OF TETRAHYDROFURANE

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The relative acidity scale of solvents which may be used in acid-base titration under defined conditions (titrant system, electrode system etc.) represents intervals of millivolt values (Es).
The range of relative acidity scale (mV) of tetrahydrofurane has been determined by the difference of semineutralization potentials of percloric acid and tetrabutyallmonium hydroxide. Semineutralizing potentials have been measured by using indicator electrodes: glass, antymon, H_2/Pd and D_2/Pd, the last two produced in our laboratory. As the standard electrode, saturated calomel electrode has been used. All measures have been carried out at the temperetaure of 25 ± 1ºC.
By using electrode pairs, the relative scale of acidity is the following: for glass - SCE 880mV, for antymon - SCE 470 mV, for H_2/Pd - SCE 1155mV, and for the pair D_2/Pd – SCE 1075mV.
Water decreases the relative acidy scale of tetrahydrofurane, the decrease being the highest with the use of H_2/Pd and D_2/Pd electrodes.

POTENTIOMETRIC TITRATION OF TWO-COMPONENT MIXTURES OF WEAK ACIDS IN TETRAHYDROFURANE BY USE OF H_2/Pd AND D_2/Pd ELECTRODES

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In this paper, the possibility of applying an H_2/Pd electrode as indicator and reference and an D_2/Pd electrode as indicator for potentiometric titration of two-component mixtures of weak acids in THF (tetrahydofurane) as solvent. Mixtures of monocarbonic acids, mixture of monocarbonic and dicarbonic acids and mixtures of dicarbonic acids (pK values of the used acids in the water range 4.2 to 9.93) have been defined. As titrants , methanol solution of potassium hydroxide and tetrabutylammonium hydroxide have been used.

By following the changes in the potential – time curves of H_2/Pd and D_2/Pd indicator electrodes in THF, a conclusion has been reached that these electrodes behave in a similar manner, and their potential changes 2-3mV per hour. The potentials of these electrodes coupled with the reference H_2/Pd electrode are quickly established during titration and at the end point. The potential jumps of the end point in potentiometric titration using investigated electrodes with standard solution of methanol solution of potassium hydroxide are 2-5 times higher than potential jumps obtained using a glass-SCE electrode pair.

The results obtained by H_2/Pd and D_2/Pd electodes as indicators, and H_2/Pd and SCE as reference deviate in the range of ± 0,3% the results obtained by potentiometric determination of the end point titration by using the pair of electrodes glass – SCE.

EFFECT OF STRUCTURE ON ELECTROCHEMICAL BEHAVIOR OF SCHIFF BASES AND THEIR COMPLEXES WITH COPPER(II) BY CYCLIC VOLTAMMETRY

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The electrochemical behaviour of six Schiff bases and corresponding copper(II) complexes was investigated. Schiff bases contained ethane-1,2-diamine or propane-1,2-diamine as the amine part and pentane-2,4-dione and/or 1-phenylbutane-1,3-dione as β-diketones part. The electrochemical experiment was carried out using a CHI760b Electrochemical Workstation potentiostat (CH Instruments, Austin, TX). Cyclic voltammetry was performed using a conventional three-electrode cell (5 mL) equipped with several working electrodes, a Ag/Ag^+ electrode as reference and a platinum wire as an auxiliary electrode. Electrochemical behavior is studied in different non-aqueous media such as dimethylsulfoxide, acetonitrile and mixture of dimethylsulfoxide/dichloromethane (50% v/v) as aprotic solvents at the surface of different solid electrodes (platinum, gold and glassy carbon) using sodium perchlorate as supporting electrolyte. The best conditions were obtained when dimethylsulfoxide was used as solvent and glassy carbon as working electrode. The cyclic voltamograms were recorded in 0.1 mol/L sodium perchlorate as supporting electrolyte in the potential range of -1.5 to 1.5 V, at sweep rates between 0.01 and 0.1 V cm^{-1}. All solutions were deoxygenated by passing the stream of nitrogen into the solution at least 10 minutes prior to recording the voltammogram.

The redox process of the ligands was highly irreversible, whereas redox process of Cu(II) complexes were observed as one electron transfer process in quasi-reversible reaction when reaction take place at central ion and completely reversible when it occurs at ligand part. These electrochemical processes are controlled by diffusion and adsorption. The effect of concentration of the analytes, solvents, type of electrodes and scan rates on the redox behavior of these compounds was investigated and effect of electrochemical behavior was discussed under optimal conditions. The steric and inductive effects of substituents and length of C-chain in diamine bridge of Schiff base ligands as well as complexes were discussed.

VOLTAMMETRIC STUDY OF HYDRAZINE ELECTROOXIDATION AT GLASSY CARBON ELECTRODE

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The anodic oxidation of hydrazine at a glassy carbon electrode (GCE) has been investigated using linear sweep voltammetry. The electrochemical pretreatment of the GCE was used to reduce the hydrazine overpotential to make it possible to obtain the electrooxidation peak of hydrazine for its quantification. It has been found that the oxidation of hydrazine occurs at a potential of about 0.35 V less positive than that of an unpretreated GCE.

For electrooxidation mechanism study a Tafel plot was obtained and the dependence of peak current on scan rate was investigated. The electron transfer coefficient, α, was also calculated. The overall number of electrons involved in the oxidation of hydrazine and the number of electrons involved in the rate-determining steps were found to be 4 and 1, respectively.

The voltammetric hydrazine detection was carried out in the base electrolyte of 0.1 M KCl. The calibration plot was linear in concentration range of 10^{-5} – 10^{-3} mol dm^{-3} and detection limit of $1.3 \cdot 10^{-5}$ mol dm^{-3} was achieved under optimal conditions. The method was successfully utilised for the hydrazine determination in real samples of waste water. The proposed method is simple, sensitive and low-cost.

VOLTAMMETRIC DETERMINATION OF PHENOL AT GOLD MICROELECTRODE ARRAY

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The anodic sweep voltammetry has been used for the determination of trace amounts of phenol in aqueous solutions. The gold microelectrode array was used as a working electrode. The microelectrode array was obtained by electrodeposition of gold from the solution of $AuCl_4^-$ on the composite support at 0.0 V for 60 seconds. The support consisted of polyethylene mixed with carbon black in 5:1 ratio. The electrode's modified surface was characterized by scanning electron microscopy and cyclic voltammetry. The electrocatalytic effect of gold particle modifying was investigated. The oxidation peak of phenol was observed at 0.68 V (vs. $Ag/AgCl_{1M\ KCl}$) in the solution of 0.1 M H_2SO_4 used as a base electrolyte. The peak current was proportional to phenol concentration in the range of 10^{-8} – 10^{-6} M. The lowest concentration of 0.01 µM was achieved for phenol. Photocatalytic UV-irradiation and ozone treatment are considered to be advantageous methods for organic pollutant destruction. The voltammetric phenol detection at gold microelectrode is proposed as a sensitive, reliable and easy handling method for the control of phenol destruction in waste waters.

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THIOPHENE BEARING 6,6“-DICARBOXY-2,2’:6’,2“-TERPYRIDINE: EVALUATION OF CARBOXY SUBSTITUENTS ON THE ELECTROCHEMICAL AND OPTICAL PROPERTIES

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Conducting polymers are a class of organic polymers with a high degree of conjugation and whose conducting properties can be modulated from insulating to conducting through the doping level[2].

In this context, we are concerned with the synthesis of conducting polythiophenes where the monomeric unit is made up by terthiophene as polymerizing fragment and N-chelating moieties (terpiridine or phenantroline) as coordinating fragment toward transition metal ions. We connected these two moieties with spacers of different nature and in particular we achieved better properties of the final material introducing an ethynyl spacer. We previously report the synthesis of such molecule, 4’-(2,2’:5’,2’’-terthien-3’-ethynyl)-2,2’:6’,2’’-terpyridine (TAT)[3] and the electrosynthesis of the relative conducting polymer. Moreover we evaluated the applicability of such polymer as active material in organic photovoltaic devices. In the perspective of using this molecule in other applicative fields we introduced in the structure some little changes, which could enable the relative complex with transition metals to be exploited in dye-sensitized solar cells (DSSC) or light-emitting devices (LED). For example, complexes of carboxy-terpyridine with Ru(II) proved to be efficient sensitizers in DSSC[4] while Eu(III) carboxy-complexes in a particular configuration enable to obtain efficient photoluminescence[5]. In this communication we describe the attempt to introduce carboxy groups on the 6,6’’ positions of terpyridine in TAT, in the multiple perspective of i) electropolymerizing and characterizing the relative conducting polymer in order to evaluate the influence of carboxy groups on the polymeric species PTAT, ii) coordinating Ru(II) and Eu(III) or other lanthanide ions and testing the complex photochemical and photophysical properties and the applicability in the fields above cited.

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POTENTIOMETRIC AND SEM/EDX STUDIES OF POLYMERIC MEMBRANES PLASTICIZED WITH IONIC LIQUIDS

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The utilization of Ion-Selective Electrodes in Potentiometry is a very useful tool for the determination of chemical species with high selectivity and accuracy, therefore receiving a wide use in clinical and quality control laboratories. In addition to traditional electrodes that incorporate conventional plasticizers as phthalates, in recent years have been becoming increasingly used another sensors made of a PVC-matrix membrane including different percentages of Ionic Liquids (Ils). The noticeable properties of the Ils, concerning to their megligible vapor pressure, and their capability to be customized by selecting the cation an anion, allow to replacing the classical plastizicers by the Ils that are capable to act simultaneously as plasticizers and ionic exchangers.

The aim of the present work was to study the potentiometric response of the ion-selective electrode made of a PVC-matrix membrane prepared with two selected ionic liquids, 1-buthyl-3-methylimidazolium hexafluorophosphate, $[bmim^+][PF_6^-]$, and trihexyltetradecylphosphonium chloride, $[THTDP^+][Cl^-]$ to the Cl^-, NO_3^- SCN^- and SO_4^{2-} anions. Additionally, the response towards a very interesting pharmaceutical anion, such as the ibuprofen anion [2-(4-isobuthylphenyl)propionate] was also analysed. Finally, the Scanning Electron Microscopy and Energy-dispersive X-ray (SEM-EDX) characterization of electrodes was accomplished in order to understand the ion-exchange mechanism involved.

The membrane plasticized with trihexyltetradecylphosphonium chloride provided a potentiometric response to the inorganic anions that followed the Hofmeister series. The response to ibuprofen was fairly good and reproducible. Likewise, the structural characterization of the membrane after being used proved an anion exchange between the chloride anion present inside the membrane and the ibuprofen anion in the sample solution. The preservation of the phosphonium cation inside the membrane, as showed by SEM characterization, explained the reproducible response of the electrode.

POTENTIOMETRIC RESPONSE OF AN ION-SELECTIVE ELECTRODE BASED ON A MEMBRANE CONTAINING AN IONIC LIQUID TO TRIIODIDE, TETRACHLOROAURATE(III) AND BIS(TRIFLUOROMETHYSULFONYL)IMIDE

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Ionic liquids can act as plasticizers for various polymers and have been considered as a new generation of stable plasticizers for poly vinyl chloride. Some ionic liquids show better resistance to leaching and migration than many conventional plasticizers. Furthermore, some ionic liquids bring ion-sensing ability to the plasticized polymeric membranes, probably due to their ion-exchange properties. For these reasons, it is found in the literature some works on ion-selective electrodes based on membranes containing ionic liquids.

In this paper we study the potentiometric response of a PVC membrane plasticized with trihexyltetradecylphosphonium chloride to the anions triiodide, tetrachloroaurate(III) and bis(trifluoromethylsulfonyl)imide. These anions were tested because of its lipophilic character. Thus, there are some ion-selective electrodes for triiodide and tetrachloroaurate(III) based on polymeric membranes containing conventional plasticizers and ion exchangers. The bis(trifluoromethylsulfonyl)imide anion was tested because it is present in the composition of several ionic liquids insoluble in water due to its hydrophobic character.

In the selected experimental conditions the electrode showed a reproducible linear potential response against logarithmic triiodide concentration in the range 1×10^{-5}-2×10^{-2} M. This response may be useful for the development of analytical methods based on yodimetry. The electrode also provided potential response for tetrachloroaurate(III) and bis(trifluoromethylsulfonyl)imide, from 1×10^{-6}M and 2×10^{-6}M respectively, although in these cases the responses showed potential drift.

A Scanning Electron Microscopy and Energy-dispersive X-ray study (SEM-EDX) made with the ISE membrane exposed to triiodide and tetrachloroaurate(III) showed the presence of these anions and remaining the trihexyltetradecylphosphonium in the membrane. This indicates that an anion exchange process between chloride and triiodide/tetrachloroaurate occurs during the exposure.

EC37 **Session A**

LTCC MICROSYSTEM BASED IN MOLECULARY IMPRINTED POLYMER AS IONOPHORES FOR THE POTENTIOMETRIC DETERMINATION OF TRIMETHOPRIM IN AQUALCULTURE WATER

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Trimethoprim (TMP) is among the synthetic antibiotics used in both human and veterinary medicines. Its routine analytical control is required, for which a selective, quick and inexpensive method would be appreciated.
Potentiometric sensors consisting on ion-selective electrodes (ISEs) are a possibilitty [1]. Increased performance may be achieved by using a TMP ionophore capable of discriminating the main ion from co-existing ones in a high extent. Molecularly-imprinted polymers (MIPs) are specifially tailored for a target analyte and may be used to design a new ionophore for TMP.
For routine applications, the ISE should be adaptated to flow methods. Accordyingly, Low Temperature Co-fired Ceramic (LTCC) technology may be used to interface microfluidic circuits with a potentiometric sensory surface. LTCC is indeed an advantageous alternative for miniaturizing analytical systems.
Thus, this work proposes an LTCC device including an ISE based on TMP-MIP ionophore. The calibration displayed 61 (±0.2) mV/decade, with linear behavior down to 0.58 µg/mL, 0.997 squared correlation coefficient, 0.18 µg/mL limit of detection, and 1.3 % relative standard deviation for a 5.8 µg/mL TMP solution (n=10 and 95% confidence). Successful application has been conducted in the analysis of water samples.

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POLY(VINYL CHLORIDE) AND POLY(VINYL CHLORIDE-CO-VINYL ACETATE) AS MEMBRANES FOR POTENTIOMETRIC SENSORS

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Most of ion-selective electrodes (ISEs) of polymeric membrane type use PVC with lipophilic salts as an organic matrix [1]. We have recently reported easily modification of polypyrrole (PPy) based on silver by immersion of the electrode in silver nitrate solution [2]. In the present work, we studied modification of PVC and PVC-co-VAc containing plasticizer and ion-exchanger by various cations and its possible sensor performance. The modified membrane was examined by cyclic voltammetry and it was found that only silver is capable for stabile modification of polymer matrix. Based on the CV recorded Ag was deposited in elemental state on the surface and near surface layers of the membrane. Membranes of various compositions of PVC and PVC-co-VAc, DOS, DBP and o-NPOE as plasticizers and TDMAC and NaTPB as ion-exchangers were prepared and investigated as silver-selective sensors. The best performance was observed with the membrane composed of PVCAc, DBP and NaTPB. Thus, optimized electrode has linear working range of 1.0 x 10^{-1} - 1.0 x 10^{-5} M, with a Nernstian slope of 60.25 mV/decade of activity and detection limit is 4.25 x 10^{-6} M. The selectivity of this sensor, evaluated with fixed interference method and matched potential method, show that the sensor is significantly selective to silver over all the interfering ions except Hg^{2+} and Fe^{3+} which strongly interfere. The proposed electrode was used as indicator electrode in the potentiometric titration of silver ions. A simple preparation procedure and extensive pH range through remarkable acidic media (pH 0.7 – 7) are advantages of this modified electrode over a number of reported silver selective electrodes based on polymer matrix containing different ionophores.

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EC41 **Session A**

ELECTROCHEMICAL SYNTHESIS AND CHARACTERIZATION OF TERPYRIDINE-TETHERED POLYTHIOPHENE BEARING ETHYNYL BRIDGE

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Organic polymers were known as not conductive material until the discovery of conducting polymers (CP), in the last Seventies. Their major characteristics are i) a high degree of conjugation; ii) the possibility to be synthesized by chemical or electrochemical way; iii) the opportunity to undergo oxidative or reductive (-p or –n) doping processes, again through both chemical or electrochemical way, promoting an increase in their conductivities. Thanks to their properties CP (like polythiophenes, polypyrroles, and so on) are studied in many application fields, like electrochromic devices[6], OLED[7], rechargeable batteries[8], corrosion protection[9], organic photovoltaic devices[10], sensoring[11], biosensoring[12] and also biomedicine[13].

Currently we are involved in the electrochemical synthesis and characterization of terthiophene-based conducting polymers, especially bearing N-chelating pendant moieties (terpyridine or phenantroline) which enable the insertion of transition metal ions along the backbone, leading to a metallopolymer. In order to improve the properties of the polymeric material, in the past we modified both the chelating moiety and the bridge (saturated or unsaturated) connecting it with the terthiophene polimerizing fragment. Keeping the fragments which brought the better improvements (terpyridine as chelating group and ethynyl as spacer), we are now interested in studying different assembling of such fragments, that is binding the ethynyl substituent on a side position of the terthiophene unit instead of the central position. In this communication we describe the synthesis and electrochemical characterization of a new high conjugated molecule where two terthiophene fragments are connected symmetrically through an ethynyl spacer on both sides of terpyridine. The electrosynthesis of the corresponding conducting polymer and its electrochemical and spectroscopic characterization is also described. The results of such characterizations are used to compare the two way of assembling, side-fashion and central-fashion, and to evaluate the influence of the different way of linking on the properties of the electrogenerated polymer.

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APPLICABILITY OF THE MANGANESE(IV)-OXIDE BULK MODIFIED CARBON PASTE ELECTRODE FOR THE AMPEROMETRIC MONITORING OF H_2O_2 CONCENTRATION IN DIFFERENT PHOTODEGRADATION SAMPLES OF CLOTHIANIDIN

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The aim of the work was to investigate the applicability of manganese(IV)-oxide bulk modified carbon paste electrode, based on graphite powder, paraffin oil and 5% MnO_2 w/w% (MnO_2-5-CPE), for the hydrodinamic amperometric monitoring of H_2O_2 concentration in different photodegradation samples of clothianidin (CLO).

The surface morphology of the MnO_2-5-CPE was characterized by scanning electron microscopy, and the local microanalysis of the surface was performed by energy dispersive spectrometry. The optimization of amperometric measurements was performed at pH 7.5 at different working potentials between 0.2 and 0.7 V (vs. SCE). Although, the highest sensitivity was obtained at 0.7 V, the optimal working potential was 0.4 V since different additional oxidation processes take place at the potentials higher than 0.5 V in the case of photodegradation samples.

Three different types of photodegradation sampes were prepared with equimolar concentration of CLO (99.87 $\mu g/cm^3$) and treated by natural insolation during one month. The first sample contained only CLO (S1), the second one H_2O_2 and CLO (S2), and the third one photo-Fenton reagent (Fe^{2+} and H_2O_2) and CLO (S3). The obtained amperometric curves showed that in the case of S1 no measurable amount of H_2O_2 was detected, in the case of the S2 the concentration of the H_2O_2 decreased slowly, but in the case of S3 a significant drop of H_2O_2 concentration was measured. In paralell with the amperometric measurements of the H_2O_2 concentrations, reverse phase HPLC/DAD measurements were performed to monitor the concentration of CLO in the same samples (S1-S3). It was found that the direct photolysis of CLO was very slow, the presence of H_2O_2 lead to the moderate degradation of CLO, and the presence of photo-Fenton reagent due to high capability for $OH^{\cdot}$ radical generation caused rapid degradation of CLO. Since H_2O_2 as a source of $OH^{\cdot}$ radicals is the promoter of degradation, it is useful to monitor its concentration in these experimets. The MnO_2-5-CPE was found convenient and reliable electrode for this purpose.

VOLTAMMETRIC DETERMINATION OF DOXORUBICIN ON GLASSY CARBON AND FULLERENOL NANOPARTICLES-MODIFIED GLASSY CARBON ELECTRODES

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Doxorubicin is a widely used anticancer drug from a class of anthracyclines. Voltammetric properties of doxorubicin have been investigated using various carbon based electrodes such as carbon paste electrode, modified and unmodified glassy carbon electrode (GCE), etc. This was done by measuring either doxorubicin directly or some other product in pharmaceutical or biological samples, with a detection limit reaching picomolar concentrations.

In this research we used two GCEs from different manufacturers, with approximately equal surfaces, as working electrodes for adsorptive stripping voltammetric determination of doxorubicin. In the 0.1 mol/dm^3 acetate buffer solution pH 4.75, in the investigated potential range from 0.0 to 0.8 V (vs. SCE), there was observed one well-defined oxidation peak around 0.5 V. The resulting signals obtained at two electrodes were of different intensity, which leads to conclusion that signals significantly depend on the type of glassy carbon electrode material. Doxorubicin strongly and irreversibly chemisorbs on the electrode surface. Moreover, it was a special challenge to recover the electrode surface after measurements, which was successfully done by immersing them in sodium hydroxide aqueous solution pH 10. In our research we confirmed the possibility of doxorubicin detection in model solution at low ppb concentration level.

We try to investigate the interactions between FNP and doxorubicin applying a highly sensitive voltammetric method. Since the mechanism of interaction between FNP, which is believed to act as a protector during chemotherapy, and doxorubicin has not been defined clearly, in our further research we will employ voltammetric measurements to get a deeper insight in the investigated area.

ELECTROCHEMICAL RESPONSE AND DETERMINATION OF NOVEL NICOTINIC RECEPTOR AGONIST – VARENICLINE AT HANGING MERCURY ELECTRODE

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Varenicline, 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)(3)benzazepine is a novel drug used for smoking cessation. Its mechanism of action, as a partial agonist, is to block the full-agonist activity of nicotine by competitive binding.

No literature data on electrochemical study of varenicline and the methods based on its electrochemical behavior were found.

The reduction of varenicline (VAR) was studied at hanging mercury drop electrode (HMDE) in a wide pH range (Britton Robinson buffer, pH 2-12), showing the electrochemical activity at all pHs investigated. In an acid medium (pH $<$ 6), two well distinguished waves were observed (with $\Delta E_{1/2} \sim 0.5$ V), and only one, well developed reversible wave in pH range from 6 to 12. This is in accordance with the electrode reaction mechanism which is proposed as two two-electron reduction of C=N bond.

Based on the reversible voltammetric peak of VAR (in BR buffer at pH 5 and 8), a robust, highly reliable adsorptive stripping square-wave voltammetric method (AdSSWV) was developed for determination of VAR in buffer solutions and spiked plasma samples.

The linearity was achieved by AdSSW voltammetry in the range from 1.0×10^{-8} – 1.6×10^{-7} M and from 2.0×10^{-8} – 1.2×10^{-7} M at pH 5 and 8, respectively, with detection limits 2.72×10^{-9} M and 4.0×10^{-9} M, and limits of quantification 9.08×10^{-9} M and 1.33×10^{-8} M. The determination of VAR in protein free spiked human plasma samples showed much better results at pH 8 within linear concentration range from 5.0×10^{-8} – 1.4×10^{-7} M, with limit of detection 1.49×10^{-8} M, and quantification limit of 4.96×10^{-8} M.

The comparison of statistically obtained parameters for VAR in plasma samples spiked with Champix® tablets by applying UPLC method as a reference one, confirmed the high accuracy of investigated voltammetric method.

ELECTROCHEMICAL DETERMINATION OF VARENICLINE AT BORON DOPED DIAMOND AND GLASSY CARBON ELECTRODES

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Varenicline is a novel and selective nicotinic receptor partial agonist and is FDA approved for smoking cessation. Because of its mixed agonist-antagonist effects, varenicline is expected to reduce the psychogenic reward associated with smoking while also relieving nicotine craving and withdrawal symptoms in abstinent subjects.
The electrochemical study of varenicline (VAR) was done in a wide pH range (2-12), at boron doped diamond electrode (BDDE) and glassy carbon electrode (GCE), using cyclic (CV) and square wave (SW) voltammetric techniques. Depending on the pH and the type of the working electrode the characteristic electrochemical behavior of varenicline was established. It was shown that the use of BDDE electrode, in the case of varenicline, is a good alternative for studing the reduction processes in cathodic regia of potentials.

Based on the obtained results the new electroanalytical method was developed for its determination in buffer solutions and plasma samples. By applying square wave voltammetry (SWV) on BDDE and GCE, at pH 3.5 and 4.0, the linear dependence in plasma samples was achieved within the concentration range from 2×10^{-6} – 1×10^{-5} M and 4×10^{-6} – 1×10^{-5} M, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as 7.1×10^{-7} M and 2.4×10^{-6} M on BDDE, and 1.0×10^{-6} M and 3.5×10^{-6} M on GCE, respectively. The recovery and RSD values obtained for VAR in plasma suggested BDDE electrode to be preferable in comparison with GCE.

The accuracy of the voltammetric method was confirmed through determination of VAR in plasma spiked with Champix® tablets and the results were statistically compared with those obtained with UPLC method, as a reference one.

VOLTAMMETRIC DETERMINATION OF 5-NITROQUINOLINE USING CARBON FILM ELECTRODE

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5-Nitroquinoline (5-NQ) belongs to the group of nitrated aromatical heterocyclic compounds and is known as a cytotoxic compound [1, 2]. This contribution focuses on search of optimized conditions for determination of 5-NQ using modern voltammetric methods, namely differential pulse voltammetry (DPV) and direct current voltammetry (DCV) at a carbon film electrode (CFE). The advantages of carbon film electrode are primarily its wide potential window in both cathodic and anodic regions (cca 3 V span), high sensitivity and low noise of measurements. Another advantage of CFE is quickly and easily renewable surface of the electrode and also non-toxicity as compared to mercury electrodes [3]. Determination of 5-NQ at CFE is based on cathodic reduction of present nitrogroup. It was confirmed that it is possible to determine 5-NQ in concentration ranges of 0.4 to 100 μmol.l^{-1} with limit of quantification (LOQ) 1 μmol.l^{-1} using DC voltammetry and 0.2 to 100 μmol.l^{-1} with LOQ 0.4 μmol.l^{-1} using DPV. All of these measurements were made in medium of Britton-Robinson buffer pH of 11.0. This work has proven practical applicability of CFE for determination of submicromolar concentrations of 5-NQ based on cathodic reduction of present nitrogroup.

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NEW POTENTIOMETRIC SENSOR FOR THE DODECYLSULPHATE DETERMINATION

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Modern methods for the sodium dodecylsulphate determination are following: chromatography, extraction-spectrophotometric or extraction-spectrofluorometric techniques and potentiometry with ion-selective electrodes – each has its advantages and lacks. Chromatography are too expensive for daily use. Spectrophotometric and spectrofluorometric methods use toxic organic solvents. The most of known dodecylsulphate-ISEs have good sensitivity, but are complicated in manufacturing, have small time of a life and low selectivity.

The liquid plasticized membrane potentiometric sensor for the sodium dodecylsulphate determination was developed. Influences of different solvents and plasticizers were studied. The ionic associate of sodium dodecylsulphate and astrafloxine FF base was used as ionophore. PVC membrane was plasticized with dibutyl phtalate (DBP), dibutyl sebacate (DBS), dioctyl phtalate (DOP), dinonyl phtalate (DNP), tricresyl phosphate (TCP). Influences of conditioning time, solutions pH, composition of internal solutions and background electrolytes were investigated. Electrode response time kinetic studies were carried out. Coefficients of selectivity were calculated for more than twenty interfering ions. Electrode has a near-Nernstian slope: 56.7 – 59.0 mV per decade over the dodecylsulphate concentration range 10^{-5} – 10^{-1} M. The limit of detection is 9×10-6 M, pH working range: 2 – 11, response time: less than 10 s, electrode life time: more than 6 month. The electrode is suitable for definition of concentration of dodecylsulphate -ions in wide range of concentrations and can serve as a reliable sensor for the control of the anionic surfactants in natural and industrial targets.

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MONITORING OF ELECTROLYTE IMPURITIES IN OIL USING ELECTROMEMBRANE METHOD

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To ensure the apparatus monitoring of electrolyte impurities entering the turbine oil system at the cooling water leakage, is applied electromembrane method of extracting impurities from oil to the aqueous phase, followed by conductometric detection. Removing electrolytes impurities that are in the form of emulsified aqueous solution in the oil (inverse emulsion), is carried out in flowing two-chamber two-electrode cell, in which the controlled oil is separated from the aqueous phase by a hydrophilic membrane. Quantitative determination of electrolyte impurities in the oil is produced by the increment of the conductivity of aqueous solution after passing through a membrane cell.

Dialysis flow of ionic components from the inverse emulsion through the membrane is quite small, which does not provide the method for the analysis of ionogenic additives in oil. This may be due to the slow kinetics of water droplets transport to the membrane and their coalescence with a film of water on the membrane surface. The imposition of the normal electric field leads to intensification of electrolytes transfer from the emulsion to aqueous phase through the membrane. Besides the direct influence of electrodialysis transfer on the transport of ions across the membrane, electric field accelerates the process of coalescence of aqueous salt solution droplets with the aqueous phase film on the membrane surface due to their polarization. Also, do not exclude the influence of electric field on acceleration of the aqueous droplets transport in the membrane surface layer of the oil phase, where their convective transport becomes weak.

The known regularities of the process (the dependence of electrolyte impurities transfer by the electric field gradient and their concentration in the oil) allow us to apply electromembrane method for analysis of electrolyte impurities in turbine oil and for tightness control of heat exchange equipment.

THE INVESTIGATION OF THE ELECTROCHEMICAL REDUCTION PATHWAY OF 1-(4'-IODOPHENYL)-3,3-DIMETHYLTRIAZENE AND ITS VOLTAMMETRIC DETERMINATION

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1-(4'-iodophenyl)-3,3-dimethyltriazene (IPDMT) is a genotoxic substance that also exhibits cancerostatic activity. On the basis of voltammetric, coulometric, spectrophotometric and chromatographic measurements, in the present paper, an attempt was made to investigate the mechanism of electrochemical reduction of the studied triazene and to postulate a corresponding reduction scheme. It was established that, for reduction of the triazene group, four electrons are required, resulting in the formation of amino- and hydrazo- compounds, and the reduction pathway was postulated. The polarographic behavior of IPDMT was investigated and the optimum conditions were found for its determination by Sampled Direct Current and Differential Pulse Polarography at a Static Mercury Drop Electrode, as well as, by Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode and an Amalgamated Silver Electrode in the concentration range 1×10^{-4} to 1×10^{-7} mol L^{-1}. The sensitivity of the determination can be improved by preliminary adsorptive accumulation of the investigated substance on the surface of the Hanging Mercury Drop Electrode. Differential Pulse Adsorptive Stripping Voltammetry can be used in the concentration range 1×10^{-6} to 1×10^{-10} mol L^{-1}. It was found that for IPDMT, at an accumulation potential of -0.3 V *vs* Saturated Calomel Electrode, with a deposition time of 10 min, the limit of determination is 2×10^{-10} mol L^{-1}.

ELECTROCHEMICAL INVESTIGATION OF IRON-PHYTATE INTERACTIONS

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Phytate's structural, chemical and physical properties are the cause for its presence in many biological systems. Since its discovery has been thoroughly researched and applications in diverse fields have been found, such as nutrition, health and conservation sciences. Strong interactions of phytates (Phy) with many metals were found and have raised the interest in studies dealing with bioavailability of minerals. Because of this property it is often regarded as antinutritient, although the same property allows phytate to act as an antioxidant. It is known that phytate forms with Fe(III) strong complexes and characterization of either chelates in solution or solid precipitates is a complex task, that is not yet fully resolved and understood. Many studies were reported on iron(III) complexation by phytate studied by ^{31}P NMR, potentiometric or spectrophotometric methods, but only a few have used voltammetric measurements.[1-3] Recently Wang et. al. has shown that phytic acid can be used for layer-by-layer assembly for fabrication of mesoporous gold film and its biosensor application.[4] We have conducted a series of experiments using polycrystalline gold electrode (PGE) and hanging mercury drop electrode (HDME) as working electrodes, since only this electrode enable identification and characterization of very strong Fe(III)-Phy complexes in solution. Using different pH values and phytate to iron(III) ratios for the solutions, we could deduce that the soluble species in solution involve more than one molecule of phytate per iron(III). It is known that in phytate-iron precipitates up to four iron(III) ions per phytate are found,[1] but if phytate is in excess precipitates completely disolve at phytate to iron(III) ratios around 6.

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DEVELOPMENT OF CARBON NANOTUBE MODIFIED MEDIATOR BIOSENSOR FOR REAL TIME MONITORING OF BLOOD GLUCOSE LEVELS IN FISH

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We have been developing biosensors for continuous blood glucose measurements in fish using the inner scleara fluid of the fish eyeball (EISF). Blood glucose concentration of EISF is higly correlated to blood glucose concentration of blood. However, these sensors used glucose oxidase (Gox), oxidizng enzyme, and therefore change of oxygen levels corresponding to condition of fish were of concern. To solve this problem, we developed mediator type biosensor: this sensor was constructed by applying ferrocene, chitosan, single-walled carbon nanotube (SWCNT), and polyethylene glycol (PEG) to the sensing cavity. A needle-type biosensor was prepared using a platinum iridium wire as the working electrode and Ag/AgCl paste as the reference/counter-electrode. SWCNT was mixed with 5% Nafion solution and the sensing cavity was first dipped in Nafion solution to form Nafion layer. Ferrocene derivative was modified to chitosan (Chit-Fc) and was mixed with Gox. PEG was also added and the final cocktail was applied to the sensing cavity (Nafion-SWCNT/Chit-Fc/Gox-PEG sensor). Nafion-SWCNT/Chit-Fc/Gox-PEG sensor with 30mg ml^{-1} of SWCNT showed good linear correlation between the sensor output current and concentration of glucose standard in the range of 3.32~400mg dl^{-1} (R=0.9987). This sensor was also compared with sensors without SWCNT and PEG, and attained higest sensitivity after seveny two hours of continous measurement in standard solution. Finally, *in vivo* measurement was perfromed. Constructed sensor was implanted in to EISF and as a result, real time monitoring was made successfully for total of seventy eight hours.

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VOLTAMMETRIC DETERMINATION OF LEAD, CADMIUM AND ZINC IN PLANTS

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In this work, the investigations of voltammetric determination of heavy metals (lead, cadmium and zinc) in plants, were described. Two types of plants were selected: accumulating plants and plants used in nutrition. Efficiency of heavy metal extraction was determined by measuring concentration of lead, cadmium and zinc before and after the sorption process by *armeria maritima* (from the soil) and *white willow* (from water). Impact of heavy metals on living organisms (humans and animals) was determined by measuring the concentration of lead, cadmium and zinc in *spinach*, cultivated on the territory of Kosovo and Metohija as well as in the *biomass of natural lawns*.
For metal determination voltammetric technique was applied – potentiometric stripping analysis (PSA). Lead, cadmium and zinc from previously dilapidated plant samples were determined as follows:

- By metal extraction due to reduction on previously formed working electrode. Working electrode is formed by depositing thin film of mercury on a glassy carbon surface under the current of 48,90 μA from the solution of mercury(II) ions.Extraction of metal on working electrode was realized at potentials of: -0,999 V for lead, -1,106 V for cadmium and -1,350 V for zinc. All the three metals were extracted simultaneously at the applied potential of -1,400 V; and
- By returning metal into the solution due to oxidation when the potential of the working electrode practically remained constant until the total amount of collected metal is oxidized. After the completed oxidation of one element, the potential of working electrode increased up to the characteristic potential at which the oxidation of another metal occured. Metal concentration is determined after the oxidation by standard addition method.

Results of this research show that *armeria maritima* may accumulate up to 1,8% of lead for the content less than 20 μg dm^{-3}, *white willow* 30,3% of lead, 53,2% of cadmium and 3.9% of zinc. Lead content in *spinach* was determined by PSA method with variation of 4%, while during simultaneous determinations in *biomass of natural lawns*, the variations were: 4,58% for lead, 1,91% for cadmium and 1,89% for zinc.

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INFLUENCE OF PROTOLYTIC EQUILIBRIA ON ELECTRO-OXIDATION POTENTIALS OF ELLAGIC ACID IN AQUEOUS METHANOL MEDIUM

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Ellagic acid is plant-derived polyphenol found in a wide variety of fruits and nuts such as raspberries, strawberries, walnuts, grapes, and black currants. It is the major phenolic constituent present in distilled beverages. It exhibits antimutagenic, antioxidant, and anti-inflammatory activity in bacterial and mammalian systems. The protective effects of ellagic acid are often attributed to antioxidant activity. Electrochemistry provides powerful tool for the study of redox reactions. However, the voltammetric methods have not been used for the analysis of ellagic acid, so far. We studied the electro-oxidation of ellagic acid at various pH in methanol-water medium using cyclic voltammetry (CV) on the glassy carbon (GC) electrode. Acidity constants (pK_{a1} and pK_{a2}) were potentiometrically determined and UV/VIS spectrophotometry was used for pK_a values evaluation.

The cyclic voltammetry study of eallagic acid (0.2 mM) was carried out in pH range 2–9 (methanol:water=1:1, *v/v*) at t=25±1 ºC. In acidic media, the oxidation of ellagic acid is a two step process (two peaks), but peaks are almost completely overlapped. The separation of peaks 1 and 2 was much better in the second scan. The oxidation potentials of both peaks depend on pH. At higher pH values (>5) the peak 2 E_p value becomes pH independent, which implies that protons no longer participate in the oxidation process. This result is in accordance with potentiometrically determined acidity constants (pK_{a1}=5.42±0.01 and pK_{a2}=6.76±0.01). According to heat of formation and electron densities calculated on semiempirical level (MOPAC 2007, MNDO-RM1 Hamiltonian) dissociation scheme was proposed. The behavior of ellagic acid was also spectrophotometrically studied and obtained results are in a good agreement with experimentally determined pK_a values.

ELECTROCHEMICAL BEHAVIOUR OF A SERIES OF AMINO ACIDS, AMINOALCOHOLS AND BIOGENIC AMINES: CORRELATION WITH THEIR ANTIOXIDANT ACTIVITY

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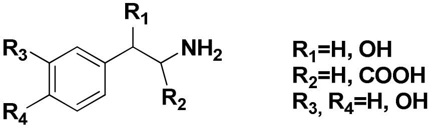
Aerobic organisms form Reactive Oxygen Species (ROS) as an unavoidable consequence of cell metabolism. The defense mechanisms that the organisms use against these highly reactive species include enzymes and naturally occurring antioxidants. However, the antioxidant activity of the physiological mechanisms can prove inefficient when the organism suffers from excessive ROS production, as in the case of many pathological conditions. As part of our research toward natural and synthetic compounds that display important antioxidant activity, the antioxidant properties of selected amino acids, amino alcohols and biogenic amines, which will be further exploited as starting materials for the synthesis of a variety of analogues, were investigated.

The series of compounds presented in this study include the amino acids phenylalanine, tyrosine and 3,4-dihydroxyphenylalanine (L-DOPA), the biogenic amine tyramine, a natural product produced by the enzymatic decarboxylation of tyrosine, phenethylamine and its derivative 2-amino-1-phenylethanol. These compounds possess the common structural feature of phenethylamine and differ on the substitution of the phenyl ring and the alkyl chain and are all reported to exhibit a variety of biological activities.



In this work, the electrochemical behavior of the compounds is studied using cyclic voltammetry (CV), a technique that can provide an insight on the mechanism of the electrochemical oxidation of the molecules under investigation. In addition, the evaluation of the antioxidant activity of these compounds using different antioxidant protocols, namely the luminol chemiluminescence assay, which evaluates the ability of the compound to scavenge H_2O_2 and the Ferrous Reducing Antioxidant Power (FRAP) assay is presented. The correlation of the oxidation potential of the investigated compounds with their antioxidant activity, as well as the relationship among structural characteristics, antioxidant activity and electrochemical properties will be discussed.

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MODERNISATION OF POST-GRADUATE STUDIES IN CHEMISTRY - AN EXAMPLE OF TEMPUS PROJECT ACTIVITIES

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With the ever increasing progress and achievement in science and technology it is evident that higher education is under pressure to continue to produce new generations of highly skilled individuals who will be capable of contributing further intellectual and technical advances in the 21st century. Therefore, higher education (HE) systems around the globe are facing an enormous challenge to develop programmes that will help produce such scientific graduate leaders and the necessary new generations of scientists and technologists.

To further this aim of developing modern scientists who are competitive on a world stage we have created a joint-project, funded by the European Union's TEMPUS programme, which aims to respond to current societal needs to develop and modernise existing Chemistry programmes in Serbia with a view of making programme outcomes consistent with best practice in the rest of Europe. To achieve this aim the following objectives and work programmes have been formulated:

- Revisit current benchmarking statements and align them with 21st century needs.
- Modernisation of the HE quality assurance (QA) system.
- Staff development – both pedagogical and scientific.
- Implementation of modern technologies in teaching practice.
- Aligning assessment criteria and methodology with new teaching strategies.

Higher education modernisation is often driven by its desire to establish the most effective ways of delivering teaching and learning. When talking about modernisation of curricula in the 21st century we often think about the use of interactive boards, public response systems and virtual learning environments. Although, it is evident that computer aided teaching and learning processes are often dominant, implementation of new teaching strategies is often dictated by:

- An effective understanding of how learners learn (teaching theories).
- The desired learning outcomes (stake holders input)
- Available tools (technologies).
- Latest scientific discoveries (research informed teaching).

SPECIAL LABORATORY COURSE FOR CHEMISTRY STUDENTS INTENDING TO GET JOB IN INDUSTRY

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Beside Industrial chemistry course for all chemistry students at our Faculty, another special course was developed in the fourth (last) year of studies (B. Sc) for undergraduate students intending to find job in industry. The practical work (six hours per week during one semester) was mostly based on small research projects with a lot of problem solving activities. During the projects students were working individually and as a team. Within the projects students were combining their previous knowledge and skills in analytical chemistry (gravimetric, volumetric and spectrophotometric determinations) with some new information from the literature. Several projects included complete qualitative and quantitative analyses of food (milk, flour) or complex commercial products (putty, sealing wax, scouring powder). The emphasis was on selection of suitable method and the interpretation of data. The programme of the course included teaching/learning about quality system based on international ISO 9000 standards in chemical laboratories, certified reference materials, interlaboratory studies and accreditation of laboratories. Information about various local and international activities were also mentioned: VAM initiative (Valid Analytical Measurement, UK), EURACHEM (A Focus for Analytical Chemistry in Europe), IMEP (International Measurement Evaluation Programme), CITAC and IUPAC. The short video “Let’s agree to agree” commissioned by the Laboratory of the Government Chemist (UK) was found both interesting and useful as teaching aid. Two round interlaboratory study was simulated in the laboratory. Each student was in the role of participating chemist and had to determine the content of chloride in river water. Very good agreement of results was obtained. Students also learned about various methods for analysis and evaluation of results of interlaboratory studies.

MULTIVARIATE ANALYSIS: TEACHING OF PRINCIPAL COMPONENT ANALYSIS BY USING DATA FROM AIR QUALITY CONTROL NETWORK OF THE MADRID REGION

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The main objective of the work is the use of computational and chemometric tools and the integration of knowledge in chemistry to face up the complexity of questions in the environmental area. Starting from a priori knowledge and multivariate nature of the data collected in the "Air Quality Control Network" of the Community of Madrid, chemometrics appeared as a powerful tool in providing information needed for drawing conclusions concerning the air quality and the influence of several climatological parameters on air pollution (i.e. wash out effect of rain or solar radiation and photochemical tranformation).

In this work, several groups of students collected information about chemical and meteorological parameters of 23 stations, located in several towns and villages of the Madrid Region, throughout a month. These stations get measurements every hour 24 hours a day. Data are presented as text file in http://gestiona.madrid.org/aireinternet/html and all this information is transformed in large data sets after elimination of "non valid" responses. The transformation of high-dimensional feature space to the space defined by a few factors was used for multidimensional data graphical representation. Influences of the variables were discussed through their significant loadings on extracted factors. These factors are related to the sources of the parameters in the studied stations. Additionally, as pattern recognition method, cluster analysis was also applied. It proved useful for finding groups within the observed dataset. All stations provide information about amounts of NO ($\mu g/m^3$), NO_2 ($\mu g/m^3$), suspension particles ($\mu g/m^3$) and ozone ($\mu g/m^3$), some of them CO ($\mu g/m^3$), SO_2 ($\mu g/m^3$), hydrocarbons ($\mu g/m^3$), benzene ($\mu g/m^3$), xilene ($\mu g/m^3$), toluene ($\mu g/m^3$) as well as climate data as temperature (ºC), air humidity (%), direction (Grd) and speed of the wind (m/s), atmospheric pressure (mbar), solar radiation (W/m^2) and precipitation (l/m^2). The quality of the air can be related not only to climatological parameters but also to population and industrial activities. Data about demographic parameters and the socioeconomic activity can be obtained from http://www.madrid.org/iestadis/fijas/estructu/demograficas/censos/icenso01.htm.

ANALYTICAL CHEMISTRY IN RUSSIAN UNIVERSITIES: REFORMS ARE STARTING

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At the beginning of the second decade of the 21st century all Russian Universities (RU) are obliged to start fulfilling the directives of the Russian ***Ministry*** of ***Education*** and ***Science*** in accordance with the Bologna Process. In autumn 2011 all entrants of the Universities will begin to study according to the novel standards and programs which have been under discussion and preparation for several years. The majority of RU will implement the two-tier system of studies, while some of them, and Moscow State University (MSU) especially, is permitted to continue to graduate specialists. However, the specialist's course at MSU will take 6 years instead of 5; besides, MSU has got programs and licenses for awarding master's degrees for the bachelors graduated from other universities.

According to the novel plans, the basic course of analytical chemistry (AC) will be taught at the 2nd year during the same number of hours (684), while specialization in AC will take almost twice more hours (936) than now (540). Such plan widens the possibilities of the AC divisions to give the students more knowledge in different areas of AC, and many new special courses are planned to be taught.

Nowadays chemistry is not as popular among the young people as it was 40-50 years ago. So RU, and MSU especially, take great efforts to attract talented schoolchildren and stimulate them to enter chemistry departments; various chemistry olympiads for schoolchildren are organized which facilitate the winners to enter the leading Russian universities. Different divisions of the chemistry departments compete with each other for best students. They organize the so called "Open Days" to show the students their research laboratories with modern equipment, tell about the most interesting achievements, etc. In this respect the AC divisions have great advantages as their professors can demonstrate good prospects of student’s participation in solving real problems of chemical analysis and tell about successful work of future analysts in the most powerful companies, analytical centers, laboratories, etc.

The activities of different AC divisions under the modern conditions are coordinated by the Commission for Teaching AC of the Scientific Council on AC of Russian Academy of Sciences. This commission promotes the co-operation between AC divisions, organizes conferences on topical problems of teaching AC, accumulates and disseminates the experience of various universities, develops the recommendations related to various aspects of teaching, etc.

A VIDEO DEMONSTRATION RELYING ON A FLOW INJECTION SYSTEM: COMPARISON OF STABILITY OF SOME CUPRATE(II) COMPLEXES

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A didactic and simple flow injection system[1] is proposed for a class demonstration. To this end, a webcam device is attached to the flow-through cuvette allowing images of the flowing sample to be real-time projected on a large screen. The demonstration involves in-line formation and monitoring of different colored cuprate(II) complexes. By taking into account the typical coloration of the projected images, it was possible to classify the complexes according to the sequence of their stability constants[2]. The cuprate(II) complexes involving water, ammonia, EDTA and DDTC, as ligants were selected. The solutions 0.1 mol L^{-1} Cu, 0.03 mol L^{-1} NH_3, 0.1 mol L^{-1} EDTA and 0.02 mol L^{-1} DDTC were inserted into the carrier stream 0.1% v/v HNO_3 by means of solenoid valves. Concepts of analytical selectivity and sensitivity are discussed. The flow system is rugged and portable, requiring minute amounts of solutions.

The stability of a complex is related to the metal ion involved and the characteristics of the ligand, that was confirmed by the formation of different complexes according to the colours associated with combinations of the involved solutions.

The inherent characteristics of the proposed flow system, especially the portability and low consumption of solutions are appropriate for its use in didatic video demonstrations. This feature makes it attractive to classes with many students due to the visibility of the projection of the images. Moreover, better student / teacher interaction can be achieved.

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ASSAYING THE DIFFERENT ASPECTS OF RED WINE

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The development of more effective and efficient methods to assess of grape and wine quality is important to the wine industry. It is desirable that these methods require minimal sample preparation and are able to produce rapid results, preferably providing information on multiple parameters simultaneously. The aim of this study was to investigate the use of Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy in the assessment of wine quality. Artificial Neural Networks were chosen as the modeling tool to correlate measured FTIR spectral data of wine samples with the following experimentally determined parameters: total phenolic content, total acidity, pH and alcohol content. A total of 20 (2 whites and 18 reds) different wines were used in this study. These came from three different states across Australia; New South Wales, Victoria and South Australia.

The total acidity of the wines ranged from 4.45 to 7.04 g/L, pH from 3.25 to 3.88, acetic acid concentration from 0.02 to 1.54 g/L, and polyphenolic content from 0.17 to 3.06 g/L. Within the FTIR fingerprint region, several absorption bands were identified as functional groups representative of those present in phenols. A plot of the values predicted by the validated ANN models showed excellent correlation with the experimentally measured values (r = 0.898-0.924). This work demonstrates the use of FTIR spectroscopy is a promising technique and provides a rapid and nondestructive method for the assessment of wine quality, with minimal sample preparation.

ANALYSIS OF CAROTENOIDS, VITAMINS AND FOLATES IN TRADITIONAL FOODS FROM BLACK SEA AREA

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Nowadays, consumers are much more aware of nutritional composition and show especially interest in compounds with putative health benefits. Therefore, in the last few years, great attention has been devoted to the study of bioactive compounds in order to promote the consumption of traditional foods.

The European project BaSeFood (Sustainable Exploitation of Bioactive Components Black Sea Area Traditional Foods) aims to study the traditional foods from the Black Sea Area, namely their nutritional and bioactive composition. In the frame of this project, the present work has analysed carotenoids, vitamins and folates in 33 traditional foods.

Carotenoids and retinol and α-tocopherol were extracted from samples with hexane/ethanol (4:3, v/v) and quantified by Ultra-high Pressure Liquid Chromatography (UPLC®) with diode array detection (DAD). Some samples required saponification with methanolic KOH prior to chromatographic analysis. In general, the most abundant carotenoid was β-carotene. Most of the samples contain α-tocopherol and do not present retinol.

In order to quantify L-ascorbic acid, samples were stabilized with perchloric acid and metaphosphoric acid in ultrapure water. After dilution with mobile phase, samples were quantified by High Performance LC (HPLC)-DAD. One of the samples with highest L-ascorbic content was fruit of the evergreen cherry laurel (29.7 mg/100 g).

Vitamin B_2 was extracted after acid hydrolysis followed by dephosphorylation and HPLC with fluorescence detection (method EN 14152:2003, accredited according to ISO 17025). The determination of the total folate content in foodstuffs was carried out by a microbiological assay (EN 14131:2003, accredited according to ISO 17025). One of the highest vitamin B_2 and total folate levels was found for roasted sunflower seeds (0.19 mg and 113 µg per 100 g of edible portion, respectively).

Acknowledgement

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**A full list of Black Sea area partners is available at the BaSeFood website* *http://www.basefood-fp7.eu/*

FA03 **Session A**

BIOACTIVE PHYTOSTEROLS AND FATTY ACIDS PROFILE OF TRADITIONAL FOODS FROM BLACK SEA AREA COUNTRIES

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Phytosterols (PS) are abundant in foods of plant origin and vegetable oils. These compounds have received particular attention due to their capability to lower serum cholesterol levels, resulting in significant reduction of the risk of heart disease. Also, the consumption of fatty acids (FA) is important because it can be associated with both negative and beneficial health effects, depending on the FA. This work was performed within the collaborative research program Sustainable exploitation of bioactive components from the Black Sea Area traditional foods (BaSeFood), funded by the European Commission. Traditional foods from Black sea region are presently being studied for their potential positive effects on human health, especially focusing on its bioactives compounds. The aim of this study was to analyse the bioactive PS, total fat and FA profile of 33 traditional foods from six Black Sea Area countries.

Total fat analysis was performed by means of an acid hydrolysis with Soxhlet extraction (AOAC 948.15, 2000, accredited method according to ISO 17025). Preparation of FA methyl esters was carried out by a combined method of methylation and transesterification. For PS content, preliminary assays were done in order to establish the optimal extraction conditions. Chromatographic analysis was performed with a gas chromatograph equipped with a mass spectrophotometer detector. A HP-88, fused silica capillary column (100 m x 0.25 mm i.d., 0.25 mm) was used for FA determination and DB-5 MS (30 m x 0.25 mm i.d., 0.25 µm) was used for PS analysis. Samples were analyzed in triplicate. The GC-MS method allows separation and identification of more than fifty FA, including some *trans*-FA and ω3 or ω6 FA.

Great variability was found in the total fat content of samples. For instance, rose jam total fat content was 0.02 g, while for roasted sunflower seeds was 58.2 g. Results are given per 100 g of edible portion. A wide range of fatty acids profile was also found.

Acknowledgement

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n.º 227118.

**A full list of Black Sea area partners is available at the BaSeFood website http://www.basefood-fp7.eu/*

PRELIMINARY STUDY OF BIOGENIC AMINES CONTENT IN CHILEAN RESERVE WINES

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Biogenic amines are well known organic nitrogenous compounds with low molecular weight; they are generally formed by bacterial decarboxylation of free amino acids. In the case of wines this kind of amines are mainly formed during malolactic fermentation. Biogenic amines are a health risk for sensitive individuals producing varied symptoms. The objective of this work was to determine the biogenic amine content in Chilean reserve wines. An HPLC method with pre-column dansylation was optimized and validated to accomplish a reliable quantification of histamine, tyramine, spermine, spermidine, putrescine, cadaverine and phenylethylamine in Chilean reserve wines. Using a central composite design derivatization and chromatographic conditions were optimized. Separation was performed on a C_{18} YMC-Pack ODS-A (150 mm x 4.6 mm, 5 µm) column at 45°C, using a binary mobile phase in gradient composed of methanol and water at a flow rate of 1.2 mL/min. Detection was performed at λ_{ex} 330 nm and λ_{em} 520 nm. Validation was established according to ICH guidelines, calibrations in matrix fitted a linear regression model with $R^2>0.998$. Repeatability (n=6) and intermediate precision (n=3) in matrix showed RSD values of 4.2-5.8% and 6.8-9.1%, respectively. Using this optimized and validated method sixty Chilean reserve varietal wines of the most important grape varieties were analyzed, i.e. Cabernet Sauvignon (n=11), Merlot (n=11), Carménère (n=11), Syrah (n=10) and Sauvignon Blanc (n=10). Additionally, organic wines were also analyzed (n=7). Total biogenic amines content ranged from 2.2 to 65.1 mg/L. No significant differences ($P>0.05$) were observed between Cabernet Sauvignon (35.6±14.5 mg/L), Merlot (38.9±9.9 mg/L) and Carménère (38.8±11.7 mg/L) but all showed statistically higher ($P<0.05$) biogenic amines concentrations than Sauvignon Blanc (8.8±4.7 mg/L). Syrah (23.2±7.1 mg/L) showed higher concentrations than Sauvignon Blanc ($P<0.05$), lower than Merlot and Carménère ($P<0.05$) and not significant different content than Cabernet Sauvignon ($P>0.05$). Organic wines (27.2±5.2 mg/L) showed statistically comparable concentrations ($P>0.05$) with Cabernet Sauvignon, Merlot and Carménère, and higher than the Sauvignon Blanc ($P<0.05$). Even when some samples showed high values, they are still very far from the proposed toxic level of 100-225 mg/kg via oral intake. Nevertheless, attention should be paid since other authors indicated slight poisoning with histamine levels of 8-40 mg, even at concentrations of 0.4 mg/L.

PRELIMINARY STUDY OF TEA FUNCTIONALLY REGARDING L-THEANINE CONTENT IN CHILEAN MARKET

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Tea infusions are one of the most consumed beverages in the world. One of the tea compounds responsible for some beneficial effects is the amino acid L-theanine, which presents an important capacity to improve the brain ability to concentrate, learn and memorize. Tea infusions could be considered as functional beverage with respect to L-theanine if an adequate number of cups of tea have the necessary content to produce the desire effect. The objective of this work was to evaluate the functionality of teas marketed in Chile regarding its L-theanine content. An HPLC/UV method was optimized and validated in order to quantify L-theanine content in tea infusions. Chromatographic conditions were optimized using a face-centered central composite design, obtaining the following optimal conditions: mixture of water and phosphoric acid (99.9: 0.1% v/v) as mobile phase, flow rate of 0.3 mL min^{-1} and column temperature of 37ºC. Separation was carried out with the above conditions using a C_{18} Purospher Hibar-STAR (250 mm x 4.6 mm, 5 µm) column detecting L-theanine at 210 nm. Validation was established according to ICH guidelines, calibration in matrix fitted a linear regression model with R^2=0.9902. Repeatability (*n*=6) and intermediate precision (*n*=3) in matrix showed RSD values of 4.5% and 1.3%, respectively. Considering the work objective, only the most representative tea brands were analyzed (80% of the market). From nine tea brands, 18 samples (14 black and 4 green teas) were analyzed in triplicate, showing a L-theanine content from 4.2 to 24.8 (2.2 to 12.4 mg g^{-1}), with a mean value of 10.1 mg in 200 mL (5.0 mg g^{-1}). Considering the effective dose reported, only one brand (black tea) presented an adequate L-theanine content to produce the desired effect (two serving cups). Therefore it is possible to conclude that most teas marketed in Chile have not an adequate L-theanine content to be considered as functional food regarding this amino acid.

FRUIT JUICES AND TEA/DIETARY SUPPLEMENTS: ANTIOXIDANT PROPERTIES AND LABEL INFORMATION

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Tea infusions and fruit juices are appreciated all over the world due to their organoleptic characteristics and antioxidant properties. Their complex composition include a mixture of bioactive compounds with different complementary mechanisms of action, providing a greater protection against free radical damage, than any single molecule [1].

Food industry provides several of these products claiming health properties. However, labels are usually not enough informative about this topic.

The aim of this work was to analyse total phenolics, total flavonoids, vitamin C and antioxidant activity (using the DPPH assay) of several commercial juice samples (*n*=10) and teas/dietary supplements (*n*=9).

A great variability was found between the juice samples, probably due to the different fruits used to prepare each commercial beverage. Moreover, juices without antioxidant claims in the label presented similar values to those with it. Within juice samples, the highest contents of total phenolics (including flavonoids) were found in mixtures with tea. Juices containing blackberry and raspberry presented the lowest vitamin C levels. In what concerns to teas/dietary supplements, a sample composed by a blend of green tea, pineapple and hibiscus presented the highest content of phenolic compounds. In a general way, the antiradical activity was highly correlated with the phenolics content.

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SPECIATION OF ARSENIC SPECIES PRESENT IN RICE SAMPLES BY ION CHROMATOGRAPHY

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The speciation of arsenic species commonly found out in the rice (*Oryza Sativa*) samples using the ion chromatography was carried out employing a solution containing 0.0019 mol L^{-1} Na_2CO_3 / 0.0024 mol L^{-1} $NaHCO_3$ / 0.001 M $Na_2B_4O_7$, pH 10, as eluent, as proposed by Ricci et al[1]. The following arsenic species were studied: arsenite, arsenate, dimethyl arsenate (DMA) and monomethyl arsonate (MMA). In order to evaluate the retention time of each arsenic specie they were first eluted separately. Then, standard solutions containing all the arsenic species studied in the following concentration levels: 20, 50 and 100 mg L^{-1} were used to verify if the retention time observed for the species remain constant. In fact, the peaks corresponding to each one of arsenic specie are shaped and well resolved, except for arsenate ion, and the total retention time required for elution of all arsenic compounds was about 20 min. Then, the separation condition was evaluated in order to obtain the effective separation of arsenic species. Thus, Na_2CO_3 was studied in the range concentration of 0.0019 to 0.004 mol L^{-1} keeping the concentration of 0.0024 mol L^{-1} $NaHCO_3$ and 0.001 mol L^{-1} $Na_2B_4O_7$ constant. In this first study, it was verified that the eluent containing 0.0035 mol L^{-1} Na_2CO_3 / 0.0024 mol L^{-1} $NaHCO_3$ / 0.001 mol L^{-1} $Na_2B_4O_7$ associated to the 0.70 L/min of flow rate allowed the effective separation of arsenic species in about 10 min. The equipment used in this study was the Suppressed Ion Chromatography, model 761 SD Compact IC, Soft Drink, Metrohm, coupled to the conductivitymeter detector, which justifies the higher concentration level employed for the standard solution of arsenic species. The column used was the Metrosep A Supp 5 150/4.0 mm anion exchange, with quaternary ammonium groups (Methrom).

Fapesp, INCTAA, CNPq

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AN UNTARGETED APPROACH APPLIED TO THE CLASSIFICATION OF SPANISH WINES

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Quality and organoleptic properties of wine are influenced by several factors. Amongst them, origin, grape variety, ageing, oenological practices and vintage play a key role. Because these factors are usually also related with the final price of the products, there is an increasing interest in relating physicochemical properties of wine with oenological features. Commonly applied approaches such as sensorial assays, which are normally established by a group of expert pannelists, could be unpractical when dealing with a large amount of samples. Alternatively, compositional profiles of naturally occurring wine components can be used for compositional purposes. Typical characterization of wines includes analysis of organic acids, volatile compounds, polyphenols, metals and biogenic amines. However, the myriad of compounds present in wines, including several hundred that have not yet been fully identified, suggests that discrimination of wines will be better accomplished when data coming from different families is simultaneously taken into account [1].

Combining data from different sources, such as UV and fluorescence detectors may result in higher performances when building classification models. Hence, this communication describes the application of a HPLC-DAD-F method [2], to classify wines according to oenological practices. This is accomplished using an untargeted approach and unsupervised (PCA) and supervised (PLS-DA) methods. Both methods allowed the classification and discrimination of Spanish wines according to their appellation.

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CHARACTERIZATION OF WINES BY ANALYSIS OF POLYPEHONOLS USING CAPILLARY ELECTROPHORESIS AND CHEMOMETRIC TECHNIQUES

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Moderate consumption of wine has been associated with reduced risk of cardiovascular disease and cancer, as well as with several beneficial effects on the immune system and cognitive functions [1]. These health-promoting properties have been correlated with the presence of phenolic compounds, in particular flavonoids and some stilbene derivatives such as resveratrol. Other phenolic compounds, such as phenolic acids and catechins play an important role in wine quality, contributing in flavor and color properties, especially on red wines [2]. The quantification of these active substances using reliable analytical methods and the evaluation of the factors affecting the composition are considered at the moment a priority.

Capillary electrophoresis [3] together with high performance liquid chromatography (HPLC) [4] with UV detection or coupled to mass spectrometry (LC-MS) are the most common techniques for wine analysis. However, in most of these works no more than 10 polyphenols are usually analyzed.

A capillary zone electrophoresis (CZE) method using UV detection for the separation and determination of 20 polyphenols in wine samples has been optimized using factorial design. As a background electrolyte a 30 mM sodium borate buffer solution at pH 9.2 containing 5% isopropanol was used. Quality parameters such as limits of detection (0.3-2.5 mg/L), linearity (r>0.998) and run-to-run and day-to-day precisions (RSD values lower than 15%) were established.

The aim of this work is to use the previously CZE optimized method for the analysis of the polyphenol to characterize wines. Chemometric methods such as principal component analysis, linear discriminant analysis and cluster analysis were used in order to determine if there is a connection between wine polyphenol content and the origin of the wine samples.

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INTRAREGIONAL CLASSIFICATION OF SOUTH AFRICAN WINE ACCORDING TO ORIGIN BY ICP-MS AND MULTIVARIATE STATISTICAL ANALYSIS.

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In recent years much progress has been made in South Africa and elsewhere in wine authentication through fingerprinting techniques, in particular provenance determination, to combat fraudulent practices such as label and blending fraud. Results indicate that wine fingerprinting methods based on multivariate statistical analysis of trace element data and isotope ratio data for selected elements, can successfully be applied to classify wines from different countries and from different regions from within one country. In our recent[1-3] work we have demonstrated that the interregional classification of South African wines from the four major wine-producing areas is possible and also verified the link between the trace element composition of the wine with that in the provenance soil.

In current work we have investigated the intraregional classification of wines from one region in order to establish the limits and reliability of the application. The main objective was to ascertain whether differentiation was possible at ward level or even at the estate level using trace element and $^{11}B/^{10}B$ isotope ratio data. The smaller the geographical demarcation the higher the demands will be on the accuracy and precision of the analytical data obtained by ICP-MS for successful classification. Ultimately the trace element distribution in the soils of the different wards and estates within wards will be the determining factor. Our results for the intraregional classification of wine from the Stellenbosch region suggest that classification of wines from different estates is indeed possible in the Stellenbosch region using multivariate statistical analysis based on a combination of principal component, cluster and discriminant analysis.

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QUANTIFICATION AND EVALUATION OF *IN VITRO* BIOACCESSIBILITY OF MICRO AND MACRO ELEMENTS IN FRUITS, VEGETABLES AND CEREALS

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Determinations were performed to evaluate the bioaccessibility (using an *in vitro* test) and the total amount of seven essential elements (Fe, Cu, Zn, Na, K, Ca e Mg) in fruits (banana, apple, cupuaçu and soursop), vegetables (lettuce and basil) and cereals (rice, corn, amaranth and quinoa), more and less known by the Brazilian population in general. It was found that the total and bio-accessible amounts of macro elements (Na, K, Ca and Mg) were higher than the corresponding quantities for microelements (Fe, Cu and Zn), in agreement with the nutritional needs of these elements in human diet. The quantities of bioaccessibility (in mg of element/100 g food) showed that the lesser-known foods have nutritional relevance with respect to micro and macro elements determined. Among the elements studied, potassium had the highest (90.8 % in average) and sodium showed the lowest (22.1 % in average) bioaccessibility in food. The vegetables had considerable amounts of calcium bio-accessible (371 ± 8 for lettuce and 822 ± 17 for basil, in mg of Ca/100 g of food), showing that they are important sources of calcium for the human diet. In general, amaranth and quinoa showed the lowest bioaccessibility for the elements analyzed (values reaching 3.57 % for Zn in the quinoa and 7.32 % for Ca in amaranth, percentage of bioaccessibility), and this fact can be correlated to the high phytate content (which have complexing ability) present in these grains.

THE INVESTIGATION OF TRACE METALS CONTENT AND STABLE ISOTOPES COMPOSITION ON THE SOIL-LEAVE-FRUIT CHAIN FROM SOME TRANSYLVANIAN AREAS

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Geographic identity of fruits is important because many consumers consider that the quality of these is related to the geographic area in which the fruit was grown. This is true, because each geographic area has its own climate, soil and water resources that affect specific plant maturation and quality of fruits.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a multielement technique suitable for the analysis of different matrix (water, soil, plant, juice, wine) in semiquantitative mode providing high selectivity, sensitivity and lower detection limits than other multi-element techniques. A simple and fast method for the determination of metals in soil, leave, fruit juices samples, by ICP-MS after digestion with HNO_3 ultrapure, HF, ultrapure water of samples is proposed.

Analyses of carbon isotopic ratios ($\delta^{13}C$) in soil organic matter (SOM) provide insights into dynamic of carbon cycle and it is specific for each area. Plants discriminated against $^{13}CO_2$ during photosynthesis and the extent of this discrimination is dependent on their photosynthetic pathway type (C_3, C_4, or CAM). Soil influences tend to be expressed indirectly through features such as heat retention, water holding capacity and nutritional status.

A preliminary study related the existing correlation among isotopic signatures and trace metals content on the soil-plant-fruit chain from different Transylvanian areas, to highlight the particularities differences among different places from isotopic viewpoint, is presented in this work.

A COMPARISON OF THREE EXTRACTION METHODS TO DETERMINE OCHRATOXIN A IN BREAD SAMPLES

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The nearly ubiquitous consumption of cereals and cereal derivatives gives these food products a very important position in human nutrition. In Portugal, one of the most consumed cereal derived products is bread. It is usually consumed in a daily basis and, sometimes, in considerable amounts by most part of the population.

The worldwide contamination of food and feed with mycotoxins poses a significant health problem. Ochratoxin A (OTA), chemically known as *N*-{[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl]-carbonyl}-3-phenyl-L-alanine, is a mycotoxin mainly produced by *Penicillium verrucosum* and some *Aspergillus* species (e.g. *A. ochraceus*, *A. carbonarius* and *A. niger*). The International Agency for Research on Cancer has classified OTA in group 2B as a possible carcinogenic compound to humans.

OTA occurs predominantly in cereal grains, cereal products, cocoa, spices, oilseeds, coffee beans and legumes. However, cereal products are the major group of food commodities where the toxin is of greatest impact. The frequency of food contamination with OTA represents an important source of daily OTA intake [1]. The maximum levels (MLs) fixed by European regulations for OTA in cereals and cereal products are 5 and 3 μg kg^{-1}, respectively [2, 3].

The ”Quick, Easy, Cheap, Effective, Rugged and Safe” extraction method (QuEChERS), microwave-assisted extraction (MAE) and immunoaffinity column extraction (IAC) were used in the analysis of ochratoxin A in different cereal based bread types (wheat, maize and rye). The extracts were analyzed by high-performance liquid chromatography with fluorescence detection and the results were compared. The developed methods, based on QuEChERS and MAE extraction, compare very favourably with the results obtained using the classical IAC extraction. Moreover these methods constitute an improvement as regards the cost and time of the analysis.

In the commercial bread samples analysed, OTA was detected in levels below the established European legal limit for cereal based products.

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BISPHENOL A RELEASING BY PLASTIC CONTAINERS WITH GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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The made-man chemical, Bisphenol A (BPA) (2,2-bis(4-hydroxyphenyl)propane) is an Endocrine Disrupting Chemical, because acting as an estrogen receptor that can interfere with the endocrine system, affecting health, growth and reproduction of animals and humans [1].
The BPA is a monomer that is mainly used in combination with other chemicals to manufacture plastics heavily used in the production of various types of food and drink containers to the quotidian use such as baby bottles, water bottles and water carboys; and epoxy resins are used in the internal coating for food and beverage cans. It has been a great concern, since it can migrate from these containers, increasing the potential risks of exposure to BPA [2]. Therefore, contamination is widespread in the environment leading to a myriad of exposures.
The aim of this study was to assess the evidence for BPA migration from different plastic containers of low density polyethylene (LDPE), polyethylene terephthalate (PETE), polypropylene (PP) and polycarbonate (PC) in order to evaluate its concentration in the contents. Optimization and validation of a method based on a solid phase extraction (SPE) methodology coupled with gas chromatography tandem mass spectrometry was used to analyze water samples. For derivatization the 2,2,2-trifluoro-N-methyl-N(trimethylsilyl)acetamide (MSTFA) was used [3]. Matrix-induced chromatographic response enhancement was avoided by using matrix-standard calibration solutions and heteroscedasticity has been overtaken by a weighted least squares linear regression model application [4]. Deuterated bisphenol A was used as a quality control internal standard, for process evaluation through recovery studies, being added in a constant amount to all analysis prior to extraction. High temperatures, as microwave heating and exposure to boiling water, as well as sequences of washing and rinsing were also tested. Concluded, BPA can be released in small amounts to into water stored in different plastic containers, including baby bottles, after simulated normal use.

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FA16 **Session A**

DETERMINATION OF RIBOFLAVIN IN PASTEURIZED AND UHT COW MILK BY HPLC WITH FLUORESCENCE DETECTION

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Riboflavin (vitamin B_2) is an essential constituent of all living cells, and is one of the most widely distributed water-soluble vitamins. The most important and common dietary sources of riboflavin are milk and milk products. In cow milk, at least 90% of the riboflavin is in the free form, but in most other sources, it occurs bound to proteins.

The aim of this paper was estimation of riboflavin content in commercial cow milk from our market. Pasteurized and UHT milk samples of most representative producers in Serbia with different fat content (0.5-2.8%) were collected from local stores. The milk samples were treated with perchloric acid (12%,v/v), mixed and allowed to stand for 15 min at room temperature protected from light. After proteins precipitation and centrifugation, 2M K_2CO_3 in 6M KOH was added to supernatant to adjust the pH value of the sample to 5.0. Then, the formed insoluble perchlorate was removed by centrifugation. An aliquot of supernatant was filtered through 0.45 μm syringe filter before HPLC analysis.

Chromatographic determination of riboflavin was achieved at 30°C using RESTEK Ultra IBD C18 column with mobile phase consisted of 36.0% methanol and 64.0% 0.005M NH_4Ac solution (pH 5.0) and fluorescence detection (λ_{ex}=440 nm, λ_{em}=520 nm). The flow rate was kept at 1.2 ml/min and 10 μl of the sample was injected.

The method was validated by means of following parameters: linear range (0.2-5.0 μg/ml), linearity (r=0.9994), detection and quantification limits (0.099 μg/ml and 0.330 μg/ml, respectively). The riboflavin contents of the milk samples were obtained by interpolation of the standard calibration curve.

According to other authors, average riboflavin content in cow milk is 1.70 μg/ml. Analyzed UHT milk samples had slightly higher amounts of riboflavin (1.68-1.92 μg/ml) than pasteurized milk (1.30-1.71 μg/ml), and these concentrations are in agreement with values found in other literature. During prolonged heating in pasteurization, sensitive components of milk such as water-soluble vitamins, may be altered or destroyed. Due to the short processing time of the high temperature treatment, there is less vitamin loss and greater nutrient retention in UHT milk compared to pasteurized milk.

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CHARACTERIZATION OF NUTRIENTS AND ACTIVES IN HERBAL SUPPLEMENTS AND NUTRICEUTICALS USING UHPLC-MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETRY – COMPARISON OF SUPPLIERS AND DOSAGE FORMS OF GREEN TEA, GINGER AND ACAI BERRY EXTRACTS

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The qualitative and quantitative analysis of the content of nutritional supplements and alternative medicinal therapies is becoming more important and regulation by the FDA is imminent. High resolution mass spectrometry provides a rapid, selective and useful mechanism of QC of extracts and finished dosage forms, producing a minable, comprehensive data set. In this application, extracts and various supplement dosage forms are introduced via direct infusion or UHPLC separation and analyzed by mass spectrometry (MS). Ultrahigh resolution MS was performed using a multi-reflecting time-of-flight MS (MR-TOFMS). Selected resolving powers and acquisition rates >100 spectra/s provided rich information sets for discrimination of various sources, quality and potential adulterants present in the extracts and the dosage forms. The analyses provided chemical fingerprints which are qualitatively and quantitatively distinct. The utility of these resolving powers and acquisition speeds for facile determination of elemental composition and resultant unknown identification is demonstrated. The utility of in source CID (isCID) to provide supplemental structural information is also presented. Comparisons of the effect of different resolving powers and acquisition speeds on the details of metabolite fingerprints are provided.

IDENTIFICATION AND QUANTITATION OF COPS IN CYPRIOT MEAT SAMPLES USING DIFFERENT SAMPLE PRE-TREATMENT METHODS IN HPLC

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The goal of this research is the quantitative and qualitative analysis of cholesterol oxidation products (COPs) in Cypriot meat products by the use of high performance liquid chromatography (HPLC). Cholesterol as an unsaturated alcohol is susceptible to oxidation in the presence of light, oxygen, and high temperatures, and it can generate numerous oxidation products commonly known as COPs. Several of COPs have been shown to possess undesirable biological effects such as atherogenicity, cytotoxicity, mutagenicity, carcinogenicity and changes in cellular membrane properties. COPs were detected in several foods of animal origin, including meat, eggs and dairy products. Therefore, nutritionists, as well as food scientists are interested in detecting sources of COPs and quantifying them accurately. These compounds are usually analyzed by gas chromatography (GC) or GC coupled to MS (GC-MS). GC, although efficient, can produce artifacts from thermal degradation of cholesterol and its oxides. It requires derivatization, making recovery very difficult. As a consequence of the limitations of GC, HPLC has become an alternative method, mainly because the analyses are carried out at ambient temperature. For the separation of six COPs (20α-hydroxycholesterol, 7-ketocholesterol, 22R-hydroxycholesterol, 25-hydroxycholesterol, 7β-hydroxycholesterol, 4-cholesten-3-one) and cholesterol optimal conditions were established by varying several parameters, such as the composition of the mobile phase, the flow rate, the injection size and the column temperature. The optimal separation conditions were then used for the identification and the quantitation of COPs in Cypriot meat products using different sample preparation procedures. Different sample pre-treatment methods were compared in order to determine the one that is the most effective, in regards to analyte recovery, time, difficulty and reproducibility.

NON-AQUEOUS CAPILLARY ELECTROPHORESIS METHOD WITH UV-VISIBLE AND FLUORESCENCE DETECTION FOR PHENOLICS COMPOUNDS IN OLIVE OIL

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Phenolic compounds are part of the minority fraction of compounds in olive oil, which constitutes approximately 2 % by weight of total. The interest in the study and analysis of these compounds is related with the fact that they act as natural antioxidants and may contribute to the prevention of human disease. Quercetin (Q), tyrosol (TY), hydroxytyrosol (HYTY), oleuropein (OI), vanillic acid (VAN), gentisic acid (GEN), cinamic acid (CIN), *o*-coumaric acid (*o*-CUM) and caffeic acid (CAF) have been chosen as a representative group of it. In this communication the necessary studies to resolve these phenolic compounds by non-aqueous capillary electrophoresis (NACE) with UV-visible and Fluorescence detection are presented.
The injection time, temperature analysis and BGE composition were optimized using a Box-Behnken design by the Response Surface Methodology (RSM) using olive samples spiked with the interest compounds. The best separation of these compounds was achieved using a BGE containing 18 mM KOH, 25 mM boric acid in a 1-propanol:methanol mixture (74:26 v:v), 6 s of injection time at -30 mbar, 35 º C and a separation voltage of 20 kV. In these conditions, the EOF velocity is very small and the analytes, which are anions, move against it and are detected in the anode. 220 nm, 254 nm, 280 nm and 400 nm were UV-visible wavelengths selected to carry out the photometric detection and 297/320 nm the fluorescence wavelengths ($\lambda_{em}/\lambda_{exc}$). Finally, identification was carried out and the analytical figures of merit were obtained under optimal conditions.

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IDENTIFICATION AND QUANTIFICATION OF SEVERAL PHENOLIC COMPOUNDS IN DIFFERENT VARIETIES OF OLIVE OIL BY NON-AQUEOUS CAPILLARY ELECTROPHORESIS WITH UV-VISIBLE AND FLUORESCENCE DETECTION

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The use of NACE technique to carry out the analysis of organic samples (as olive oil) presents, among others, the advantage of reduce the pretreatment sample stages. With this purpose, a simple and rapid method to determine some phenolic compounds in extra virgin olive oil samples have been developed. The analysis was performed using hydrodynamic injection (6 s at -30 mbar), 35º C capillary temperature, 20 kV of separation voltage and a BGE containing 18 mM KOH, 25 mM boric acid in a 1-propanol:methanol mixture (74:26 v:v). To quantification the phenolic compounds which are present in higher concentrations in olive oil, such as tyrosol (TY) and hydroxytyrosol (HYTY), direct injection of olive oil samples diluted 1:1 with 1-propanol was carried out. In the case of compounds in minor concentrations a pre-concentration stage is necessary. Different extract solvents and phase relations were tested and, finally, an extraction of 5 g of extra virgin olive oil with 1 mL of ethanol was selected and the ethanolic extract phase was directly injected in the capillary. The peak identification was carried out using the migration time and by comparing the apex spectra of pure analytes with those peaks in the mixture. This procedure was applied to the analysis of phenolic compounds in extra virgin olive oil from different Spanish olive varieties (*cornicabra, picual, arbequina, hojiblanca, manzanilla cacereña and morisca*) in order to characterize them and quantify some of the phenols, therefore being able to differentiate the oils depending on the olive variety.

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A NOVEL MICROEXTRACTION BY PACKED SORBENT-BASED METHODOLOGY COMBINED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF SIGNIFICANT BIOACTIVE METABOLITES IN WINES

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The current research study is dedicated towards the development and validation of a novel sensitive, fast and accurate approach for the simultaneous determination of bioactive metabolites in wines. The new approach offers decreased sample preparation and analysis time as compared to traditional methodologies. This approach was based on a miniaturized extraction technique, the microextraction by packed sorbent (MEPS), combined with ultra-high performance liquid chromatography (UPLC) equipped with a PDA detector. Important factors affecting the performance of MEPS such as the type of sorbent material (C2, C8, C18, Sil, and M1), number of extraction cycles (extract-discard), and sample volume, were studied. The optimal conditions of MEPS extraction were obtained using C8 sorbent and small sample volumes in one extraction cycle and in a short time period. The wine bioactive metabolites were eluted using 250 µL of the mixture containing 95% methanol and 5% water, and the separation was carried out on a HSS T3 column (100 mm × 2.1 mm, 1.7 µm particle size) using a binary mobile phase composed of aqueous 0.1% formic acid (eluent A) and methanol (eluent B) in the gradient elution mode (10 min of total analysis).

The method validated in terms of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), extraction yield, accuracy and inter- and intra-day precision, gives satisfactory results in terms of linearity (r^2*-values* > 0.996) within the established concentration range. The LOD varied from 0.14 to 5.95 ng mL^{-1} and the LOQ from 0.48 to 19.8 ng mL^{-1}. The extraction yields of the $MEPS_{C8}$/UPLC-PDA methodology was between 87.4 % (syringic acid) and 100 % (gentisic acid and (-)-epicatechin). The inter-day precision (*n=3* days), expressed as the relative standard deviation (RSD%), varied between 0.4 % (kaempferol) and 8.3 % (gentisic acid) while the intra-day precision between 0.2% ((-)-catechin) and 10.3 % (gentisic acid).

DETERMINATION OF PESTICIDES RESIDUES IN PROCESSED CEREAL SAMPLES

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Cereal grains comprise nowadays one of the most consumed foods worldwide because of their global importance in the diet associated with their role as energy source and with their high content in essential fatty acids, nutritious proteins and dietary fiber. Such extent necessity of cereals and derivatives to feed the ever growing population of the world requires efforts to improve and maximize production yields for which pesticide usage has become an important practice.

In this work a group of fifteen pesticides including some of their metabolites (disulfoton sulfoxide, ethoprophos, cadusafos, dimethoate, terbufos, disulfoton, chlorpyrifos-methyl, malaoxon, fenitrothion, pirimiphos-methyl, malathion, chlorpyrifos, terbufos sulfone, disulfoton sulfone and fensulfothion) has been analyzed in wheat and maize gofio, which is a roasted flour widely consumed in the Canary Islands and in different countries around the world, as well as in wheat flour and baby cereals. The QuEChERS (*Quick, Easy, Cheap, Effective, Rugged* and *Safe*) methodology was studied and its dispersive solid-phase extraction procedure was optimized in depth by means of an experimental design with the aim of reducing the amount of coextracted lipids and obtaining a clean extract. Gas chromatography with nitrogen phosphorus detection was used as separation and detection techniques, respectively. The studied method was validated in terms of selectivity, recoveries, calibration, precision and accuracy as well as in terms of matrix effects. Limits of detection achieved were between 0.07 and 34.8 µg/kg with recovery values in the range 71-110% (relative standard deviation values were below 9%). A total of 40 samples of different origin were analyzed. Residues of pirimiphos-methyl were found in six of them at concentrations in the range 0.08-0.47 mg/kg, below maximum residue levels established for this pesticide in cereal grains. Tandem mass spectrometry confirmation was also carried out in order to unequivocally identify the presence of this pesticide.

FEASIBILITY STUDY ON THE USE OF NEAR INFRARED SPECTROSCOPY FOR THE DETERMINATION OF TOTAL CARBON, TOTAL NITROGEN AND $\delta^{13}C$ IN CHILEAN WHEAT

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Near infrared spectroscopy (NIRS) is an accurate and fast technique that can be employed as a replacement for time-consuming methods.
The aim of this study was to evaluate the potential of NIRS technology to determine total carbon, total nitrogen and $\delta^{13}C$ in Chilean wheat.
Seventy-eight samples of wheat were collected from three Chilean geographic zones: 18, 33 and 27 samples from VII Region, VII Region and IX Region respectively. Samples were milled and their corresponding flours were used for the NIRS analysis. Fifty-five samples were allocated into the calibration set and the remaining 23 samples into the validation set. Calibration was performed by modified partial least squares regression (MPLS) using the spectral data and the corresponding reference values for the determination of total carbon, total nitrogen and $\delta^{13}C$ in wheat flour samples.
The obtained models presented multiple correlation coefficients (RSQ) and corrected standard error of cross-validation (SECV) of 0.84 and 0.20% for total carbon; 0.99 and 0.02% for total nitrogen; 0.71 and 0.76 ‰ for $\delta^{13}C$.
The robustness of the method was checked applying NIRS technology models to the 23 samples that did not belong to the calibration group. The relative differences between the reference methods and the NIRS technique in the external validation were 0.5% for total carbon, 1.3% for total nitrogen and 2.5% for $\delta^{13}C$.
The aforementioned models allow the determination of these parameters in Chilean wheat in the following ranges: total carbon 40.0 to 42.4%; total nitrogen: 0.7 to 2.8% and $\delta^{13}C$:-23.4 to -28.5 ‰.

Acknowledgements
This work has been supported by Programa de Cooperación Interuniversitaria e Investigación Científica entre España e Iberoamérica (PCI Iberoamaérica, A/031295/10).

USE OF NEAR INFRARED SPECTROSCOPY TO DETERMINE WET GLUTEN IN CHILEAN WHEAT

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About 250000 hectares of wheat (*Triticum aestivum* L.) are cultivated in Chile and around 88% correspond to the Maule (VII Region), the Bío Bío (VIII Region) and the La Araucanía (IX Region) regions. The need for a system of accreditation of wheat quality in the national Chilean milling sector involves the use of quality parameters such as wet gluten (Chilean Norm NCh1237-2000), which is already the most usual parameter in wheat commercial transactions.This requires having rapid methods of analysis available with minimal or no sample preparation.

Near Infrared Spectroscopy (NIRS) provides fast, accurate, direct and non-destructive analysis reducing costs compared to wet chemical analysis and without generating waste. Therefore, this technique coupled with chemometric tools could provide an alternative method to undertake the analysis of wet gluten in Chilean wheat.

Two hundred and forty nine samples of wheat (2 different showing seasons and 14 varieties) were collected from the three aforementioned geographic zones. Samples were milled and their corresponding flours were used for the NIRS analysis. One hundred and ninety nine samples were allocated into the calibration set and the remaining 50 samples into the validation set. Calibration was performed by modified partial least squares regression (MPLS) using the spectral data and the wet gluten reference values (Norm ICC 137/1).

The robustness of the method was checked applying NIRS technology model to the 50 samples that did not belong to the calibration group. The relative difference between the wet gluten reference method and the NIRS technique in the external validation was 9.9%.

The results of this work show that the model developed using NIRS technology together with chemometric tools allows the wet gluten to be determined in Chilean wheat.

Acknowledgements

This work has been supported by Programa de Cooperación Interuniversitaria e Investigación Científica entre España e Iberoamérica (PCI Iberoamaérica, A/031295/10).

PESTICIDES IN ORGANIC AND CONVENTIONAL FOOD PRODUCTS: ANALYTICAL METHODS FOR MULTI-RESIDUE SCREENING

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Pesticides, including mainly herbicides, fungicides and insecticides, are widely used for crop production of fruits, vegetable and cereals, either before or after harvesting. Since the last decades, many monitoring campaigns have pointed out the presence of pesticide residues in raw or processed products, which is now considered as a major issue in food safety. For that reason, and despite their evident efficiency in protecting crops, the use of pesticides is now becoming widely unpopular, which leads to a growing interest of customers for organic products. Nevertheless, as a number of external sources may cause cross-contamination, even organic raw or processed food products may contain pesticides residues, generally at a lower level than non-organic food.

The analysis of pesticide residues in food items is highly demanding, due to the very low detection limits required and to the wide range of potential contaminants. This study describes the development of a multi-residue analytical protocol for the quantification of various classes of pesticides, with the aim of detecting potential traces of pesticides in conventional and organic food products. To assess the robustness of the methods, five different food matrices were investigated, namely apple, tomato, lettuce, wheat flour and honey.

The proposed method combines a generic extraction procedure using dispersive solid-phase extraction with highly sensitive analytical techniques based on chromatography and triple-quadrupole mass spectrometry, namely GC-MS/MS and LC-MS/MS. On the whole, nearly 40 molecules, including triazine herbicides, strobilurin fungicides and chlorinated insecticides, among others, can be quantified at the low ng/g level. The first results obtained on selected food products showed the presence of trace amounts of fungicides. As expected, despite the fact that organic products are sometimes contaminated with pesticides, the contamination level is significantly lower than in conventional food.

FATTY ACIDS ANALYSIS IN PLANT MATRIX BY DIRECT TRANSMETHYLATION WITHOUT PRIOR EXTRACTION

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Fatty acids may be found in scarce amounts in free form in plant but, in general they are combined in more complex molecules through ester bonds. The analysis of total fatty acids from biological materials is a complex task and precautions should be taken at all times to prevent or minimize the effects of degradation/oxidation by long time manipulation of samples. A one-step extraction and derivatization method has been proposed, essentially based on a dispersive solid-liquid/liquid microextraction and transmethylation with BF_3/methanol as derivatization reagent followed by GC MS analysis. Briefly, 2.5 mL of boron trifluoride–methanol reagent were added to weighed amounts of sample (0.5 grams) in a 20 mL centrifuge tube provided with a Teflon-lined screw cap under the nitrogen. The tube was closed, heated at 70 °C for 30 min (ultrasound bad) and cooled. 1 mL of a NaCl saturated aqueous solution and 2.5 mL of n-hexane were added. The mixture was vigorously shaken and phase separation was achieved by centrifugation. 1.5 mL of clear supernatant was transferred into an autosampler vial for GC MS analysis. This simple and fast method using small amount samples, BF_3/metanol as derivatization reagent and hexane as FAMEs extractor reagent was applied with great accuracy and sensitivity for the determination of free or combined fatty acids (C14-C22, saturated and unsaturated) in different plant matrix.

POTENTIAL OF NEAR INFRARED SPECTROSCOPY FOR THE ANALYSIS OF MAIN MINERAL COMPOSITION IN CHILEAN WHEAT

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Cereal products are among the most important staple foods of mankind. Nutrients provided by bread consumption in industrial countries are close to 50% of the daily requirements of carbohydrates, one third of proteins and 50-60% of vitamin B. Moreover, cereal products are also a source of minerals and trace elements.

The aim of this study was to evaluate the potential of near infrared spectroscopy (NIRS) technology to determine the main mineral composition (Ca, K, P and Mg) in Chilean wheat.

One hundred and fourteen samples of Chilean wheat (different showing seasons and varieties) were collected from two different geographic zones of Chile. Samples were milled and their corresponding flours were also used for the NIRS analysis.

Samples were allocated into the calibration or validation sets by a principal components analysis (PCA)–Mahalanobis distance method using the spectral data of intact wheat or wheat flour. Calibrations were performed by modified partial least squares regression (MPLS) using the spectral data and the main mineral composition obtained by ICP-optic as reference values.

The robustness of the method was checked applying NIRS technology models to the remaining samples which had not been allocated in the calibration groups. The differences between the ICP-optic values and the NIRS technique in the external validation ranged from 10 to 18% for intact wheat and from 10 to 13% for wheat flour. The best results were generally obtained using wheat flour, as expected.

The results of this work show that the models developed using NIRS technology together with chemometric tools allows the main mineral composition (Ca, K, P and Mg) to be determined in Chilean intact wheat or wheat flour.

Acknowledgements

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EXPLORATORY DATA ANALYSIS APPLIED TO SENSORY AND PHENOLIC COMPOSITION OF GRACIANO GRAPE SKINS. UNDERSTANDING THEIR RELATIONSHIP

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The aim of this work was to evaluate sensory changes in *Vitis vinifera* L. cv. Graciano skins during ripening and to obtain a correlation with their phenolic composition. Flavan-3-ols (monomers and dimers), prodelfinidins, hidroxibenzoic acids, hidroxicinamic acids, anthocyanins and flavonols were determined by HPLC-DAD-MS. In addition, skin dilaceration, tannic intensity, astringency, acidity, aroma intensity and type of aroma were evaluated. Multivariate analysis showed the evolution of these samples throughout ripening and the relationship among sensory scores and phenolic composition of grapes skins. Skin dilaceration, type of aroma and to a lesser extent aroma intensity tend to increase throughout ripening. Phenolic compounds in grape skins present a similar pattern. Nevertheless, tannic intensity, astringency, dryness and acidity present an opposite pattern which indicates a decrease of these sensory scores throughout ripening.

The relationship between some studied sensory parameters and phenolic compounds may be indirect and can not be easily described by their phenolic composition. Therefore, a wide study could be useful in order to explain the variation in the aforementioned sensory attributes taking into account not only the phenolic composition but also volatile composition, etc.

Acknowledgments

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DETERMINATION OF QUINOLONE RESIDUES IN INFANT AND YOUNG CHILDREN POWDERED MILK COMBINING SOLID-PHASE EXTRACTION AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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During the last decade, special concern has arisen in the food safety field on infant foods. The wide variety of infant food products nowadays available, as well as the high number of target analytes to be analyzed, demands the development of ultra-fast analytical techniques able to provide results in a short time with extremely low detection limits. In this sense, ultra-performance liquid chromatography (UPLC) usually provides lower analysis time and higher efficiency and sensitivity compared to conventional HPLC.

This work describes a method based on solid-phase extraction (SPE) and UPLC-MS/MS for the simultaneous determination of three quinolones (pipemidic acid, oxolinic acid and flumequine) and twelve fluoroquinolones (marbofloxacin, fleroxacin, pefloxacin, levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, danofloxacin, lomefloxacin, difloxacin, sarafloxacin, and moxifloxacin) in different infant and young children powdered milks. Quinolones constitute an important family of antibiotics with a broad spectrum activity which are widespread used worldwide. After suitable deproteination of the reconstituted powdered samples, a SPE procedure was developed providing recovery values higher than 84% (RSDs lower than 13%) for all the analytes, with limits of detection between 0.04 and 0.52 µg/kg. UPLC-MS/MS analyses were carried out in less than 10 minutes. Sixteen infant and young children powdered milk samples of different origin, type and composition bought at Spanish markets were analyzed. Residues of the selected antibiotics were not detected in any of the selected samples.

APPLICATION OF ^{31}P NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN DETERMINATION OF PHOSPHATES ADDITIVES IN MEAT PRODUCTS

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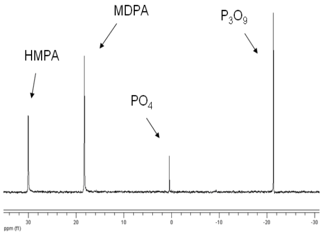
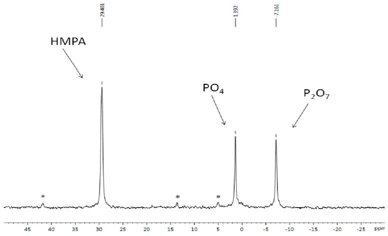
The development of ^{31}P MAS and HR MAS NMR resulted in application of these techniques for speciation analysis of polyphosphates in different matrices.

Due to the extensive food additives applications, such as polyphosphates ($Na_3P_3O_9$, $Na_5P_3O_{10}$, $Na_2H_2P_2O_7$, $Na_4P_2O_7$,) in food industry, new analytical methods of phosphates speciation analysis are worth study. The NMR technique seems to be one of the most appropriate in determination of phosphates anionic forms. New methods of polyphosphates determination in meat products were elaborated by using the external reference standard MDPA (Methylenediphosphonic Acid) and internal standard HMPA (Hexamethylphosphoramide) procedure[14,2]. High recoveries (in the range of 95 - 99%), accuracy and precision (RSD<5%) parameters were confirmed on standard solutions and applied in meat products (tinned pork foods, luncheon pork meat, smoked pork ham). The polyphosphates were determined with reduced analysis time in relation to official methods PN-ISO 13730, 1999 (spectrophotometric vanadate-molybdate method). However, much more improved NMR method, without the special procedure of sample preparation, based on MAS and HR MAS ^{31}P NMR measurements was studied and results are presented at figures (*vide infra)*.



^{31}P NMR spectrum of meat extract with $Na_3P_3O_9$



MAS ^{31}P NMR spectrum of laboratory sample with $K_4P_2O_7$

High recovery level (95–99%) for all NMR methods and the possibility of different phosphate ions determination in single analytical run suggest application of this method in analysis of meat products. Described NMR methods can be used for control of the added phosphates level during production process and in food quality assessment.

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NEW ELECTROLYTE SYSTEM FOR ISOTACHOPHORETIC DETERMINATION OF SELECTED BIOGENIC AMINES IN MEAT SAMPLES

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Capillary isotachophoresis (ITP) has been studied recently as a new approach to food analysis. The reason for the increased acceptance of this method is short analytical time, uncomplicated and economical with reduced to minimum the sample pretreatment procedure and small sample requirements.

In the presented work selective and sensitive ITP method with conductometric detection in which selected biogenic amines were determined after simple sample preparation was described. The level of cadaverine, putrescine, spermine and spermidine in meat samples (beef, pork and poultry) was determined. The meat samples were purchased from local markets.

We proposed new electrolyte system consisting of leading electrolyte (LE): 5 mM $Ba(OH)_2$ with 1% hydroxyethylcellulose (HEC) and 15 mM valine to pH =9 and 0.02 M histamine as terminating electrolyte (TE) for determination of selected biogenic amines in meat samples. The food samples were extracted with trichloroacetic acid (TCA) (3%) using an orbital shaker for 30 min. The extracts were separated using centrifuge at 9000 rpm for 30 min, followed by double filtration. All extracts were transferred into 50 mL volumetric flasks, made up to the mark and analysed with ITP.

The cadaverine, putrescine, spermine and spermidine were identified using the relative step height (RSH) parameter. Precision was evaluated as the within-day and between-days coefficient of variation (CV) and obtained results (CV $< 5\%$) were satisfactory. Furthermore, proposed method characterised satisfactory accuracy – obtained recovery ranged from 95% to 99%.

The proposed method permitted on selective, sensitive and reproducible determination of biogenic amines after simple sample preparation method

Acknowledgement

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CHANGES IN SELECTED BIOGENIC AMINES IN MEAT SAMPLES DURING STORAGE LIFE

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Biogenic amines occurrence in food, especially in fish, cheese and meat products, varies by a great extent depending on technological processes and microbial factors. In fact their formation mainly depends on the actions of microbial decarboxylases and in a minor role on the endogenous amino acid decarboxylase activities.

In this work we applied capillary isotachophoretic method (ITP) for determination of biogenic amines (histamine, tyramine, cadaverine, putrescine, spermine and spermidine) in food samples after simple sample preparation. The meat samples (different kind of beef, pork and poultry) were purchased from local slaughterhouse. The meat samples were homogenized and storage in refrigerator by 5 days (temperature about 4^0C). The samples were determined every day. Sample preparation included extraction with 3% trichloroacetic acid (TCA) (30 min), separation with centrifuge at 9000 rpm for 30 min and double filtration.

The used method permitted on simple and accurate determination of tested amines without derivatization stage. Analysis of biogenic amines were performed with electrolyte system: leading electrolyte (LE): 5 mM $Ba(OH)_2$ with 1% hydroxyethylcellulose (HEC) and 15 mM valine to pH =9 and 0.02 M tris(hydroxymethyl)aminomethane (TRIS) with 1 M HCl to pH = 8.3.

According to our results, spermine and spermidine appear to be the natural amines in beef, pork and poultry meat, while amount of tyramine, histamine, putrescine and cadaverine increased during the storage. It should be noted, that contents of selected amines were dependent on kind of meat and the highest total content of all detected amines was observed for pork meat.

Acknowledgement

The authors acknowledge the grant from Polish Ministry of Science and Higher Education: grant No. NN 312 465640 (4656/B/P01/2011/40)

INFRARED SPECTROSCOPY FOR THE DETERMINATINO OF POLYMERIZED TRIGLYCERIDES IN DEEP-FRYING VEGETABLE OILS

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The first part of the study deals with the determination of polymerized triacylglycerides (PTGs) generated during thermal treatment of oil. Spectral changes observed in the attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectra of heated olive oil samples could be correlated with the PTG content in oil samples by partial least squares (PLS) regression. To optimize the PLS results, three different approaches for selection of the spectral regions used to build the PLS model were tested and compared: (i) variable selection based on expert knowledge, (ii) uninformative variable elimination PLS (UVE-PLS) and (iii) interval PLS (iPLS). The PLS model provided excellent performance ($R^2 = 0.991$) and low calibration, cross validation and prediction errors of 1.14, 1.21 and 1.40% w/w, respectively. Moreover it could be verified that the determination of PTGs was not influenced by the type of foodstuff fried in the olive oil.

In a second study, different types of vegetable oil (olive, sunflower and corn oil) were investigated to extend the range of applications of the developed method. The use of a one-class-classifier Partial Least Squares – Discriminant Analysis (PLS-DA) and a rooted binary directed acyclic graph (DAG) tree provided accurate oil classification for vegetable oils fried without foodstuff. Class separation was less evident for samples fried with foodstuff. The combined use of permutation testing with double cross model validation was employed to validate the obtained PLS-DA classification models confirming the results. After class separation, the PTG content was determined calculating PLS models based on the previously selected classes. Root-mean-square-error-of-prediction (RMSEP) could be significantly improved using PLS models based on the selected calibration sets using PLS-DA, ranging between 1.06 and 2.91% w/w.

The presented approach could be a useful tool for quality control of oil samples as it is suitable for cheap high-throughput analysis and provides accurate and precise results.

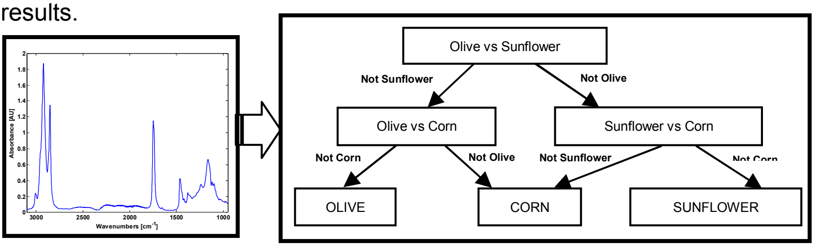

Figure 1. Schematic view of the process of oil classification and PTG determination.

FA38 **Session A**

ANALYSIS OF POLYPHOSPHATES IN FOOD USING ION CHROMATOGRAPHY

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A method for the determination of polyphosphates (di-tri –and higher polyphosphates), in food product by ion chromatography (IC) was developed. The polyphosphates was extracted with deionized water and then filtered twice. The separation was carried out on an IonPac AS16 analytical column (250 mm x 4 mm) and an IonPac AS 16 guard column (50 mm x 4 mm) using 30 - 80 mmol/L NaOH gradient elution at a flow rate of 1.0 mL/min at 30 degrees C, coupled with an ASRS (Anion Self Regenerating Suppression) and a conductivity detector. Under optimum conditions, the measurement could be completed within 40 min. Standard of ortho-phosphate, pyrosphate, trimetaphosphate, and triphosphate were used to calibrate the method. The method was applied to the determination of anions in meat and dairy product with good recoveries. The method offered high selectivity, sensitivity, and gave a satisfactory results for real sample analysis. The proposed method will be thoroughly validated in terms of linearity, LODs, LOQs precision and accuracy (recoveries) repeatability (by the analysis of six replicate) and reproducibility (by the analysis of twelve replication performed by two operators).

LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION COUPLED TO ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY FOR THE DETERMINATION OF MELAMINE AND ITS DERIVATIVES IN FOODS

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Melamine (MEL) is a nitrogen-rich compound used for the manufacture of plastic and resins, which is illegally added to various food products to increase the apparent protein content. A liquid chromatographic method with diode array detection coupled to atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) is proposed for the determination of MEL and structurally related compounds, including ammeline (AML), ammelide (AMD) and cyanuric acid (CA). The procedure is based on the direct analysis by ion-exchange LC using isocratic elution with a mobile phase of 10 mM ammonium acetate (pH 6.5) at 0.5 mL min^{-1}. The LC was hyphenated with two different detection systems, photodiode-array (DAD) and APCI-MS in positive mode for MEL and AMN and in negative mode for AMD and CA. The opposite polarities required necessitate the use of two different runs to improve sensitivity. The elution order was: CA (2.3 min, ion 128), AMD (2.5 min, 127), AMN (3.5 min, 128) and MEL (6.8 min, 127). The method using LC-DAD was less selective requiring intensive validation to ensure that there are no interferences. Samples were extracted by homogenization with a 5/4/1 acetonitrile/water/diethylamine mixture. The method was validated for linearity, detection and quantification limits, selectivity, accuracy and precision. Specificity was demonstrated by the retention characteristics and UV and MS spectra, by comparing with commercial standards. The recoveries obtained for spiked samples were satisfactory for all the analytes. Matrix effects have been studied in the different type of samples analyzed to evaluate the signal suppression and calibration must be carried out by the standard additions method. The proposed procedure was successfully applied to the analysis of different baby foods, as infant formula (starting, follow-on and prebiotic follow-on) and breakfast cereal (multicereals with honey), and other samples of rice flour, glutinous rice flour, potato starch, soya and coconut drinks. The analytes were not detected in any of the samples above the detection limits, confirming that non-proteinaceous sources of nitrogen have not been added to foods.

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BACTERIAL SENSORS FOR FOOD SAFETY BASED ON BIOSILICA IMMOBILIZATION FOR LABEL-FREE OWLS SENSORS

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In the last years enzymatically active proteins, so called silicateins, were studied and identified, which form the axial filaments of the spicules of the siliceous sponges, consisting of amorphous silica and are able to catalyze the poly-condensation and deposition of silica under mild conditions (low temperature, physiological pH)(1,2). Silicateins can be expressed in *E. coli*. The resulting recombinant proteins are able to catalyze the formation of polysilicate nanoshell around the cells. The advantage of silica-encapsulation could be the easier handling of bacteria and an increase in their chemical and mechanical stability(3). The encapsulation of bacteria makes possible the preparation of novel bioreactors and biosensors by attaching them to sensor chips of Optical Waveguide Lightmode Spectroscopy(4) (OWLS). High specificity/selectivity of the SiO_2 surface sensitized by bacteria coupled with high sensitivity of OWLS detection gives the possibility to develop sensors with in most cases definitely lower detection limit than traditionally used assays.

The surface modification of SiO_2-type chips by using silicatein and the immobilization process of the silica-encapsulated *E. coli* cells were studied and demonstrated. After optimization of the immobilization process (buffer concentration, pH, temperature, reaction time, etc.) the biological properties, e.g. inhibitory effect of stressors/environmental pollutants on the biosilica-encapsulated biosensors were investigated in real time and compared to the traditional methods.

The effect of oxidative stress was studied by exposing the sensors with modified *E. coli* cells to different concentrations of hydrogen peroxide. The effect of different antibiotics was tested by chloramphenicol (CAP) being effective against a wide variety of Gram-positive and Gram-negative bacteria by inhibiting bacterial protein synthesis and by penicillin G destroying their cell wall. After the treatment with penicillin G spheroplasts are only present causing the cell to lose control over its shape, so even if the original bacterium were rod-shaped, the sphereoplast is generally spherical. This effect could be probably followed with the novel bacterial sensor. The inhibition by carbofuran (CF) was also tested; CF is one of the most toxic carbamate pesticides which inhibit cholinesterase enzymes. When different concentrations of stressors were used, the inhibiting effect could be in each case observed, and the signals can be distinguish according the increasing concentration of them.

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DISPOSABLE OPTICAL MULTISENSOR FOR HEAVY METALS

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Due to environmental considerations there is an increasing demand of simple techniques for the analysis of heavy metals in water, industrial wastes, landfills and other sources of pollution capable to degrade close ground water. The toxicity of heavy metals is attributed to harmful or even lethal effects on the human body, particularly on the central nervous system, causing mental disorders. For this reason, it is necessary the development of cheap and rapid analytical toxicity tests that can provide rapid information about the toxicity impact of an effluent. A recent trend to obviate the difficulties of obtaining single analyte sensors based on specific receptors – problems of selectivity – is to combine non-specific sensors in an array that produces a set of analytical signals (electronic tongue for gaseous species/electronic nose for species in solution). This information together with higher dimensionality and encoded in the overlapping measurements, can be processed in order to extract qualitative and quantitative information through advanced mathematical procedures of pattern recognition and/or multivariate analysis, improving in this way the performance of the sensors [1]. Most different optical approaches used for tongues are based on the measurement of the colour using different colour spaces obtained from the image captured mainly by imaging devices, like CCD cameras or scanners. So far, the most commonly used space is RGB, whose coordinates are used for processing with multivariate techniques. However, in the present work, we used the HSV colour space, whose main characteristic is that it represents the useful information of the colour in one single parameter, the coordinate H (hue) [2]. We approached the analysis of heavy metals using the concept of an optical tongue using an array of membranes placed on a transparent support that contains immobilized conventional chromogenic reagents. Their usual drawback, i.e. their lack of selectivity, here is used as the source of non-selective information about the contained metals, considering their usual good sensitivity. Diverse chromogenic reagents for heavy metals were tested for immobilization using different strategies such as ion-exchange, adsorption, and entrapment using as criteria for selecting the no leaching conditions, the change in tonal colour coordinate by reaction and non-selective behavior. The array of sensing membranes prepared on a plastic support, allowed us to obtain the hue component of the HSV colour space using imaging techniques (scanner, CCD camera) of each one in order to determinate the concentration of metals using multicomponent analysis.

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SENSOR ARRAY-BASED COLORIMETRIC PORTABLE INSTRUMENT FOR pH DETERMINATION IN DIFFERENT MATRICES

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A portable measurement system for accurate full-range pH determination (0-14) has been developed using a disposable sensor array. The system is based on the measurement of colour changes of an optical multimembrane pH sensor based on 11 sensing elements with immobilized pH indicators. In previous work, the same acid-base indicators were individually characterized by means of spectrophotometry and scannometry[1]. Now, we have used a portable instrument[2]. In the last to cases, imaging techniques have been used to acquire the tonal information (H coordinate) of the HSV colour space.

The different pH membranes were selected according to the conditions of no leaching, changing in the tonal colour coordinate by reaction and covering the full pH range by overlapping between the responses. The membranes were prepared by coating technique from THF solutions containing different types and amounts of acid-base indicator, membrane polymer, plasticizer, humectant, if necessary, and lipophilic salt.

The instrument is compounded by an organic light-emitting diodes display acting as programmable light source and twelve digital colour sensors S9706 aligned with the sensor array. The emitted light by the display, which is configured to be similar to D65 illuminant, illuminates the multimembrane pH sensor (4x3 positions) and the transmitted light reaches the colour sensors (Figure 1). The instrument also counts with an integrated EEPROM memory, which allows to store measurements, and a keypad, for measurement configuration. Finally, the results obtained are displayed in a LCD screen or can be transmitted to a computer via USB port. The array response was evaluated each 0.1 pH units. After measurement, the HSV coordinates were calculated from RGB acquired by digital colour sensors of the instrument.

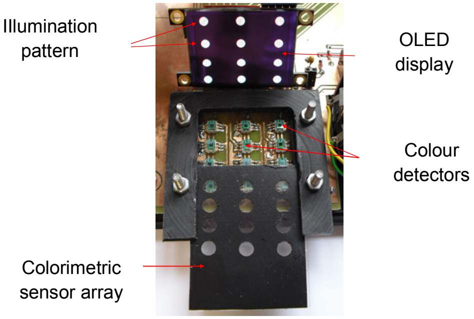

Figure 1. Sensor module photograph

The results obtained with the portable instrument are very similar to that obtained using imaging techniques to acquire the tonal information or absorbance measurements (Figure 2). The pH of food, clinical, personal care and cleaning samples has been determinated, such as orange, tangerine, tomato or pineapple juices; orange, lemon or cola soft drinks; milk; tap, river, mineral or carbonated waters; vinegar; lemon; alcohol-free or draught beers; ammonia solution; shampoo; washing-up liquid; urine; saliva; etc. validating results against a pH-meter.

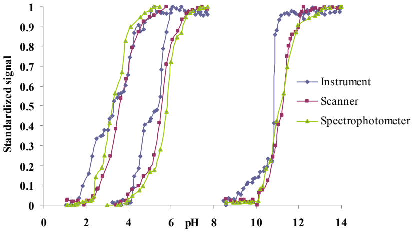

Figure 2. Comparison between different membranes using different devices

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AMPEROMETRIC GLUCOSE BIOSENSOR BASED ON CARBON PASTE ELECTRODE MODIFIED ZINC OXIDE NANOPARTICLES AND VARIOUS MEDIATORS

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The determination of glucose is significant in many areas such as clinics, human health, diabetes, biological analysis, environment and food industries [1]. Amperometric biosensors have been widely used for measuring glucose due to their selectivity, low cost, storage stability and miniaturization [2]. ZnO nanoparticles have excellent properties, such as biocompability, chemical stability, and high electrocatalytic activity to acidic proteins by electrostatic interactions [3-5].

In this study an amperometric biosensor using glucose oxidase (GOx) was constructed for determination glucose in serum samples. GOx catalyzes the direct electron transfer from glucose to oxygen to produce gluconolactone and hydrogen peroxide.

The carbon paste was prepared by mechanically mixing with graphite powder, ZnO nanoparticles, mediators such as benzoquinone, GOx and paraffin oil. The prepared modified carbon paste was placed into a Teflon tube cavity and electrode surface was smoothed on a weighing paper. Glucose determination was performed by oxidation of enzimatically produced hydrogen peroxide at +0.40 V versus Ag/AgCl reference electrode.

Amount of enzyme, working pH, temperature were optimized. Reusability, life time, effects of ascorbic and uric acid, sensitivity, working range and detection limit were investigated. ZnO nanoparticles modified carbon paste electrode was compared with unmodified carbon paste electrode. Nanoparticles modified carbon paste electrode showed high sensitivity and wide working range. The effects of mediators such as benzoquinone on electrode response were also investigated. Proposed biosensor was used for determination glucose in human serum samples.

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LABEL-FREE OPTICAL DETECTION OF CYSTATIN-C AS A PARAMETER FOR RENAL FUNCTION

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Optical biosensors provide the possibility to measure clinical parameters fast and cost efficient. Especially for Point-of-Care (POC) applications, it is necessary to have fast and robust detection methods available.

In the presented work we show an assay which allows for quantifying Cystatin-C, a serum parameter indicating changes in renal function. By measuring the concentration of Cystatin-C in human serum it is possible to determine the glomerular filtration rate (GFR). The GFR is defined as the volume of fluid that is filtered by both kidney in a unit of time and it will decrease in case of renal dysfunction. Compared to the established parameter for GFR creatinine, Cystatin-C shows several advantages. Above all its serum level is independent of age, nutrition, muscle mass or gender of the patient which is not the case for creatinine[1].

Using Reflectometric Interference Spectroscopy (RIfS) it is possible to quantify biomolecular interaction label-free, time-resolved and nearly temperature independent[2]. This method has been used to characterize surface-modification of glass transducers and for quantification of Cystatin-C.

We were able to immobilize Cystatin-C on the transducer surface and to calibrate using antigen-antibody interaction with a binding-inhibition test. As for a binding-inhibition test only one type of antibody is needed, the measurements can be kept simple and cheap. Different types of monoclonal antibodies were compared due to their specificity and dynamical range of the received calibration curve. We tested and optimized different surface-modifications to receive high and selective signals. The sensor-chips could be fully regenerated several times, so every calibration could be done on a single chip. With these calibrations it is possible to quantify Cystatin-C in buffer and human serum within the relevant clinical ranges.

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DISPOSABLE AMPEROMETRIC MAGNETOSENSORS FOR THE SPECIFIC DETECTION OF CLINICALLY RELEVANT BACTERIA

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Streptococcus pneumoniae is the leading cause of bacterial meningitis and responsible for around 800,000 annual deaths of children throughout the world. The rapid and specific diagnosis of pneumococcal infection is needed for a prompt implementation of an effective therapy.

Three different approaches based on the use of magnetic beads (MBs) and gold screen-printed electrodes (AuSPEs) are reported here for the specific detection of *S. pneumoniae*.

The first approach implies a sandwich immunoassay using appropriate capture and detection antibodies on Protein A-modified MBs.

The second strategy involves the use of a specific probe for detection of a characteristic region of the pneumococcal *lytA* gene. Streptavidin-modified MBs are modified with a biotinylated capture probe and hybridization and labelling was allowed to proceed with the predominantly single-stranded biotinylated amplicons obtained by asymmetric direct PCR (DAPCR) and a streptavidin-peroxidase polymer, respectively.

The third procedure combines the previous ones. The amplified products prepared by DAPCR from bacteria attached to capture antibody modified MBs are specifically detected on MBs modified with the specific capture probe.

In all cases, the modified MBs are captured by a magnetic field on the surface of tetrathiafulvalene (TTF)-modified AuSPEs and the amperometric response at -0.15 V upon H_2O_2 addition was measured.

The approaches show a good analytical performance (LODs between 100 and 1.5×10^4 CFU mL^{-1}) for the specific *S. pneumoniae* detection, without preconcentration steps. The short analysis time and small sample volumes required, and easy adaptability to detect other relevant bacteria, make the proposed methodologies promising alternatives for clinical diagnosis.

AZOLE-SUBSTITUTED SPIRONAPHTHOPYRANS: TOWARDS PHOTODRIVEN CHEMICAL SENSORS

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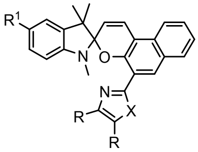
The elaboration of new sensitive elements and investigation of their properties play an essential role in sensor design. The detection process of new generation chemical sensors is to be reversible not only chemically but also under the external physical action [1]. The latter becomes achievable by employment of bistable molecular systems that can exist in several thermodynamically stable isomeric states having different affinity to analyte. The switching between passive and active states may be provided by different wavelength light irradiation. Spironaphthopyrane are perspective bistable organic compounds. Recently we have reported on the synthesis of photochromic 5’-(4,5-diphenyl-1,3-oxazole-2-yl)substitutedspiro[indolinenaphtho-pyran], the merocyanine isomer of which reversibly forms complexes with bivalent metal ions [2].



R^1 = Alk, OAlk, Cl, NO_2

X = O, S

R = Ph, benzo

The present work is devoted to the investigation of the photo-, thermo- and cation induced reactions of the series of spironaphthopyrans bearing azole fragment in the side chain in order to construct the photodriven chemosensors.

The introduction of the azole substituents in position 5´ results in the additional longwavelength CT absorption band of the spiroforms spectra and to luminescence observed not only for the merocyanine form but also for the spiroform.

It has been revealed that in solutions, merocyanine isomers form intensively colored complexes with metal ions in the dark. Binding constants of the complexes have been determined (logK = 1.7-12.0). Nature of a heterocyclic substituent in position 5´ of the naphthopyran fragment influences complexes composition and stability. In the case of compounds having the same coordination centre the electronic properties variation of the substituent in position 5 of the indoline part of the molecule allows realizing of fine tuning of selectivity to metal ion binding.

The complexation reaction with diamagnetic cations is photochemically reversible. Complexes photodissociation quantum yields (φ = 0,01-0,3) have been calculated for the first time. Effects of different factors on these values have been analyzed.

Acknowledgements

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DESIGN OF A VERSATILE FLOW-THROUGH PAIRED EMITTER-DETECTOR DIODE-BASED OPTRODE

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An affordable, fiberless, mechanically resistant and versatile mesofluidic biooptrode has been constructed of two ordinary light emitting diodes in a Paired Emitter-Detector Diode (PEDD) configuration, integrating a sensing film, as a result of which a complete optosensor is generated. The film had been coated with Prussian Blue dye as a model chemosensor that allows sensing of reducing and oxidizing species. Ascorbic acid and hydrogen peroxide were employed as model analytes.

The fixation of an oxidase (in our case, glucose oxidase, GOx) onto the chemoreceptor allowed the detection of enzyme substrates (here glucose) since the reaction in most cases yields hydrogen peroxide, which is sensed by the film. A full factorial design followed by a second-order Doehlert matrix-based response surface method was exploited for multivariate optimization of the PEDD-sensor to attain maximum signal response. The intensity of the emitting LED and the surface concentration of GOx in the film were regarded as the most significant variables in sensor's response. The response surface demonstrated the robustness of the biosensor since the intensity can be shifted by 18.5% of the optimal value, and the superficial concentration of GOx by 17% with decrease in sensitivity in all instances < 5%.

Measurement trueness of (bio)chemosensor was investigated comparing the results of Vitamin C containing drugs against the iodometic titration reference method, beside the analysis of human serum certified samples against the Trinder I method for glucose. Detection and quantification limits (3 and 10 σ-criteria) for glucose were within the submilimolar range, that is, LOD = 0.078 mM and LOQ = 0.26 mM.

The PEDD-based model sensor demonstrated in this work be easily applied to other kinds of optical sensors based on absorbance sensing films.

SENSITIVE AND RAPID AMPEROMETRIC MAGNETOIMMUNOSENSOR FOR THE DETERMINATION OF *Staphylococcus aureus*

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Staphylococcus aureus (*S. aureus*) is an important pathogen that constitutes a potential danger to human health. It exists as a harmless commensal in approximately 25 % of humans but also can cause several infections, illnesses, and even sepsis. Consequently, there is an increasing interest in the development of rapid methods for its detection.

In this work we report the development of a competitive magnetoimmunosensor for the detection of protein A (protA)-bearing *S. aureus*. The developed immunosensor is fabricated by immobilizing the anti-protA antibody onto protA-modified magnetic beads, and the immunoassay is based on the competition between protA antigen and protA labelled with HRP (protA-HRP). The electrochemical detection of the enzyme product is carried out at a disposable gold screen-printed electrode, using tetrathiafulvalene (TTF) as electron transfer mediator and H_2O_2 as the enzyme substrate.

Current changes have been observed related to the variation of the protA solution concentration. The incubation of the antibody-modified MBs with a *S. aureus* cells solution results in the binding of the protA present in the cell wall to the antibody. Thus, when the *S. aureus* concentration in the sample increases, the access of the protA-HRP to the specific antibody is inhibited leading to a decrease in the catalytic effect of the HRP to the oxidation of TTF by H_2O_2.

The developed sensor is able to detect the protA quantity corresponding to only 0.076 cell mL^{-1} (after bacterial lysis), showing a dynamic range within 1.0-1.0x10^7 cell mL^{-1}. A good reproducibility for ten different immunosensors constructed in the same manner was obtained at *S. aureus* concentration levels of 10 and 1 cell mL^{-1} (RSD, 4.7 % and 7.2 %, respectively). The analysis of six milk samples spiked at concentration levels of 10 cell mL^{-1} and 100 cell mL^{-1} yielded *S. aureus* contents of (11.2±0.9) and (106±8) cell mL^{-1}, respectively, thus showing the applicability of the developed sensor to the analysis of real samples.

A PORTABLE BIOSENSOR PLATFORM FOR VETERINARY DIAGNOSTICS

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Fast, cheap and reliable analytical methods in the domain of veterinary diagnostics are more and more in demand. For example, to prevent the transfer of infections that pass between animal and human (zoonoses), control of food originating from animals is necessary. Furthermore, veterinary diagnostics play an important role for surveillance in live stock, as early detection of infected animals may avoid larger economical damages. For this reason, monitoring the immune response of working animals is not only important due to health regulations but also in regard to economical issues.

For analysis, most samples have to be sent to centralized laboratories. Thus, results are obtained at the earliest after several hours or days. Additional costs because of aftereffects, e.g. spreading of infections in live stock, can occur as only after a confirmed diagnosis appropriate measures can be introduced.

To quantify relevant parameters out of animal serum, a new miniaturized biosensor platform has been set up and promising measurements have been carried out.

The new miniaturized biosensor platform contains a modified, commercially available LED unit as well as a photodiode and is based on 1-lambda Reflectometric Interference Spectroscopy (1-lambda RIfS). RIfS [1] is a direct optical method based on interference of light beams at thin layers. Due to its small size, its robustness and simple handling, the new miniaturized biosensor platform can be used for getting rapid results on-site.

In comparison to standard commercial tests like Enzyme Linked Immunosorbent Assays (ELISAs), the developed miniaturized biosensor platform achieves feasible automation without the need of expensive labeling reagents. For this reason, costs per test as well as the length of analysis time stay low.

Exemplarily, parameters out of the domain of working animals (salmonella infections) as well as companion animals (toxoplasmosis) are determined. For the detection of more parameters at the same time, the detection module is situated on a movable bar so that different spots can be approached one after another.

The technology of the biosensor platform can also be used for human diagnostics. Therefore, an assay for detection of the inflammation marker C- reactive protein was established.

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MOLECULARLY IMPRINTED POLYMER FOR DIPHENYLAMINE SENSING

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Molecular imprinting has gained popularity during last decades as a technique of synthesizing polymer materials with chemically selective recognition sites. Due to the high stability, low cost, easy preparation and possibility to synthesize receptors for a very wide range of analytes, several applications of molecularly imprinted polymers (MIP) as a stationary phase for HPLC, sorbents and sensing materials have been reported.

The present study deals with synthesis of the MIPs imprinted by diphenylamine (DPA) and their application as active substances for potentiometric sensors. DPA is widely used as a pre- and post-harvest scald inhibitor in apples and pears. It is a toxic compound that appears in the third list of EU priority pollutants. Though DPA content is drastically reduced during fruit storage and washing, residual amounts can remain on the peel and especially in the pulp due to its capability to be absorbed through the peel. Development of chemical sensors capable of rapid detection of DPA residues in fruits is therefore of practical interest. No reports on the chemical sensors for DPA detection were found.

A series of polymer microparticles imprinted with DPA and respective non imprinted polymers were synthesized by thermal precipitation polymerization using methacrylic acid (MMA) a monomer, trimethylolpropane trimethacrylate (TTMA) as a cross-linker, 2,2'-Azobis(2-methylpropionitrile) as a catalyst and acetonitril as a solvent. Amount of template and reaction times were varied with the aim to optimize polymer selectivity. Synthesized polymers were characterized using FTIR-ATR spectroscopy, scanning electronic microscopy (SEM) and equilibrium batch re-binding experiments. Binding behavior of the polymers was described using Freundlich isotherm. Based on the MIP characterization results polymers more suitable for sensing applications i.e. with higher selectivity and binding capacity were selected and used for the preparation of plasticied PVC membranes for potentiometric chemical sensors.

THE PROPERTIES OF METAL COMPLEXES WITH PHENANTROLINE LIGANDS. ANALITICAL METHODS BASED ON THESE PROPERTIES

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The properties of complexes, formed by metals and N containing aromatic ligands, are widely used in chemical technology, biochemistry, and especially analysis. The presence of 14π electrons delocalized over the aromatic ring of phenantroline ligand confers spectroscopic, photochemical, and electrochemical properties to phenanthroline-based transition metal complexes. Detailed consideration is given to the possibilities and limitations of analytical methods based on various properties of these complexes (light absorption, luminescence, solubility, redox properties, etc). The tendency for stacking interactions of these complexes is important for using these complexes in various fields.

The study is based on the crystal structures archived in the Cambridge Structural Database (CSD), and the crystal structures involving phenanthroline complexes with coordination number 4 were screened for intermolecular contacts. In the CSD 172 stacking interactions of phen square-planar complexes were found [1]. In most of the interactions two interacting complexes are oriented “head-to-tail”, with the large area of phen ligand involved in the overlap.

Phen complexes show a large range of different overlap geometries in stacking interactions, however, short metal-metal distances were not observed. Based on the combination of geometric parameters we have two major groups. Diferences between these two groups are based on the type of overlaping of phenanthroline ligands. First group have two or more rings of phenanthroline overlaping, and second group have mainly terminal pyridine ring of phenanthroline ligands overlaping. The geometry of the interaction is very often influenced by two ligands coordinated at the third and fourth coordination position, or by ions in the crystal structure. Stacking interactions of phen square-planar complexes form chains and dimers in crystal structures. Stacking chains with alternating overlaps are the predominant type of packing.

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A SIMPLE AND VERSATILE ANALYTICAL METHOD FOR DETECTING mRNA BY ROLLING CIRCLE AMPLIFICATION WITH RNaseH

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Prevailing microbial detections conventionally depend upon cultivations in selective media, biochemical analyses like enzyme-linked immunosorbent assay (ELISA), or molecular biological analyses like PCR-based methods including reverse transcription PCR. The cultivation method can be applied to microbes that can be grown in media. Although ELISA is a rapid analytical method for detecting microbes, it is known to often give false-positive signals. PCR-based methods also rapidly identify target microbes, however they still suffer from disadvantages such as complicated procedure and high running cost. Therefore, a novel analytical method, which could simply and rapidly identify microbes, has been expected for practical uses.

Here, RNA-primed rolling circle amplification (RPRCA) using phi29 DNA polymerase, a precircularized probe and SYBR Green II, achieved real-time detection of specific mRNA from living microbes. When in vitro transcribed green fluorescent protein (GFP) mRNA was used as a primer, RPRCA could specifically detect at least 1 fmol mRNA in the presence of a circular probe that had a sequence complementary to the 3'-end of the mRNA without reverse transcription. This circular DNA probe could also identify expressed GFP mRNA present in 10 ng of total RNA isolated from *Escherichia coli* without DNA digestion. On the other hand, when a circular DNA probe, which was complementary to a sequence in the middle of GFP mRNA, was applied, faithful RPRCA reaction was found only in the presence of RNaseH, which digested RNA strand in DNA-RNA hybrid, in the reaction mixture. RPRCA did not require an expensive device such as a thermal cycler because RPRCA was an isothermal reaction. These suggest that RPRCA could be a simple and versatile analytical method for identifying living microbe by means of direct detection of mRNA with partially known sequences.

A FIBER-OPTIC BIOSENSOR WITH UV-LED EXCITATION FOR MONITORING OF GASEOUS FORMALDEHYDE

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Formaldehyde (FA) is a volatile organic compound, which can cause serious health effects. FA has been linked to cancer deaths. Exposure to high FA levels were at increased risk for leukemia. Indoor, non-industrial exposure to FA can occur continuously at low levels, contributing to headaches, eye irritation. Effective detection of chemicals, such as FA, in environment requires a sensitive and selective analytical sensor. Such devices could continously monitor our surrounding and give us warnings about the level of toxic chemicals in environment. In this study, a highly sensitive fiber-optic bio-sniffer using formaldehyde dehydrogenase (FALDH) immobilized membrane for gaseous FA monitoring was developed. Gaseous FA was measured using the bio-sniffer as fluorescence of nicotinamide adenine dinucleotide (NADH), which is the product of FALDH reaction. The bio-sniffer device was constructed by attaching a flow cell with a FALDH immobilized membrane onto a fiber-optic NADH measurement system. The NADH measurement system utilizes an ultraviolet-light emitting diode (UV-LED) with peak emission of 335 nm as an excitation light source. The excitation light was introduced to an optical fiber probe, and fluorescence emission of neighboring NADH, which was produced by applying gaseous FA to the FALDH membrane, was concentrically measured with a photomultiplier tube. Assessment of the bio-sniffer was carried out using a standard gas generator. Response, calibration range and selectivity to other chemical substances were investigated. Circulating phosphate buffer, which contained NAD^+, was available for continuous monitoring of gaseous FA. The calibration range of the bio-sniffer was 2.5 ppb to 10 ppm, which covers the guideline value of the World Health Organization (80 ppb). High selectivity to other gaseous substances due to specific activity of FALDH was also confirmed. Considering its high sensitivity, a possible application of the bio-sniffer is continuous monitoring of indoor FA to provide healthy residential atmosphere.

ELECTROCHEMICAL IMMUNOSENSOR FOR ADRENOCORTICOTROPIN HORMONE

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ACTH is a 39 amino acids polypeptide hormone produced by pituitary gland. It acts stimulating adrenal cortex where glucocorticoids are secreted. Its determination allows to diagnose diseases as Addinson or Cushing syndrome, with an excessive increasing of cortisol concentration. From the point of view of sport doping, external ACTH administration is forbidden by the World Antidoping Agency (WADA), because it stimulates the production of corticosteroids and generates euphory feelings [1]. Normal levels of this hormone in plasma are between 10 and 60 pg/mL, decreasing throughout the day. Radioimmunoassay techniques (RIA) have been used for ACTH determination, but the sensitivity in not adequate for hormone levels lower than normal values. Thus, nowadays, immunometric assays with antibodies binding N- or C- terminal hormone epitopes are preferred.

This work describes for the first time an electrochemical immunosensor for the determination of ACTH in serum which uses aminophenylboronic acid to immobilize anti-ACTH directly to the electrode surface. A competitive immunoassay between the antigen and biotinylated hormone for the binding sites of antibody was established. The electrochemical response was generated using streptavidin marked with alkaline phosphatase, by measurement of the oxidation current of 1-naphtyl phosphate enzyme reaction product by differential pulse voltammetry at a screen printed carbon electrodes

Once optimized all variables involved in the immunosensor preparation and in ACTH detection, a calibration curve with a linear range between 25 pg/mL y 1ng/mL (r=0.995) and EC50= 0.10 ± 0.02 ng/mL was obtained. The limit of detection was 18 pg/mL and reproducibility RSD = 3.3 % (n=8). Cross-reactivity studies with other hormones (cortisol, estradiol, testosterone, progesterone, hGH and prolactin) demonstrated a good selectivity, with CR values lower than 0.001 % except for cortisol, with CR = 0.02 %.

It must be remarked the high sensitivity of the developed immunosensor, which compites very favourably with the method described in [1] for ACTH using LC/MS/MS, with a detection limit of 0.1 pmol/mL (500 pg/mL).

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DISPOSABLE MAGNETOIMMUNOSENSOR BASED ON STREPTAVIDIN-MAGNETIC BEADS FOR HUMAN CARDIAC TROPONIN T DETECTION

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Troponin T (TnT) is a useful biomarker for the diagnosis of myocardial cell damage used as an aid in the differential diagnosis of acute myocardial infarction because of its high sensitivity and specificity. Several methods have been described to detect TnT; however, they involve several steps and are time consuming.

In this work, an amperometric magnetoimmunosensor to detect TnT is developed based on a sandwich format. The binding of streptavidin magnetic beads (strp-MBs) to a specific biotinylated TnT antibody is used and, after TnT incubation, a HRP-labelled TnT antibody (anti TnT-HRP) is employed. The electrochemical detection of the enzyme product is carried out at disposable gold screen-printed electrodes (Au/SPE), using 3,3′,5,5′-tetramethylbenzidine (TMB) as electron transfer mediator and H_2O_2 as the enzyme substrate.

A suspension of 5 µL of strp-MBs is transferred to a 1.5 mL tube, washed twice with 100 µL B&W buffer solution to remove the NaN_3 preservative and resuspended in 100 µL of the biotinylated TnT antibody solution (2 µg mL^{-1}). The antibody is captured onto the beads for 30 min at 37 °C with shaking. Subsequently, the antibody-modified MBs are resuspended in 100 µL of a biotin solution. After 15 min at 37 °C under stirring, the modified-MBs are incubated with the TnT solution for 30 min at 37 °C. After the recognition reaction with antiTnT-HRP (45 min, 37 °C), the resulting modified-MBs are washed five times with 0.1 M sodium phosphate buffer solution (pH 7.0) and resuspended in 45 µL of the same buffer solution. Finally, the MBs are transferred to the pretreated Au/SPE and the amperometric response is recorded.

In order to obtain the best amperometric signals and, consequently, the maximum sensitivity in the determination of TnT, the experimental conditions of the developed procedure were optimized. Also, the analytical characteristics of the developed method were determined.

Fe_3O_4 NANOPARTICLES MODIFIED CARBON PASTE ENZYME ELECTRODES FOR AMPEROMETRIC DETERMINATION OF CREATININE AND CREATINE

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Creatinine is the final product of creatine metabolism in mammals and is a clinically important index of the renal glomerular filtration rate [1]. Creatine is extracted from the body as creatinine, thus the amount of creatinine in urine is proportional to the amount of creatine and is used to predict the total muscle mass [2]. The creatine level in serum is a marker of muscle damage. Therefore, fast and reliable determination of creatinine and creatine in biological fluids is routinely required for diagnosis and treatment. For creatinine and creatine detection, the most commonly used biosensor principle was described by Tsuchida and Yoda [3]. A three-enzyme sequence consisting of creatininase, creatinase, and sarcosine oxidase catalyzes the conversion of creatinine via creatine and sarcosine to glycine, formaldehyde and hydrogen peroxide. Recently, applications of nanomaterials to biosensors have aroused much interest. Among the various nanomaterials, magnetite nanoparticles have recently gained increased interest due to biocompatibility, strong superparamagnetic property and low toxicity [4].

In this study, two new amperometric carbon paste enzyme electrodes including Fe_3O_4 nanoparticles were developed for creatinine and creatine determination. Creatininase, creatinase and sarcosine oxidase or creatinase and sarcosine oxidase were immobilized into the carbon paste electrode by crosslinking with bovine serum albumin and glutaraldehyte. Determination of creatinine and creatine was performed by oxidation of enzymatically generated H_2O_2. The optimum working parameters of the enzyme electrodes have been investigated in detail. Optimum buffer concentration, pH and working potential were found to be 0.05 M; 7.0 and +0.30 V vs. Ag/AgCl. The linear working range of the Fe_3O_4 nanoparticles modified enzyme electrode was $1.9\times10^{-7}-1.3\times10^{-4}$ M and detection limit was 1.9×10^{-7} M for creatine. The repeatability, storage stability of enzyme electrodes and effects of interferences were studied. The performance of the enzyme electrode was tested on real samples.

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BIENZYMATIC AMPEROMETRIC BIOSENSOR BASED ON SELF-ASEMBLED MONOLAYERS AND GOLD NANOPARTICLES FOR LACTATE DETECTION

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Lactic acid is a metabolic product generated by lactic fermentation and it accumulates in muscular cells after intense anaerobic exercise. It is also the main carboxylic acid present in dairy products where it is produced by fermentation of lactose by lactic acid bacteria. Also, it is present in red wines as a product of malolactic fermentation, enhancing the flavor of wines because it have a lower acidity than malic acid. For this reason it requires rapid analytical methods for lactate monitoring, particularly in food analytical chemistry [1].

In this work we developed a bienzyme amperometric biosensor for lactate determination, based on modification of a gold electrode with self-assembled cysteamine monolayer, on which was immobilized colloidal gold. We used lactate dehydrogenase (LDH) and glutamate pyruvate transaminase (GPT) enzymes, glutamate and Meldola Blue (MB) to catalyze lactate oxidation[2]. LDH and GPT were immobilized by depositing 5 microliters of a solution containing 1 U / uL of LDH and 2 U / uL of GPT and then evaporate under nitrogen stream. Subsequently, the enzymes were retained onto electrode surface by crosslinking with glutaraldehyde.

The reactions catalyzed by immobilized enzymes are:

$$Lactate + NAD+ = pyruvate + NADH$$
$$Pyruvate + glutamate = alanine + alpha\text{-}ketoglutarate$$

GPT is included in the developed biosensor, to shift equilibrium of lactate oxidation to product formation and generate NADH.

In the presence of redox mediator MB, NADH is reoxydized, according:

$$NADH + MBox = NAD + MBred$$

Reduced Meldola blue is determined by direct amperometry to E = 200 mV, by using enzyme biosensor as indicator electrode, reference electrode Ag / AgCl and auxiliary electrode a platinum wire. The measurements were carried out with a potentiostat CH842 (CHInstruments). We obtained a highly selective lactate biosensor, with a linear response range of 0.45 mgL^{-1} - 10 mgL^{-1}, analytical sensitivity $3\cdot10^{-9}$ $Almg^{-1}$, LOD 0.2 mgL^{-1}, LOQ 0.6 mgL^{-1}. The biosensor is possible to apply for 8 days, with 10 measurements per day, with a loss of activity of 10% of initial signal. The biosensor has no significant interference in the presence of sugars (glucose and lactose) and other organic acids (acetic and malic), and it was applied in lactate determination in red wines.

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ELECTROCHEMILUMINESCENT DISPOSABLE BIOSENSOR BASED ON LUMINOL COPOLYMER FOR URIC ACID DETERMINATION IN URINE

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The use of electrochemiluminescence (ECL) could offer clear advantages as controlling the launch of reaction and good detection limits. The immobilization of reagents on disposable cells is a mayor issue, being one of the ways the polymerization of luminol on screen printed electrodes (SPE) by an electropolymerization process [1]. In this work, a electrochemiluminescent disposable biosensor for uric acid is presented by the formation of two layers on SPE cells. A first detection layer is based on the formation of a copolymer of luminol with 3,3',5,5'-tetramethylbenzidine (TMB), which offers improved characteristics such as better adherence and ECL intensity than polyluminol. One of the advantages using an immobilized copolymer by electropolymerization is the better emissive characteristics than the conventional entrapment by using cellulosic polymers as Methocel [2]. A second recognition layer contains the enzyme uricase using chitosan to entrap the protein. The good mechanical and improved electroluminescent characteristics of the new copolymer poly(luminol-TMB) make it possible to determine uric acid by measuring the growing ECL emission with the analyte concentration. The combination of enzymatic selectivity with ECL sensitivity results in a disposable analytical device with a linear range for uric acid from $1.5\cdot10^{-6}$ M to $1.0\cdot10^{-4}$ M, a limit of detection of $4.4\cdot10^{-7}$ M and an accuracy of 13.1 % ($1.0\cdot10^{-5}$ M, n = 10) as relative standard deviation. Satisfactory results were obtained for uric acid determination in 24 h-urine samples compared to a reference procedure. This uric acid biosensor can be used as a low-cost alternative to conventional methods.

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LABEL-FREE DETECTION OF TESTOSTERONE IN MILK AS MODEL SYSTEM FOR FOOD QUALITY MONITORING

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Milk is an important agricultural product and part of the everyday nutrition of humans. Besides being an essential food commodity itself, it is also the basis of many food products like butter, cheese or yoghurt. However milk is prone to contamination by a multitude of substances like environmental pollutants [1], hormonally-active substances, microorganisms [2] or mycotoxins [3].

In order to protect consumers health and prevent disease development, it is vital to develop reliable tools for detecting contaminants in milk before it reaches the consumer. The detection procedure should be sensitive, simple, cost-effective and fast to perform. In this work the label-free and time-resolved optical sensing method Reflectometric Interference Spectroscopy (RIfS) has been used. RIfS is based on white light interference at thin solid films. Binding of biomolecules to the sensitive layer is monitored by changes of the optical thickness.

Pasteurized whole milk is a complex matrix consisting of a variety of components such as proteins, fat and lactose which can interact with the sensor surface. Thus it is essential to investigate matrix effects of milk in the first instance. To do so, a testosterone assay in milk has been established. Testosterone has been used because it allows for very reliable quantitative measurements in buffer. The measuring program itself and the data evaluation had to be optimised because of disturbing effects like the Tyndall scattering or unspecific binding of milk contaminants on the sensor surface.

Testosterone has been quantified in milk using an binding inhibition assay. The calibration curve yielded LOD and LOQ values of 87,9 ng/L and 217,1 ng/L, respectively. Furthermore, very good recovery rates have been determined, which point out the great potential of this optical sensing system for analysis in milk.

Our gained knowledge will be used for the development of assays against different contaminants of milk like hormonally aktive substances, staphylococcal enterotoxins or polychlorinated biphenyls by means of RIfS.

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A SOLID-PHASE FLUORESCENT OPTICAL BIOSENSOR FOR THE DETERMINATION OF PHENOLIC COMPOUNDS

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Numerous spectrophotometric and fluorescent sensors are described in literature. However, their sensitivity and selectivity are not sufficient to solve many analytical problems. In this report we present the data on developing a novel biosensor of a simple construction which provides the sensitive and rapid determination of different phenolic compounds.

The basic element of the biosensor is a mirror plate covered with a sensitive layer which consists of the film-forming polymer chitosan labeled by fluorescence agent Rhodamine B isothiocyanate and the enzyme - horseradish peroxidase.

The operation of the proposed sensor is based on two subsequent reactions:

i) peroxidase-catalyzed oxidation of a phenolic compound;

ii) interaction of the oxidized product with labeled chitosan.

The analytical signal is an emission spectrum (λex and λem are 550 and 580 nm, respectively) of the sensitive layer after its contact with the tested solution and air-drying. Fluorescence quenching is observed as a result of the analyte oxidation in the presence of the biocatalyst.

A number of isomers of disubstituted phenolic compounds (hydroquinone, catechol, and resorcinol) and catecholamines (dopamine and adrenaline) were tested as the model analytes for the evaluation of the sensor performance. It has been shown that the detection limits of the considered compounds are in the range of 0.1 – 10 µM. The sensor is characterized by good reproducibility and stability and provides the low-cost and simple determination of the listed analytes.

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DEVELOPMENT OF A SYSTEM FOR THE PARALLEL DETECTION OF PEPTIDE-ANTIBODY INTERACTION IN AUTOIMMUNE DISEASES

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In the diagnosis of autoimmune diseases the detection of autoantibodies plays an major role. Characterization and detection of such autoantibodies are important to improve the diagnosis, to help predicting the course of a disease and to allow a decision regarding individualized antigen-specific therapies. It has been shown that peptide arrays can be used as a tool for detecting autoantibodies and for epitope mapping[15].

Here we present the characterization of peptide-antibody interaction by imaging Reflectometric Interference Spectroscopy (iRIfS). This method has been developed on the basis of the direct-optical method Reflectometric Interference Spectroscopy (RIfS)[16] and enables parallelized biomolecular interaction analysis in array format up to 1000 spots per 1.5cm². RIfS has been used for a variety of applications such as antibody characterization[17] and analysis of regulatory proteins e.g. the estrogen receptor alpha[18]. Being time-resolved methods, RIfS and iRIfS allow the determination of kinetic and thermodynamic constants and thus to characterize biomolecular interactions. Even the detection of low-affine antigen-antibody interactions is possible. Both points are favorable in diagnosis and surveillance of autoimmune diseases. As an example we choose celiac disease. One pattern of celiac disease is the formation autoantibodies against Type2-Transglutaminase, an enzyme found in the intestine. We examined the interaction of different peptides with anti-transglutaminase antibodies.

Using a direct-optical method, firstly, a sophisticated and well optimized surface chemistry was needed to minimize non-specific binding to the sensor surface. Secondly, for parallel analysis of peptides we needed to establish a robust spotting procedure in order to obtain reproducible peptide arrays. Both were the basis for the following parallelized detection of peptide-antibody interactions. As a model system we spotted arrays of five sequential peptides, partially covering the antibody's epitope. The measurements revealed differences in the binding signals which coincided with the different affinities of the peptides to the antibody. Additionally, we performed measurements in real patient samples and were able to differentiate between binding characteristics of positive and negative samples. Future plans are the parallelized analysis of kinetic parameters and affinity constants of peptide-antibody interactions.

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DISPOSABLE ELECTROCHEMICAL MAGNETOIMMUNOSENSOR FOR THE DETERMINATION OF HUMAN GROWTH HORMONE (hGH)

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Human growth hormone (hGH) is a polypeptide hormone secreted by the anterior pituitary gland which is essential for normal growth and development [1]. The determination of hGH in serum is necessary to diagnose various disorders occurring in childhood as well as the existence of pituitary tumours. Furthermore, detection of hGH is also important in sport medicine because this hormone is used to increase performance in some disciplines [2]. Normal hGH levels in blood are between 50 and 100 ng/mL, but minumum levels of 0.03 ng/mL can also be detected. Methods described for the determination of hGH are mainly based on radioimmunoassay (RIA) or ELISA. Very recently, an impedimetric biosensor [3] and various designs based on surface plasmon resonance (SPR) [1] have been reported.

In this work we describe for the first time the preparation and application of an electrochemical immunosensor for hGH. This is based on the use of tosyl-activated magnetic microparticles (tosyl-MBs) to covalently immobilize monoclonal mAb-hHG antibody. A sandwich type immunoassay with a secondary pAb-hGH antibody and anti-IgG labeled with alkaline phosphatase (AP) was developed. Tosyl-MBs-mAb-hGH/hGH-pAb-hGH-anti-IgG-AP conjugate was further deposited onto the surface of a screen printed gold electrode and detection was performed using square-wave voltammetry (SWV) after the addition of 4-aminophenyl phosphate.

Once immobilized all the variables involved in the preparation of conjugate and the immunoassay protocol, a calibration curve was obtained with a linear range between 0.01 and 100 ng/ mL (r= 0.998) and a limit of detection of 0.005 ng/mL. A good repeatability, with RSD = 3% (n= 10) at the 1 ng/ mL hGH level was also obtained. Cross-reactivity studies with other hormones similar to hGH demonstrated a good selectivity. Finally, the application of the biosensor to the analysis of human serum spiked with hGH at the 4 ng/mL gave a medium recovery of 96 □ 6 %.

The analytical characteristics of the immunosensor are better than those from other reported configurations. For example, the limit of detection is approximately three orders of magnitude lower than that obtained in [1] (4 ng/mL). This advantage is due to the high capacity for immunoreagents preconcentration from MBs combined with the use of a high sensitivity electrochemical technique

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LABEL-FREE DNA DETECTION BY USING NANO-SPASED ELECTRODE

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Molecular electronic devices promise a straightforwardness in signal interfacing and in the fabrication of nanosized integrated systems. We have assembled a nano-gapped electrode system using a gold particle and successfully applied this system to a label-free electronic DNA sensing.

A nano-gapped electrode was prepared as follows: An interdigital Pt microelectrode was electrochemically cleaned by repeated potential sweeps. The electrode was immersed in 5 mM ethanolic decanedithiol for 30 min, then immersed in the Au dispersion for 10 min to anchor the Au particle on the electrode. The modified electrode was immersed again in the dithiol solution and then in the dispersion to obtain a film on the glass. The procedures were repeated until a desired resistance (~200 ohm) was obtained.

The particle film was modified with thiolated probe DNA by applying a TE buffer containing 500 pmol of a 12-base single-strand DNA (5'-HS-TCT CAA CTC GTA-3') on the Au film, and then the electrode was allowed to stand for 30 min. The electrode was thoroughly rinsed with a TE buffer to remove the excess probe, and dried under a N_2 stream. After the resistance became steady, a TE buffer involving sample single-strand DNA (500 pmol) was added over the film for hybridization. The resistance was monitored at room temperature with a digital multimeter (applied current: 1 mA) using a standard two-probe configuration. The sensor was immersed in water at 353K for 3 min to denature the hybridized DNA for the next measurement. All the experiments were repeated at least 3 times and the resistance values reported for the sensing had an accuracy of ±0.01 ohm.

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NON-INVASIVE CHOLESTEROL DETECTION BY USING MOLECULARLY IMPRINTED ELECTRODE

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Control of the total cholesterol level in the body plays an important role for preventing life-style related diseases. The conventional method is troublesome due to the necessity of going to a hospital for invasive blood collection and using the enzyme reaction through many procedures. On the contrary, about 11 percent of the body's cholesterol is found in the skin at the same rate as in the blood, according to the FDA. Therefore, we focus on a simple and non-invasive measurement for cholesterol using a molecularly imprinted self-assembled monolayer (SAM).

A gold electrode was immersed in an ethanol solution containing cholesterol and stearylmercaptan, and then washed in ethanol in order to extract the cholesterol as a template molecule. The extraction of cholesterol molecules creates shape-complementary cavities on the SAM, and the detection of electro-inactive cholesterol is achieved using an electrochemical method with potassium ferrocyanide as the redox marker. The change in the oxidation peak current shows a linear relationship with the cholesterol concentration. The change of current is related to the cavity concentration for the mass-transport of the redox marker on the molecularly imprinted SAM. When the cholesterol-sensitive SAM recognizes cholesterol, the current decreases due to marker diffusion rejection to the gold electrode surface. On the contrary, when the SAM extracts cholesterol, the marker diffuses to the electrode surface and the current increases. The sensing properties of the molecularly imprinted SAM, such as sensitivity, selectivity, and reproducibility, have been examined, and it has been applied for simple and speedy electrochemical sensor development.

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ELECTROCHEMICAL CHARACTERIZATION AND STUDY OF THE ANTIOXIDANT PROPERTIES OF PHENOLIC ACIDS USING A dsDNA ELECTROCHEMICAL BIOSENSOR. APPLICATION ON AQUEOUS HERB EXTRACTS.

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The last decade, the scientific community has been trying intensively to understand and explain the connection between ROS, DNA oxidative damage, various disorders and diseases and antioxidants [1]. Electrochemical DNA biosensors are promising tools for fast, inexpensive and simple in vitro analysis for the determination of free radicals and antioxidants [2]. Reactive Oxygen Spieces (ROS) and especially hydroxyl radicals ($^{\bullet}$OH), are known to damage DNA by oxidation of the bases, which results in their destruction, release or attack of the deoxyribose moieties by strand breaking. These lesions are related to the aging process, cancer, Alzheimer's disease and atherosclerosis. In living systems, hydroxyl radicals are generated from the metal (ferrous or cupric ions) ion-dependent breakdown of hydrogen peroxide by Fenton' s reaction, which is an important mediator of oxidative damage in vivo. Antioxidants such as phenolic acids and plant extracts containing high amounts of them, act as free radical terminators and reduce the effect of the oxidative damage on dsDNA [3].

In the present work, we studied the electrochemical behaviour of three representative phenolic acids, caffeic acid, gallic acid and trolox using cyclic voltammetry. We investigated the determination of the above antioxidants and the optimized conditions (scan rate, deposition potential and time) using differential pulse voltammetry. Moreover, we performed in vitro studies upon their effect on the oxidative damage on dsDNA, using adsorptive transfer stripping voltammetry. We used a dsDNA modified carbon paste electrode, a Pt wire counter electrode, a Ag/AgCl reference electrode and Fenton's system, by $FeSO_4$ and H_2O_2, as a strong oxidative system. Our conclusions were based on the oxidative signal of guanine and adenine bases before and after the addition of certain amounts of the antioxidants. This biosensor has been applied as a screening antioxidant test in order to estimate the antioxidant capacity of aqueous herb extracts.

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BIOSENSOR BASED ON OVEROXIDIZED POLYPYRROLE FILM

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Although medicine and healthcare have made fundamental and tremendous progress to cure infectious illness, microorganism contamination is still substantial threat for human being. Therefore, hygiene control in food industry and medical front has become very important from a viewpoint of food safety and public helth. Effective detection of microorganisms in food items or medical equipments carries weight to prevent foodborn diseases and hospital infections. Although there have been many methods to detect microorganisms such as cultural procedure, sandwich immunoassay formats, and ATP detection, these methods are time-consuming and labor-intensive. Moreover, no groundbreaking way has appear to solve the sensitivity and selectivity. In this study, we have effectively utilized Molecular Imprinted Polymer (MIP) film consisted of polypyrrole (PPy) to detect microorganism specifically and rapidly [1-3]. The microorganism detection has been performed using overoxidized polypyrrole (OPPy) film based on Quartz Crystal Microbalance (QCM) sensing coupled with electrophoresis method. PPy film was prepared on the quartz crystal (Au) by applying a constant potential (+0.98 V vs. Ag/AgCl) in a phosphate buffer solution (0.2 M, pH=2.6) including pyrrole (0.1 M) and Pseudomonas aeruginosa (1.5×10^9 CFU/mL). A constant potential (+0.98 V vs. Ag/AgCl) was then applied in an aqueous sodium hydroxide (0.1 M) to overoxidize PPy film, which induces to remove Pseudomonas aeruginosa taken in the PPy film. The resonance frequency change of the OPPy modified electrode, having Pseudomonas aeruginosa cavities, was monitored while electric field (frequency: 100 MHz, voltage: 20 Vpp) was impresed to the electrode in ultrapure water including Pseudomonas aeruginosa as a target. Pseudomonas aeruginosa was taken up in the PPy film in one direction, according to the SEM observation. After the overoxidation treatment of PPy film in the alkaline solution, many cavities were formed on the OPPy film due to the removal of Pseudomonas aeruginosa. To clarify the selectivity of this OPPy film for Pseudomonas aeruginosa, the experiments was conducted using Acinetobacter calcoaceticus and blank solution. The response was the highest for the Pseudomonas aeruginosa. Meanwhile the change in the freuency was hardly observed for the Acinetobacter calcoaceticus and blank solution. It indicates that the OPPy film which has the microorganism cavities specifically recognize the target species.

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Poster presentations
Session B

Analytical Chemistry in Cultural Heritage	AH01-AH15
Advanced Material Analysis	AM01-AM09
Spectroscopy in Analytics	AS01-AS35
Environmental Analysis - Part I	EN01-EN55
Food Analysis – Part II	FA41-FA83
Forensic Challenges for Analytical Chemists	FO01-FO07
Analytical Instrumentation Development	ID01-ID07
Mass Spectrometry	MS01-MS21
Process Analysis	PA01-PA03
Radioanalytical Chemistry	RC01

INVESTIGATION OF XVIII CENTURY ROBERT HUBERT'S PAINTING WITH EDXRF, SEM-EDX AND OM ANALYTICAL TECHNIQUES

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During conservation work on the Robert Hubert 's painting „Staircases in the Park of Villa Farnese in Caprarola” dated in the XVIII century it has been noticed that the original dimensions of the painting have been changed by adding strips of the canvas along of all four edges of the original painting.

Several samples of cross sections from the original painting and added strips were investigated with optic microscope in reflected light and under the UV light and also SEM EDX elemental analysis was performed on some samples. These analyses revealed different ground layers on the original painting and on the strips and the presence of two layers of the paint on some parts of the strips separated with varnish.

The question of the period in which these strips have been added and over painted is emphasized and further detailed analysis was applied. We used portable EDXRF spectrometer specially designed for analysis of paintings and frescoes, equipped with motorized platform with measuring head which consists of AMPTEK X123 spectrometer with Si-Pin Detector and focused Rh X ray tube. In that way we have been able to nondestructively analyze a large number of points with different colors (pigments) on this large scale painting (148x217 cm) including points from original size painting and from added strips.

Analyses have shown the presence of the some elements characteristic for specific pigments only on the strips such as zinc, copper, chromium, cobalt and arsenic. Since the copper and the arsenic are showing linear correlation in some points it is concluded that one of the green pigments on the strips is emerald pigment dated not earlier than year 1814. Also blue and green pigments on the stripes are different from those from original painting and permanent presence of Zn peak in every spectrum recorded on added strips indicates usage of zinc white pigment. Identified pigments on the original painting correspond with known period in which painting was made (1768-69). On the other hand, identified pigments on the added stripes can be dated later in the XIX century. Presence of two layers of paint on the some parts of the added strips is a major difficulty in process of the establishing period of the changes on the original painting.

A NEW WAY OF IDENTIFYING STARCH FROM DIFFERENT NATURAL SOURCES IN ANCIENT DOCUMENTS USING CAPILLARY ELECTROPHORESIS

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Starch is a natural polysaccharide obtained from different sources such as wheat, maize and rice. This polysaccharide has been used in the manufacture of Arabic paper[19] (a procedure called *sizing*) to make it more resistant to the application of the inks, and to give it a greater consistency. There are some references that correlate the presence of starch in the paper to its Arabic fabrication[20].

For an analytical bibliographer or any other scholar interested in mediaeval Arab texts it is essential to be able to analyse the substances contained in the components of these documents. The detection of starch in the manuscripts of some collections located in Granada is important both in their preservation and their classification as belonging to the al-Andalus period or not. It is of equal importance in knowing how to go about their restoration when necessary.

Samples available for analysis of these documents are extremely limited and so any such study needs to be highly selective and sensitive. We have found that capillary electrophoresis is a very suitable technique in these circumstances. We have developed a new methodology to identify this polysaccharide in paper, since the determination of these substances has so far been mainly used in food samples[21]. Moreover, we have carried out an artificial ageing study of starch to observe how the passing of time can affect to the detection of this analyte. Despite the complexity of the analyte and its study in paper, we have obtained good results which will allow us to classify the ancient documents properly.

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ELECTROTHERMAL VAPORIZATION INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY FOR CHARACTERIZATION OF ARCHAEOLOGICAL GLASSES EXCAVATED IN WEST BULGARIA

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Glass was used on Bulgarian territories since the end of 6th cent. BC. Together with intense import from Egypt, Byzantium and the Near East, during the first millennium local glassmaking was also developed. Only a small part of the archaeological glasses found in Bulgaria is studied in detail so far, while new, uninvestigated artefacts, e.g. window glasses, vessels and jewellery are discovered recently. Object of the present work are fragments of ancient Roman windows and medieval glass bracelets excavated in West Bulgaria.

Electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP-OES) was applied for determination of major, minor and trace elements in the investigated archaeological glasses. The finely ground glass samples were analyzed directly and CHF_3 was added as evaporation and transport modifier. Certified reference materials with different matrix (soda lime glass, fly ash and stream sediment) and evaporated aqueous standard solutions were used as calibration standards as well as for method validation. Under the optimized conditions only negligible matrix effects were observed. Precision and accuracy were comparable to conventional analytical methods after sample decomposition. ETV-ICP-OES results were in good agreement with those obtained by means of an independent method - flame atomic absorption spectrometry of digested samples.

The analytical data were used to establish the type of glass, the fluxing agents, the typical colouring and decolouring elements and the recipe norm. ETV-ICP-OES proved to be a reliable, rapid and low-cost method for characterization of archaeological glasses.

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CLASSIFICATION OF NEOLITHIC CERAMICS USING EDXRF SPECTROMETRY AND MULTIVARIATE STATISTICAL ANALYSIS

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As a nondestructive technique, Energy Dispersive X-Ray fluorescence (EDXRF) is widely used for characterization of archaeological artifacts. Elemental composition of investigated artifacts, obtained by EDXRF, has a great importance in ascertainment of ceramic provenience. In this work, the results of investigation of 67 ceramic findings are presented. Different ceramic artifacts (pottery pieces, ceramic sherds, figurines, altars) from three different Neolithic sites (Vinča, Bubanj and Pločnik) located on the teritory of Serbia were analized. Principal component analysis (PCA) and discriminant analysis (DA) were used for multivariate statistical analysis in order to classify examined ceramic pieces. The aim of classification is to distinguish the local production and ceramics which were produced on the other locations. The comparison of obtained clasiffication results have shown that DA is more efficient method for this purpose.

NON-DESTRUCTIVE ANALYSIS OF MUSEUM ARTEFACTS

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Determination of quantitative and qualitative chemical composition has become an important tool for characterization of archaeological and muzeum artefacts. The main requirement to the methods used in this case is their non-destructiveness. Moreover, any possibility of damage to the object investigated should be eliminated as a rule.

In this work, a number of non-destructive methods were used for the determination of precious metals content in coins, medals and museum jewellery of 18 - 20th century. The items investigated were: Russian platinum coins minted in 1828-1845 and in 1977-1980, Russian gold and silver medals of 19th century, Fabergé platinum rings and several other jewellery. The items were complex-shaped and varied in size (from several millimeters to tens centimeters).

The method used were:
- X-ray fluorescence spectrometry (XRF);
- ICP atomic emission spectrometry (ICP-AES) combined with touchstone test;
- Spark ablation ICP atomic emission spectrometry (SA-ICP-AES).

Besides, density and acoustic measurements of the items were carried out to support the conclusions about their chemical composition.

All the techniques were tested with certified reference materials of jewellery alloys. The results showed good agreement with the certified values. As the used methods can be applied only for the analysis of surface layers of the artefacts the bulk analysis was performed for some items from which were allowed to take samples for wet analysis. In this case, solution ICP-AES method was used.

The main investigation method was XRF performed using two portable X-ray spectrometers: PRIZMA-M Au (Russia) and InnovX-System Omega (USA). The portable spectrometers were especially suitable for the purpose because of the possibility to use them *in situ*, i.e. in museum premises. ICP-AES measurements were performed at ICP atomic emission spectrometer ICAP 61E SSEA (USA) equipped with spark ablation chamber.

In old platinum coins, investigated platinum content was 96-99%, the main impurity elements being Ir (up to 2%), Fe (up to 2%), Cu (up to 0.5 %), Rh and Pd (about 0,1%). This chemical composition correlated with the density values 19,7 – 21,2 g /cm^3 which were less than a density value for modern platinum coins and for pure platinum (21,45 g /cm^3). The results were consistent with ones obtained earlier [1] and could be explained by peculiarities of the early 19th century platinum refining process.

Some of the investigated old gold and silver medals showed chemical composition inconsistent with their measured density. The content of precious metals in gold items were: 70 – 99 % (Au), 0,1 – 24 % (Ag).

The results of non-destructive analysis of precios metals artefacts were used in the investigation of their autentity and provenance.

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LC-ESI MS/MS STUDY OF HISTORICAL TEXTILES - NEW ANTHRAQUINONE COLORANTS

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Pre-Columbian cultures had been developed in South America until they were conquered or significantly influenced by Europeans. One of the main territory, the ancient Peru, for thousands of years had been inhabited by successive great indigenous civilizations, such as Chavin, Nazca, Chimú and Chancay. In the 15th century most of the contemporary cultures joined the Incas, which formed the largest empire in pre-Columbian America, ended in 1532, after the Spanish conquest. Nowadays, textiles left behind all of these cultures are the best known artefacts dominated by the red-pink shade of cochineal. It is observed because of the presence of carminic acid, kermesic acid, flavokermesic acid, dcII, dcIV and other anthraquinones, of structures so far unknown.

The present study concerns identification of new cochineal colorants extracted from pre-Columbian Peruvian textiles. The identification was based mainly on the ESI MS/MS spectra obtained in the negative ion modes. This detector was combined with high performance liquid chromatography (HPLC), which enabled separation of the analyzed compounds. Spectra registered for each of the examined colorants allowed to identify their lost fragments, which in consequence made possible reconstruction of their molecular structure.

AH10 **Session B**

ARCHAEOLOGICAL GLASS BEADS DECORATED WITH METAL FOIL – A CHALLENGE FOR ANALYTICAL CHEMIST

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The glass beads decorated with a metal foil are occasionally found at archaeological sites. They have a complex multilayer structure, including colorful or colorless glass body covered with thin metal foil and outermost overlay of colorless glass. There are insufficient evidence on their provenance and production technology, therefore the knowledge about the elemental composition of such objects could be important for supporting studies of their typology. Suitable analytical methods which can be used for such investigations must fulfill a number of requirements. Usually the possibility of performing direct determination from solid artifacts, without any sample preparation is verbalized as the main one among them. This is crucial because historic glasses represent great cultural value for historical and archaeological research. Moreover, the stratified structure of the beads should be analyzed using methods that enable to examine not only the surface layer, but also subsurface domains, without necessity to cut objects and examine their cross-sectioning. Techniques, which are routinely used in historical glass analysis, like: EPMA, SEM-EDS, or less frequently PIXE/PIGE and NAA do not fulfill the last mentioned requirement.

In this work exemplary glass beads decorated with metal foil and excavated at the archaeological site in Lubień (Poland) were investigated. The elemental composition of body and overlay glasses was of interest, as well as the kind of metal foil used for beads decoration. The Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) was used, while it allows direct, multi-elemental analysis of solids' bulk and/or surface composition. The depth of ablation was controlled by choosing different laser parameters and optimization of the whole laser micro-sampling strategy.

It was established that the examined beads bodies were made of different types of glass. Moreover, despite the visual similarity, the different types of foil were found to be used for their decoration.

The results of this research will be presented and the usefulness of the LA-ICP-MS technique in such kind of investigation will be discussed.

A MULTI-TECHNIQUE APPROACH FOR CHEMICAL CHARACTERIZATION OF AMBER

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Amber is a fossil resin, extremely appreciated for its colour and beauty, and used as a gemstone from very early times. There are many amber species and varieties; the most well known are the Baltic amber, the Dominican amber, the Sicilian amber named simetit and the amber from Romanian Carpathians named Romanite. In the present work, we used X-ray photoelectron spectroscopy (XPS) and micro-Raman techniques for analytical characterization of amber samples both on controlled (geological) and unknown (archaeological) origin.

Small fragments were investigated by XPS in order to determine their surface elemental composition, obtain evidence of the presence and content of sulphur species and to evaluate possible differences among the geological and archaeological amber types. The amber samples from the Baltic region are similar in C, H, and O contents and differ in S concentrations from the romanite samples. The latter type of amber contains a rather wide range of trace elements, Al, K, Ca, Fe, that are lower in content or absent on the Baltic amber surfaces.

The micro-Raman technique was used to characterize amber from the bulk chemical composition point of view. The differences in the spectral profile of amber samples from various origins are related to differences in level of maturation rather than geographical sources.

We establish several definite criteria which will differentiate various types of amber to certify the Romanite or Baltic origin of the materials found in archaeological sites on the Romanian territory.

IDENTIFICATION OF PROTEIN BINDERS IN ARTIST'S PAINTS BY MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

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In the present work, we attempted to identify natural proteinaceous binders usually found in artworks by using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) peptide mass fingerprint (PMF) approach. MALDI-TOF MS has proven to be a valuable tool for protein binder identification in recent years. Sample preparation is not extensive and thus sample losses are minimal. PMF approach usually involves database search for comparing the resulting mass spectra with calculated peptide masses based on protein cleavage *in silico*. These studies, sometimes gave few results for paint binders, because of complexity of samples. Nevertheless, additional informations obtained from MALDI-TOF/TOF- experiments were the key for solving this problem and confirmed presence of different proteins in artworks samples.

As reference materials, we used proteinaceous binders from egg (egg yolk and white), milk (cheese and casein powder) and different collagens (rabbit, bovine, porcine and sturgeon). The mass fingerprint of each studied binding medium were established. The effect of pigments on the identification of proteinaceous binders was evaluated through the analysis of paint replicas prepared with proteinaceous materials named above. High concentration of inorganic pigments interfere the correct identification of the proteins. C_{18} pipette tip purification procedure was adapted for cleaning up peptides after tryptic hydrolysis step, thus eliminating signal suppression due to salts and inorganic compounds.

Tryptic digests of reference materials and model samples were analyzed by Bruker Daltonics Autoflex III Smartbeam MALDI-TOF mass spectrometer. *Biotools* 3.2 software connected to the *Mascot* search engine was used for analysis of MS data and protein identification by interrogating the SwissProt database. Peptides that can be used as molecular markers for animal glues, milk and egg proteins were identified, and their assumed sequences are confirmed by MS/MS analysis and Mascot database search. MALDI-TOF/TOF was for the first time applied for analysis of proteinaceous binders from cultural heritage.

INVESTIGATION OF A LATE ANTIQUE WINDOW GLASS FROM PERNIK, BULGARIA: A CASE STUDY

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At the beginning of the first millennium the territories of modern Bulgaria were part of the Roman provinces Moesia and Thracia. In the middle of 3rd cent. AD these regions of the Roman Empire were affected by barbaric invasion and the Balkans became an arena of social changes. Thus the material culture and art were modified. The destruction of buildings and acceptance of Christianity as an official religion led to new building. Glassmaking has been developed to cover the needs of flat window glass for churches and luxury homes. From the Roman period three glassmaking workshops (in *Oeskus, Nove* and Varna) have been discovered by archaeological excavations on the territories of modern Bulgaria so far.

Object of the present study were fragments of a window glass excavated in Roman late antique villa (Pernik, Bulgaria) and dated from 5th cent. AD. Visible spectra were recorded and DTA was applied to determine some technological parameters of glass manufacturing. The microstructure and chemical composition of glass surface were investigated by SEM-EDS. Bulk chemical analysis was performed according to standard procedure. Gravimetric chemical analysis was used for silica assessment. Flame photometry was applied to determine sodium and potassium content while aluminium, calcium, magnesium, iron and cobalt were determined by flame atomic absorption spectrometry after dissolution with a mixture of acids. There is a good agreement between results from bulk and surface analyses which is a proof for the high homogeneity of the investigated glass.

Regarding its chemical composition and literature data, the artefact was related to the soda-lime-silica group and was made according to the so-called Roman-province recipe norm. The present study contributes to estimate the technological level of glassmaking during the Roman period.

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SURFACE QUALITY MEASUREMENT OF Ni - Al_2O_3 COMPOSITE COATINGS

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Nickel composite coatings were prepared by electrochemical deposition of nickel from conventional nickel sulphate bath in which γ--Al_2O_3-nanoparticles were suspended. Composite coatings were obtained at different current densities and different concentration of suspended particles in the bath but at a constant temperature and stirring rate. Depositing time was estimated based on the applied current density to get coatings of approximately the same thickness. Copper plates were used as a substrate in preparing the composite coatings.

Surface topography and the structure of coatings were then investigated by using optical and SEM microscopy as well as metallographic analysis of cross-sectional areas of coatings and by X-ray analysis. To get better insight of coating surface, amplitude surface roughness parameters as: Ra, Rp, Rv, Rz were determined, as well as hybrid roughness parameters like Rpk, Rvk, Abbott – Firestone curves, Mr1, Mr2 were also measured. As a referent sample for comparison coatings obtained from nickel bath without particles but at the same other experimental conditions were used. It was found that the embedded alumina particles significantly affect the surface morphology as well as microstructure of composite coatings. Increase of the particles captured in coating causes increase change of its properties. Obtained results have shown an increase in surface roughness parameters with increasing both depositing current density and alumina particles concentration in the bath. Produced composite coatings have had a relatively flat (smooth) surface area (a low fraction of peaks, parameter Mr1), but with a lot of dents on the surface area (high value of parameter Mr2). Also, the values of other measured surface roughness amplitude parameters as well as the Abbott-Firestone curves, confirmed this findings.

Optical and SEM microscopy analysis supported results obtained by measuring the surface roughness parameters. X-ray analysis proved the presence of Al_2O_3 particles in the coatings while the metallographic analysis has shown uniform distribution of particles within the whole coating depth.

THE DISSIMILARITY ESTIMATION OF BINDING SITES IN ORGANICALLY MODIFIED MULTIFUNCTIONAL POROUS SILICA

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During past decade's publication was devoted to preparation and variety of applications silica-based materials. Most of the chemical properties of these materials depend on the condition of their synthesis and the support morphology. The reason for such dependence is multifunctional nature of the interfacial layer. These composite materials often have several types of the organic groups immobilized of the same support and real composition of interfacial layer is unknown. Common chemical analysis is, in other words presence is on the surface of silica of a few types of functional groups. Quantitative determination of which on the surface of functionalizing silica well-known physico-chemical methods is difficult problems. For example, elemental and thermogravimetric analysis is performed that information about general amount of bonded groups, and using the method XPS, you can establish the valence of all components of the sample, but their contents to determine only semi quantitative.

In this work the study was focused on analysis interfacial layer silica with bonded acidic or basic groups which were synthesized multistage. For this purpose, silica with bonded acidic (pyridine carboxylic (SiO_2-PyC), iminodiacetic (SiO_2-IdA), methylaminophosphonic (SiO_2-Ph)) and basic (amino- and ethylenediaminopropyl) groups were chosen. As a research method was suggested conductometric titration. It was shown that these method is promising not only concentration of functional groups and for estimating number target and residual groups in interfacial layer silica. The residual groups can bind protons of the dissociated phosphonic acid present in the immobilized layer. For this reason, part of the immobilized groups of aminophosphonic acid will be on the surface in the acid-form $(\overline{H_2L})$ of and the other part, in the deprotonated form $(\overline{HL^-})$. It turned out that conductometric titration allowed the concentration of each of these forms were determined. Thus, interfacial layer various samples SiO_2-Ph consist of 53-81% methylphosphonic acidic groups and 47-19 % of residual aminopropyl groups. By comparison interfacial layer of samples SiO_2-PyC consist of about 50 % residual aminopropyl groups. The analysis of suspension SiO_2-IdA demonstrated that, of the total amount of bonded iminodiacetic acid groups about 75% were occupied in $(\overline{HL^-})$ form.

POSSIBILITIES FOR ANALYSIS OF WASTE WOOD ASH WITH TOTAL X-RAY FLUORESCENCE ANALYSIS

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The ash from burning of wood wastes in pulp and paper production is big part from the generated production wastes in the factories. The utilization of the ash like secondary raw material, help for decreasing of the expenditure of the factories for situation and for improvement of the ecological situation in region. The secondary use of this type of waste, enforce full characterization of its chemical composition and properties, with a view of protection of the environment and the human's health. The aim of the current paper is characterization with the Total x-ray fluorescence (TXRF) technique of the wood ash separated from the pulp and paper production. By this method, with minimal sample preparation and decreased time of analysis, with high accuracy and reproducibility the concentrations of larger part from the harmful inorganic substances can be determined. In four ash samples were established the concentrations of the analyte elements: Cl, K, Ca, Mn, Zn, Br, Rb, Fe, Ni, Cu, Sr and Ba.

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SEQUENCE ANALYSIS OF THERMOPLASTIC POLYURETHANES BASED ON POLY(ε-CAPOLACTONE)-*b*-POLY(DIMETHYLSILOXANE)-*b*-POLY(ε-CAPOLACTONE) BY QUANTITATIVE ^{13}C NMR SPECTROSCOPY

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The analysis of the microstructure, i.e. distribution of hard segment length (number of repeating 4,4'-methylenediphenyl diisocyanate - 1,4-butandiol units, (MDI-BD)$_n$) in thermoplastic polyurethanes based on poly(ε-capolactone)-*b*-poly(dimethylsiloxane)-*b*-poly(ε-capolactone) prepolymer by quantitative ^{13}C NMR spectroscopy is reported. This was accomplished using the signals arising from nonprotonated aromatic carbons, which appear to be sensitive to sequence length distribution. The structure and composition of the investigated polyurethanes were determined by ^{1}H, ^{13}C NMR and two-dimensional correlation (COSY, HSQC and HMBC) spectroscopy. The composition of the polyurethanes, *i.e.*, the content of hard (MDI-BD) and soft (PCL-PDMS-PCL) segments, was calculated from the ^{1}H NMR spectra as the relative intensities of the methyl proton signals from the $–SiC\mathbf{H}_3$ groups and MDI aromatic proton signals. These results show that the weight fractions of the hard segments were in the range from 8.5 to 62.6 wt%. It could be concluded that the composition of the copolymers were in good agreement with those expected from the composition of the feed. The hard segment length was calculated by ^{13}C NMR spectra from the ratio of the integral of aromatic carbon signal from the MDI connected to 1,4-butanediol and that of the aromatic carbon signal from the MDI connected to ε-caprolactone. The average hard segment length varied from 1.2 to 14.4 MDI-BD units and was lower compared to the statistically calculated values. According to the obtained results, ^{13}C NMR analysis provides a relatively simple and reliable method to determine the microstructure of polyurethanes.

CHARACTERIZATION OF POLYURETHANE CROSSLINKED STRUCTURES BASED ON HYPERBRANCHED POLYESTER

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The novel polyurethane crosslinked structures based on α,ω-dihydroxy-(ethylene oxide-poly(dimethylsiloxane)-ethylene oxide) (EO-PDMS-EO), 4,4'-methylenediphenyl diisocyanate and Boltorn® hyperbranched polyester of the second pseudo generation were characterized by infrared spectroscopy, scanning electron microscopy and thermogravimetric analysis. The chemical structure and hydrogen-bond interactions of these polymers were investigated by infrared spectroscopy. The carbonyl region was fitted by the Gaussian deconvolution technique, using the PeakFit program, resulting in the determination of locations and areas of each band. The polyurethanes exhibited five absorbance peaks in carbonyl region: hydrogen-bonded carbonyl groups in ordered hard domains at 1690 cm^{-1}, free (non-bonded) carbonyl groups at 1735 cm^{-1}, hydrogen-bonded carbonyl groups in disordered domains at 1715 cm^{-1}, free carbonyl groups from ester bonds at 1725 cm^{-1} and hydrogen-bonded carbonyl groups from ester bonds at 1650 cm^{-1}. The deconvolution procedure showed very good agreement between observed and generated values. The fit standard error was in the range from 0.00013 to 0.0028 and $r^2 > 0.994$. The hydrogen bonds formation between urethane groups and between urethane –NH and ester carbonyl groups in polyurethanes increases with the decrease of EO-PDMS-EO content. The EDX analyses, performed to indentify the nature of the atoms present in the samples at a depth of 100-1000 nm from the surfaces, revealed the presence of all expected elements (C, O, Si and N). The Si percentage detected by EDX on the surface of polyurethanes increases with increasing PDMS content. The surface morphology of the polyurethanes indicated that the separation of the micro-domains was improved by increasing EO-PDMS-EO content. Thermal stability of polyurethanes increased with increase of EO-PDMS-EO content up to the temperature corresponding to the approximately 50 % of weight loss. However, at higher temperatures thermal degradation became slower for samples with lower EO-PDMS-EO content.

EMISSION MEASUREMENT OF POLYOLEFINS USING GAS CHROMATOGRAPHY

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Gas chromatography in combination with static headspace, dynamic headspace or thermodesorption injection systems can be used to describe the emission behaviour of polymers. These tests are especially important for materials used for interior automotive applications, because volatile substances can affect the well-being of the passengers. Another important effect is caused by condensing semi-volatile substances on the windshield: the so called fogging.

It is also possible to investigate the migration potential of materials with these techniques, which are less time consuming and more environmental friendly as migration tests based on organic solvents. Food packaging materials are the major application for this kind of measurements.

This work describes the specifics of such tests for polyolefin based materials. The investigated parameters include sample and sampling influences, material conversion, storage conditions and the influence of different test equipment and conditions.

STABILIZER DISTRIBUTION IN PLASTIC MATERIALS DETERMINED BY HPLC ANALYSIS OF MICROTOME SLICES

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The role of polyolefins in everyday life is constantly growing as more and more articles of daily use are made from plastics. However, polyolefins include tertiary carbons which can be involved in oxidative degradation. Very often these reactions are UV-light induced and involve radical mechanisms. To prevent the material from deterioration, protecting substances are added during the production process. Such stabilizers are acting as UV light absorbers, radical scavengers, or due to their consumption of oxygen. In this way stabilization ensures the applicability of the product over a long time-range without affecting the optical appearence or mechanical properties of the material.

In order to ensure appropiate quality control of plastic productes an accurate analysis of stabilizers is essential. But the total amount of stabilizer present in a polymer sample is only one point of interest. Since the mentioned degradation reactions mainly occur at the sample surface, the distribution of stabilizers within a piece of polymeric material is of great importance. Information about the stabilizer content within the first few microns of a bulk sample could help to understand processes which lead to a depletion of stabilizer (e.g. by migration processes).

In this work the stabilizer distribution was investigated by combining a method for micro sampling with HPLC-UV analysis of low sample amounts. A microtome was used for micro sampling of the polymer specimen. Microtomic cuts with a thickness of 10 µm were performed parallel to the surface of the polymer specimen, followed by extraction and analysis employing HPLC-UV. The method is suitable to determine stabilizer concentrations in a range of 0.1 – 1 mg stabilizer/g polymer (for Irganox 1010 and Irgafos 168) with a lateral resolution of only 10 µm. Furthermore stabilizers in very low sample amounts (less than 5 mg) can be quantified with sufficient precision. Thereby a proper characterization of the single slices from the microtome is possible, allowing the investigation of the stabilizer distribution on the micrometer scale within a polymeric sample.

MAGNETIC CHARACTERIZATION OF SINGLE MICROPARTICLE IN LIQUID

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Modern analytical chemistry is now required to develop the analytical method of microparticles, because it is important subject in various fields of bio-science, environmental chemistry and nano-technology.

1. Magnetic susceptibility measurement of single microparticles

The migration velocity of microparticles in liquid media under the high magnetic field gradient has been developed as the magnetophoretic velocimetry. The measurement of the magnetophoretic velocity of diamagnetic particles in paramagnetic manganese(II) chloride solution in a silica capillary allowed us to determine the magnetic susceptibility of the single particle, by knowing the size with an independent method, and the interfacial magnetic susceptibility of the particles was determined as well from the size dependence of the magnetic susceptibility. The size of the particles could be determined by the nano-gap method which was made by stacked three cover glasses and well applicable to micrometer sized particles, or by the Brownian motion analysis for sub-microparticles by employing fluorescence detection for smaller particles. From the interfacial magnetic susceptibility, the interfacial concentration of a paramagnetic ion, for example Mn(II) ion, was absolutely determined by using molar susceptibility. A small magnetic circuit could be used to generate a high gradient of magnetic flux density and to measure the magnetophoretic velocity under an optical microscope. These techniques were applied to red blood cells, HPLC particles and SPM particles.

2. Force analysis of chemical bonding and chemical equilibria by using microparticle

Magnetic or electromagnetic force was used to pull the molecules bound to a microparticles and capillary or glass wall as linker molecules. The dissociation or conformational change of the linker molecules was measured by the detachment of the particle or SERS spectra, and the bond dissociation dynamics and conformational equilibrium shift of the linker molecules were analyzed. From these measurements, an importance of force measurement in chemical analysis was recognized.

DETERMINATION OF THE VOC SORPTION MECHANISM OF PHTHALOCYANINES USING VIBRATIONAL SPECTROSCOPY

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Different phthalocyanine (Pc) molecules have been used for a long time as sensitive layers for sorption based gas sensors such as the Quartz Crystal Microbalance (QCM). However, in the literature only a few experimental or quantum chemical studies attempting to understand the adsorption mechanism can be found, thus, the reaction mechanism has not still been clarified in all details up to now.

A systematic and extensive study on VOC sorption into fluoroalkoxy Pcs was performed using gas sensors based on the QCM principle and two types of complementary vibrational spectroscopies. Phthalocyanines (Pc) with fluoroalkyloxy substituents and nickel and cobalt metal centres were synthesized and studied as thin films using Attenuated Total Reflection (ATR) Fourier Transform Infrared (FT-IR) and Raman spectroscopy during exposure to a variety of volatile organic compounds (VOCs) with different physicochemical properties such as acidity, polarity. The influence of humidity on the sorption process was also examined. The results demonstrate for the first time that the Pc molecules have different active centres capable of interacting with the analytes besides the metal such as the aromatic system of the Pc core and aromatic C-H groups. Additionally, parts of the substituent group are identified as active centres e.g. the ether bridge and the C-H of CF_2H. In the difference ATR FT-IR and RAMAN spectra calculated from the spectra before and during exposure changes in the position and intensity of bands assigned to these parts of the Pc molecules can be seen. It was found that the activity of these centres depends on the chemical properties of the analyte molecules. Finally, it was found that the sensor sensitivity increases with humidity, even though humidity uptake is quite low and the spectroscopic investigations were able to explain the influence of humidity on analyte sorption.

PREPARATION AND CHARACTERIZATION OF CARBOXYLATED LOCUST BEAN GUM

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Galactomannans are neutral polysaccharides that occur in substantial amounts in the endosperm of the seeds of some leguminous plants. Structurally they consist of a β(1-4)-D-mannose backbone to which galactose units are attached α(1-6). Of the number of galactomannans known, guar gum (GG), locust bean gum (LBG), and tara gum (TG) are the most used in applications in, for example, the food, pharmaceutical, and chemical industries as thickening agents or stabilizers due mainly to the high viscosity they give at low w/w concentrations. While guar gum commonly has a mannose-to-galactose ratio (M/G) of approximately 2, M/G is 3 and 4 for TG and LBG respectively [1]. Their functional and physical properties (including solubility, gelling behavior, and viscosity) are related to the molecular structure, sugar composition, degree and distribution of branching, and polymerization[2]. The chemical modification of polysaccharides by introducing substituent on polysaccharidic chains can often improve and change their physical and chemical properties and also helps to widening their application areas.

In this study, carboxylated LBG polymer was prepared by a technique which known as TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl)[3]. Carboxylation experiments were achieved in the presence of NaClO in acidic conditions at 4 ^{o}C. Characterization of raw and carboxylated LBG was examined by spectroscopic and chromatographic techniques. Chemical structure analyses of carboxylated LBG (CLBG) was performed by FTIR and ^{13}C-NMR analyses. Carboxylation degree of LBG was determined by potantiometric titrations. The effect of carboxylation on the molecular weight of LBG was defined by using a Gel Permeation Chromatograpy system equipped with a refractive index detector and Light Scattering detector. End of all these experiments optimum synthesis conditions i.e. optimum NaClO concentration, reaction time and pH of the reaction medium for the preparation of CLBG was determined.

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SUBSTITUENT AND SOLVENT EFFECTS ON THE UV ABSORPTION SPECTRA OF N-(SUBSTITUTED PHENYL)-CYANO-ACETAMIDES

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Phenylacetamide derivatives are a group of organic compounds having a wide range of activity and turout to be particularly important for pharmaceutical, agriculture and toxicological application. Several phenyl- acetamides displayed potent anticonvulsant, antileishmanial, antimicrobial and antifungal activity. Because of widely biological activities of phenylacetamide derivatives, synthesized N-(substituted phenyl) cyano-acetamides become interesting for different purpose. The aim of this paper is to investigate substituent and solvent effects on the UV absorption spectra of N-(substituted phenyl)-cyano-acetamides.

Absorption spectra of ten N-(substituted phenyl)-cyano-acetamide derivatives have been recorded in 4 protic and 4 aprotic solvents in the range 200-320 nm. The substituents at the phenyl nucleus are as follows: H, CH_3, I, Br, NO_2, OH, COOH, SO_3H, $COCH_3$ and C_2H_5. The effects of substituents on the absorption spectra of these new derivatives of acetamide are interpreted by correlation of absorption maximum with Hammett substituent constant, σ. The solute-solvent interactions were clarified on the basis of linear solvation energy relationships (LSER) concept proposed by Kamlet and Taft. The multiple linear regression equation electronic spectra data correlated with solvatochromic parameter π^*, α and β. It was found that the absorption frequencies for investigated derivatives in used solvents show satisfactory correlation with solvatochromic parameters. The quantitative relationship between the molecular structure and the chemical reactivity is discussed, as well as the effect of geometry on the reactivity of the examined molecules.

DETERMINATION OF THALLIUM AT TRACE LEVELS BY FLOW INJECTION VOLATILE SPECIES GENERATION ATOMIC ABSORPTION SPECTROMETRY AND CHARACTERIZATION OF VOLATILE SPECIES

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In literature, Thallium (Tl) determination by volatile species generation method was not investigated in detail because of the fact that the formation of volatile form of this element is a difficult task [1]. The generation of an analytically useful volatile form of Thallium (Tl) has been studied. Presence of 0.005% (m/v) Rhodamine B increased the volatile species generation efficiency of Tl [2]. In this system, the flow injection volatile species generation technique was performed in a dedicated generator consisting of a special mixing apparatus and gas–liquid separator (GLS) design in the presence of Rhodamine B. The mixing of reagent solutions are realized in a specially designed gas-liquid separator [3] and proceeds in a very small volume of about 3.0 mL. The main characteristic of the system is the fast separation of Tl volatile species from the liquid solution. The on-line atomization in the flame heated quartz tube atomizer for atomic absorption (AAS) detection has been employed as the convenient atomization/detection means. In order to improve sensitivity of the system, the flow rates of Tl and reductant solutions (1 mL/min), concentration of reductant solution (3.0% m/v $NaBH_4$), Ar flow rate (50 mL/min) and concentration of carrier solution (0.50 mol/L HCl) have been optimized. Furthermore, Tl^+ and Tl^{3+} have been prepared in their stock solutions in both HCl and HNO_3 medium, separately. The experimental results revealed that efficiency of the formation of volatile Tl species was better when Tl^+ was prepared in the HNO_3 medium. The optimization of generation and atomization conditions resulted in an analytical procedure yielding a detection limit of 12 ng/mL and characteristic concentration of 13 ng/mL for 0.40 mL analyte volume; the dynamic range was ranged from 100 to 2000 ng/mL. Transmission electron microscopic (TEM) measurements proved the presence of Tl nanoparticles in volatile form transported to the atomizer by the effect of carrier Ar gas.

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ELECTROLYTICAL PRECONCENTRATION FOR GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

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The superior detection power of graphite furnace atomic absorption spectroscopy (GF AAS) predestines the method for trace analysis of metals and semi-metals. Yet, in some instances concentrations below the detection limit should be measured and a pre-concentration step has to be used. The sample matrix may deteriorate the sensitivity and the detection limits and again, a pre-separation of the analyte should be carried out. The paper presents a simple but effective way for on-line pre-concentration of As, Se, Sb, and Hg by making use of flow-through electrodeposition on the surface of a porous electrode. The deposit is stripped into a small volume of suitable electrolyte and transported by air flow directly into the graphite furnace for measurement. The detection limits for As, Se, Sb, and Hg were found to be 4.0, 10, 20, and 1.5 ng/L, respectively (10 mL sample pre-concentrated). The duration of a complete analysis was 5-10 min depending on the sample volume pre-concentrated.

AS07 **Session B**

SPECIATION OF CHROMIUM IN WATERS BY ULTRASOUND ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

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The speciation of chromium in environmental and biological systems is of considerable interest because the toxicity of this metal depends on its oxidation state. Cr(III) and Cr(VI) enter the waterways mainly as a result of discharges from cooling towers, electroplating and tanning industries, oxidative dyeing, and leachates from sanitary landfills. Cr(VI) may also enter to the drinking water distribution system as a result of the corrosion inhibitors used in water pipes. In any case, the element occurs in the aquatic environment at very low concentrations, and due to the severe toxicity of Cr(VI), the EU Directive, WHO and US EPA have set the maximum concentration of this species in domestic water supplies as 50 μg/L.

Because of the low concentrations to be determined in waters, separation and preconcentration steps are often required. Conventional liquid-liquid extraction could be employed but miniaturized approaches such as those based in a liquid phase microextraction process are advantageous due to the high extraction efficiency that can be achieved with a low consumption of toxic organic solvent. Dispersive liquid-liquid microextraction (DLLME) is one of such miniaturized techniques particularly useful for the purpose, since the large contact surface between the organic extractant droplets and the aqueous phase allows the equilibrium to be reached in a few seconds. In addition, and due to the fact that only a few microliters of organic solvent are used, large preconcentration factors are obtained. The most usual way to carry out a DLLME procedure is to use a third solvent to obtain dispersion but ultrasounds can be used instead.

In this communication tri-n-butyl phosphate (TBP) is used for the speciation of very low amounts of chromium in waters. TBP plays a double role as it originates a neutral compound with Cr(VI) which is extracted into the excess of reagent, ultrasounds being used to produce the dispersion of the phases. The metal is then determined by graphite furnace atomic absorption spectrometry. The trivalent species is determined in a different aliquot after an oxidizing process. The detection limit is as low as 0.05 μg/L chromium. The practical usefulness of the procedure is verified by determining extremely low concentrations of the metal in drinking, mineral and seawaters.

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SPECTRAL CHARACTERIZATION OF SOME SCHIFF BASES DERIVED FROM 4-METHYL-2-AMINOPHENOL AND VARIOUS DISUBSTITUTED BENZALDEHYDES

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There has been a steady growth of interest in the synthesis, structure, and reactivity of Schiff bases due to their potential applications in biological modeling, catalysis, design of molecular magnets and materials chemistry [1]. Consequently, a significant effort has been devoted to the synthesis of Schiff base derivatives [2]. Structural information is useful when investigating the coordination properties of Schiff bases functioning as ligands [3].

In this study six Schiff bases, *N*-(5-methyl-2-hydroxyphenyl)-(2/3/4/5-substituted)-benzaldimines (**I**–**VI**), were synthesized using appropriate synthetic route. Their structures were characterized by FT-IR, UV-Visible, ESI-MS, ^{1}H- and ^{13}C-NMR spectroscopic techniques and analytical methods. Relationship between the melting points and the structures of the compounds were examined. The tautomeric equilibrium in the Schiff bases was monitored by UV-visible spectroscopy. The mole peaks of the Schiff bases are determined in the mass spectra. There are a lot of isotopic patterns in the ESI-MS spectra of the compounds **I**–**III** because of presence of chlorine or bromine atoms. On the other hand, the crystal structure of *N*-(5-methyl-2-hydroxyphenyl)-2,3-dimethoxybenzaldimine (**VI**) is determined by X-ray diffraction at room temperature. Crystal system is monoclinic and space group $P2_1$. There is an N···H–O type intramolecular hydrogen bonding in the molecule.

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ELECTROCHEMICALLY PREPARED SERS-ACTIVE SUBSTRATES: CAN WE RATIONALIZE METAL SELECTION WITH RESPECT TO EXCITATION WAVELENGTH?

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Nowadays, the surface-enhanced Raman scattering (SERS) spectroscopy is a powerful tool for detection and identification of trace amount of various analytes. It possesses enhancements of several order of magnitude of acquired signal from compounds attached to or situated in the proximity of a surface of nanostructured SERS-active substrate. Therefore, it is necessary to prepare a suitable SERS-active substrate with respect to the excitation wavelength, used analyte and its initial matrix. The key factors that should be taken into account is a used metal material (Ag, Au, Cu etc.) and the physical properties of the substrate (hydrosol, organosol, electrode, interfacial film).

In this study we focus on electrochemically prepared large-area SERS-active substrates (ca. 80 mm^2) of coin metals (Ag, Au and Cu) to compare their effectiveness for detection of model analytes (mainly thiols) under the same excitation conditions. Morphological characterization of prepared substrates was performed mainly using two methods of microscopy (Scanning Electron Microscopy [SEM] and Atomic Force Microscopy [AFM]). SERS-activity of substrates has been evaluated based on Raman band intensities of model analytes recorded with a series of excitation wavelengths, i.e. 1064, 785, 633 and 488 nm.

We used chemometric analysis to evaluate mainly the dependency of measured spectral intensity on used metal material. The preparation procedure parameters were also taken into account. Therefore, the possibility to harmonize the substrate for individual excitation wavelength by selection of corresponding material was tested. Comparison of various electrochemically prepared SERS-active substrates by chemometric evaluation of the results from spectroscopic and/or morphologic techniques provides the information to define the relationships among individual parameters and obtained intensities.

It is evident from the results that the used metal material play only partial role in obtained spectral intensity and thus other aspects have to be taken into account.

FTIR SPECTROSCOPIC CHARACTERIZATION OF BITUMINOUS LIMESTONE: MAGANIK MOUNTAIN, MONTENEGRO

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Fourier Transform Infrared spectroscopy (FTIR) is widespread used tool for molecular structure identification of the geological organic constituents. This technique provides fine determination of various organic and inorganic functional groups, as well as aliphatic and aromatic groups.

In this study FTIR spectroscopy has been used to detailed study of bituminous limestone samples from two different locations at Maganik Mountain (Montenegro). Maganik bituminous limestone occurs as carbonate sediments in three levels and belongs to the early Cretaceous. Our specimens come from the oldest sediments of this limestone belonging to Barremian age. The bituminous limestone samples have been collected from the locality Rekočica - sample 132 and from the locality Tuvertina greda - sample 425.

The isolation of organic matter from source rocks has been performed using successively treatment with HCl and HF due to the presence of carbonates and clay minerals. The separation of asphaltenes from the insoluble residue after demineralization was performed in Soxhlet apparatus by extraction firstly with methanol, then by n-hexane and benzene. The kerogen was purified with mixture of methanol/acetone/benzene (1:1:3, v/v).

The FTIR spectrum of the untreated sample 425 featured the following bands at: 2500, 1800, 1415, 875 and 712 cm^{-1} characteristic for carbonate minerals (calcite with minor aragonite). These bands are absent in the FTIR spectrum of untreated sample 132, indicating the inorganic fraction is composed mainly of silicate and aluminosilicate minerals.

Crucial for understanding the molecular composition of asphaltenes and kerogens is the nature of their aliphatic and aromatic structures, so the attention was paid to the absorptions in the 3000-2800 cm^{-1} and 900-700 cm^{-1} regions that can provide useful information. The curve-fitting analysis was performed to determine the intensity ratio between the bands at 2955 cm^{-1} ($-CH_3$ group) and at 2925 cm^{-1} ($-CH_2-$ group) indicating the length and branching of aliphatic structure.

USING MICRO-FTIR SPECTROSCOPY FOR INVESTIGATION OF BIOLOGICAL MINERAL TISSUES AND HISTOPATHOLOGICAL MATERIALS

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Fourier-transform infrared (FTIR) spectroscopy is considered to be one of the most used experimental techniques for the analysis and the characterization of molecular compositions of mineral biological tissues. It represents a very powerful and relatively cheap method to characterize the mineral phase in tissues (physiological as well as pathological). At the last decade, discovery of mercury-cadmium-telluride (HgCdTe, MCT) detector made possible development of FTIR micro-spectroscopy, a non destructive technique which enables simultaneous investigation of the organic and mineral tissues with visualization of studied area. In addition, FTIR micro-spectroscopy can provide spatially resolved information in the micrometer range on the modification of the chemical composition and of the structure of studied materials.

FTIR micro-spectroscopic analysis were carried out with a globar source in reflection mode using a Bruker Tensor 27 coupled to a Hyperion 1000 IR microscope equipped with a 15× objective. This system incorporates MCT array detector for imaging applications. Micro-FTIR spectra were recorded on individual specimens in the region of 4000–400 cm^{-1} with the co-addition of 320 scans at a spectral resolution of 4 cm^{-1}.

Structural changes in the bones of Wister rats due to treating with heavy metals (Pb, Cd) in vivo have been investigated. The differences in FTIR spectra were observed for specimens treated only with Pb or Cd and for specimens treated with these metals plus supplements as glutathione or lipoic acid.

Also, studies of biological minerals formed during pathological states such as kidney stones and breast cancerogenous excretion have been performed using FTIR micro-spectroscopy. Crystalline calcium oxalates of studied kidney stones have been found in two different hydration states, either as the monohydrate (whewellite, $CaC_2O_4 \times H_2O$) or as the dihydrate (weddellite, $CaC_2O_4 \times 2H_2O$). For crystals excreted through the breast skin due to the breast cancer, the micro-FTIR analyses found that is mainly formed of lactic acid.

IN SITU vs LABORATORY SPECTROSCOPIC CHARACTERISATION OF SEVERAL POLLUTANTS IN ABANDONED IRON-COPPER AND ZINC-LEAD MINES

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Industrial activities in the Basque Country have been closely linked to mining throughout its history. Although the main extracted ores contained mainly iron, more risky pollutants were also extracted: i.e. copper, zinc, lead, etc. Such activities have brought great prosperity to the country but now are a constant source of heavy metal leakage and, thus, environmental concern.

The present work was focused on two mining concessions located in the Valley of Karrantza and in Arrazola, both in Biscay (the north of Spain). The first was a blende-galena mine that operated for scarcely three decades of the last century, while the second was an iron and copper mine that worked from Roman times to the 1920s.

The main aim of the present research was the determination of the molecular composition of different compounds found in the studied areas, which is closely related with the environmental risk assessment derived from their presence. Thus, after a comprehensive bibliographical study (i.e. geology) and representative sampling, the materials found were analysed by means of several micro-Raman spectrometers: BWTEK (785nm laser), Renishaw A100 (785nm laser) and In Via Renishaw (532nm and 785nm lasers).

Some of the minerals found under the different measurement conditions were, i.e., pyrite, chalcopyrite, sphalerite, smithsonite, syderite, olivenite, claudetite, mimetite, malachite, hematite, arsenopyrite, etc.

The relationship between the geological information (closely related to the local bedrock composition) with the huge amount of minerals found, is a clue for the interpretation of different weathering processes that have been probably taking place. Considering that the leached elements are mainly hazardous heavy metals, both the identification of the elements and the molecules in which they are included and the knowledge of the weathering processes are useful in order to take actions for controlling the pollution of surrounding environments that may be at risk.

Acknowledgements: This work has been financially supported by the Basque Government through the Berrilur III project (Ref. IE09-0242). N. Goienaga and J.A Carrero acknowledge their pre-doctoral fellowship from the UPV/EHU. Technical and human support provided by the Raman-LASPEA Laboratory of the SGIker (UPV/EHU, MICINN, GV/EJ, ERDF and ESF) is also gratefully acknowledged.

ELECTROCHEMICAL GENERATION OF VOLATILE FORM OF ZINC IN ATOMIC ABSORPTION SPECTROMETRY

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The electrochemical generation of typical hydride forming elements (such as As, Se, Sb, Te,...) is nowadays sufficiently examined. On the other hand, there are many other analytical useful volatile compounds (especially transitions and noble metals) for electrochemical generation, which have not been investigated. This study is focused on the possibilities of electrochemical generation of volatile form of zinc. Experimental setup consists of flow-through thin-layer electrolytic cell with ion-exchange membrane to generate the volatile form of zinc. Atomic absorption spectrometry was used as a detection technique. Preliminary experiments were performed to optimize relevant working parameters (material of generated electrode, type of electrolyte, carrier gas flow rate, electrolyte flow rate, electric current). Under the optimal working parameters the calibration and other characteristics were measured. All experiments were carried out in on-line continuous mode.

In this work, lead, Pb-Sn alloy (75/25) and platinum as a cathode material were tested. The best signal was observed when Pb-Sn alloy was used. The optimal catholyte was chosen 1 mol dm^{-3} HCl. The optimal value 60 ml min^{-1} for carried gas (Ar) and 1.25 A for electric current were found. It was necessary to introduce the carrier gas into the catholyte flow before the entrance to the electrolytic cell. Under the optimal working parameters the limit of detection 0.75 μg ml^{-1} was obtained for electrochemical generation in continuous mode. Hydrostatic gas liquid separator with sintered glass was used for characterization of volatile form. Simultaneously the chemical hydride generation was carried out. In the end of this work the influence of oxygen introduced in different part of experimental setup was studied. The sensitivity of zinc determination measured under the oxygen influence was more than 5 times better.

The work was supported by the Project SVV 261204 and by the MSMT CR (research project: MSM0021620857 and 14/63).

FRACTIONATION AND SPECTROSCOPY CHARACTERIZATION OF KOSTOLAC LIGNITE FLY ASH: MEDICAL PERSPECTIVE

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Fly ash is a complicated heterogeneous material that requires a detailed knowledge of its physical and chemical characteristics because it could represent a health or environmental hazard depending on its constituents. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy have been used to investigate the content of Kostolac lignite fly ash revealing important details about mineral and elemental content. Research was focused on minerals and potential toxic hard metals (Pb, Cd, Co, Cr, Ni, Cu, Zn), using a procedure of selective leaching to determine a basic geochemical association. The sequence of leaching steps used was adopted so that researched metals associated with various parts of would be removed in following order: (step 1) exchangeable metals and the fraction of carbonate-hosted metals; (step 2) the metals primarily associated with metal oxides, hydroxides, monosulfides; (step 3) the metals associated with acid soluble silicates; and (step 4) the metals associated with acid insoluble silicates. ATR-FTIR analyses of the demineralized fractions of fly ash also confirmed that the dissolution was essentially complete and that a good selectivity was obtained at each stage of demineralization. ICP-OES results show that quantities of tested heavy metals are below the safety limit proposed by Environmental Protection Agency (EPA) and does not represent a threat for local population. In addition, fractionation showed that most of these metals are associated to silicate structure, which makes them stable in a natural environment. Contrary, fractionation and ATR-FTIR analyze indicate the high presence of cristaline silica (more than 70 %), which inhalation during the years may cause pulmonary damage and degenerative lung disease, resulting in silicosis.

INVESTIGATION ON THE INTERACTION OF ORGANIC CATIONS WITH GLYCOSAMINOGLYCANS – THE SPECTROPHOTOMETRIC AND RADIOLYTIC STUDY

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Glycosaminoglycans are linear, sulphated, negatively charged polysaccharides present commonly in biological material. They are exposed on animal cell surfaces and in the extracellular matrix. Some of them are known to bind and regulate a number of biologically important proteins. On the other hand, heparin is a well-known anticoagulant and antithrombotic agent.

Glycosaminoglycans present on endothelial cell surface allow an interaction with the underlying subendothelium and blood components, but also with some xenobiotics, especially of cationic character. Here we present the results of the investigation on the interaction of such negatively charged polysaccharides with methylene blue and other organic cations. The results obtained by means of steady-state spectrophotometry and pulse radiolysis techniques are compared and discussed.

THE MULTI-COMPONENT NEAR INFRARED SPECTROMETRY OF POLYDISPERSIONS: THE MILK EXAMPLE

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The multi-component dispersions, such as blood, milk, fat-protein nutrients, surfactants, etc. interact with radiation producing the spectra that are reflect both chemical making, and physical parameters of disperse phases. The special reasons for interest to milk near infrared (NIR) spectrometry are:
- Milk is the optical phantom (model) of blood and fabrics for medicine;
- Milk is the major foodstuff, and the dairy market is a competition arena for quality between manufacturers;
- NIR spectrometer is rather cheap and operative device for unmanned quality testing.

Optical properties of milk in light and NIR ranges are characterized by primary dispersion of radiation by the particles of two types: fatty globules (the average size 0.5 - 6 microns) and casein micelles (0.1 - 0.2 microns), smoothly falling down to a middle IR. And, considerable distinction in the sizes of disseminating particles leads to various types of dispersion by them: diffusing - by micelles and strongly anisotropic - by globules. In this work we present result of fat, protein and fat-free total solids (FFTS) prediction using the calibration built with the combined spectra: transmission and back scattering.

As the NIR spectrometry is the indirect method, so the chemometrical approach is used, as a rule, namely, the multi-component calibration of the spectrometer is performed with the sets of the standardized samples. Measurement of the spectra for a range of 800-1060 nanometers is executed with the spectrometer BIKAN created at Institute of spectroscopy and modified for registration of transmission and back scattering spectra simultaneously. Calibration and testing sample sets are made at Test Laboratory «MILK» of All Russian Milk Institute. Calibration models are executed on a method of projections to latent structures by the self-made program.

The conclusion follows: error of fat and protein maintenance prediction in milk manages to be lowered, and reliability – to raise if to use the transmission and back scattering spectra for PLS calibration

SURFACE-ENHANCED VIBRATIONAL SPECTROSCOPY OF B VITAMINS: WHAT IS THE EFFECT OF SERS-ACTIVE METAL USED?

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Surface-enhanced Raman scattering (SERS) spectroscopy and surface-enhanced infrared absorption (SEIRA) spectroscopy are analytical tools suitable to detect small amounts of various analytes adsorbed on metal surfaces. During recent years, these two spectroscopic methods became more and more important in the investigation of absorption of biomolecules and pharmaceuticals on nanostructured metal surfaces.

In this work, the adsorption of B-group vitamins pyridoxine, nicotinic acid and riboflavin has been investigated on electrochemically prepared massive gold and silver substrates, using SERS-spectroscopy at excitation wavelength 1064 nm. Both gold and silver substrates were prepared by cathodic reduction on massive platinum targets. In the case of gold substrates, oxidation-reduction cycles were applied to increase the enhancement factor of gold surface.

It has been found out that SERS-spectra of riboflavin, nicotinic acid and pyridoxine adsorbed on silver substrates differ significantly from SERS-spectra of these B-group vitamins adsorbed on gold substrates. The analysis of SERS-spectra reveals that each of B-vitamins investigated in this work interacts with gold substrate through different mechanism of adsorption than with silver substrate.

In the case of riboflavin adsorbed on silver substrate, the interpretation of SEIRA-spectrum was also helpful in elucidation of the absorption mechanism of riboflavin.

MULTIVARIATE APPROACHES FOR BACKGROUND CORRECTION OF DATA OBTAIEND FROM ON-LINE LIQUID CHROMATOGRAPHY WITH INFRARED SPECTROMETRIC DETECTION

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Two straightforward background correction approaches based on Simple-to-use Interactive Self-modeling Mixture Analysis (SIMPLISMA) and Principal Component Analysis (PCA) are proposed. The contribution of the background absorption to overall Liquid Chromatograph – Infrared (LC-IR) signals was compensated using the spectral information obtained during the equilibration of the LC system. Both approaches have been evaluated on reversed phase gradient LC-IR data sets obtained during the analysis of carbohydrate and nitrophenol mixtures.

Furthermore the use of multivariate curve resolution – alternating least squares (MCR-ALS) in LC-IR is discussed. When directly applying MCR-ALS to uncorrected on-line gradient LC-IR data, the resolution of spectra and concentration profiles of eluting analytes is hindered by the intense background absorption changes during gradient elution. In contrast, when applying MCR-ALS after subtraction of the calculated background signal, chemical rank selection and both, peak and spectral resolution could be achieved providing improved signal-to-noise ratios. Additionally, remaining signal variation due to background absorption and detector drifts could also be compensated. The potential of the assessed approach is illustrated by the results found for both model examples.

The present approach enables easy-to-use background correction and facilitates the use of MCR-ALS in on-line LC-IR, even when gradient conditions are employed and only poor chromatographic resolution is achieved. This method shows great potential to facilitate the full exploitation of the advantages of on-line IR detection that provides the possibility of simultaneous quantification and identification of a vast amount of analytes, making new exciting applications more accessible.

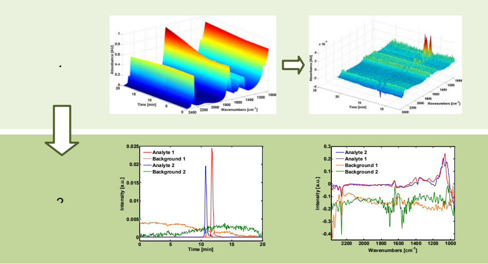

Figure 1. Top: data obtained during the injection of a soft drink sample before (left) and after background correction (right) and bottom: concentration (left) and spectral profiles (right) obtained employing MCR-ALS on the background corrected data.

ANALYTICAL CHARACTERIZATION OF NOVEL COPPER NANOANTIMICROBIALS

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A new type of nanomaterial has been developed as antimicrobial additive for food packaging applications. This nanocomposite is composed of copper nanoparticles (CuNPs) embedded in polylactic acid (PLA), combining the antimicrobial properties of CuNPs with the biodegradability of the polymer matrix.

Copper nanostructures were synthesized by three different routes: wet-chemical reduction, sacrificial anode electrolysis, and laser ablation.

The first two approaches provide stabilized NPs with a core-shell structure. The peculiarity of these materials consists in the stabilizing shell, which allows a gradual and controlled release of copper ions, when the nanostructure (either pristine or dispersed in a nanocoating) is exposed to aqueous solutions.

On the other hand, laser ablation of solid copper target in liquids is a novel route to generate bare nanoparticles even in absence of surfactants/stabilizers.

As prepared core-shell or bare CuNPs suspensions were then easily mixed to a PLA solution. The resulting hybrid solutions with different CuNPs loadings were directly spin-coated or cast on several substrates, thus obtaining antimicrobial self-standing packaging or antimicrobial coatings.

All samples were characterized by UV-Vis, FTIR, and X-ray Photoelectron Spectroscopies, and by electron microscopies.

The kinetics of copper release in aqueous solution from antimicrobial nanomaterials were studied by means of Electro-Thermal Atomic Absorption Spectroscopy.

Ion release data were finally matched with bioactivity tests, performed on *Pseudomonas spp* by JIS methods (Japanese Industrial Standard, JIS Z 2801:2000).

AS23 **Session B**

PREPARATION OF LONG-CHAIN POLYYNES OF $C_{28}H_2$ AND $C_{30}H_2$ BY LIQUID-PHASE LASER ABLATION

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Polyynes, sp-hybridized linear carbon chains connected by a sequence of alternating triple and single bonds, are known to exist as interstellar materials. The typical one-dimensional electronic structure of polyyne has attracted considerable attention for its band gap and nonlinear optical properties. Liquid-phase laser ablation has been used to produce materials such as polyynes. This method has some advantages; the cost is comparatively low because expensive instruments such as a vacuum apparatus are unnecessary, and the recovery of the product is higher than laser ablation in gas phase. Moreover, ablation surroundings can be easily changed by replacing solvent.

Here we report the preparation of polyynes up to $C_{30}H_2$ by liquid-phase laser ablation of graphite rod. To produce long-chain polyynes, experimental conditions were improved as follows. In the previous studies, we used a silica optical cell (1 x 1 cm^2) as a reaction container and graphite pellet as target, while in the present study, a glass bottle of 3 cm in diameter and graphite rod were used. The ablation time was 180 min and the laser wavelength was 1064 nm. In the detection of long-chain polyynes, the mobile phase (hexane : ethanol = 600 : 1) and a long column (4.6 mm x 250 mm) were used for chromatography. Absorption peaks (358, 372, and 406 nm) and a chromatogram of $C_{30}H_2$ were observed for the first time.

DETERMINATION OF OZONE IN WATER AND AIR USING A FLUORESCENCE TECHNIQUE

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Air pollution characterized by formation of ozone is a common urban and regional phenomenon in many parts of the world. Ozone is also employed as a strong oxidant for the disinfection of drinking water and water used for recreation, amongst other usages. The analytical determination of ozone is problematic due to its inherent instability and the lack of a readily-available standard reference material, and ozone concentrations are often estimated from the degree of decoloration of indigo dye. In this work, we propose the use of indigo dye as a reagent, but measure the concentration of fluorescent secondary species that present emission at 410 nm when excited at 260 nm. For measurements of ozone in water, the method was linear in the concentration range 0-5.0 μmol L^{-1}, with a precision of 3.7% and an accuracy of 87-113%. The detection limit achieved (0.014 μmol L^{-1}) was around 100 times better than that provided by the colorimetric method. The same reaction was applied in the hanging drop technique used to determine concentrations of ozone in ambient air. The spectroscopic behavior of the fluorescent species was firstly examined in order to identify potential excitation wavelengths of the molecule formed, and it was found that these species could be excited at 220, 260, 325 and 340 nm. The method was adapted for measurements of atmospheric ozone using a He-Cd laser emitting at 325 nm. This is a source that is widely available in optics laboratories, with an additional advantage in that it minimizes the degree of light scattering within the drop. The results showed that it was possible to measure ozone concentrations in air at ppb levels, using sampling times of 2 minutes or less.

DEVELOPMENT OF KINETIC-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LEAD TRACES

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Development of simple and rapid kinetic-spectrophotometric method for the determination of trace amounts of Pb(II), is presented in this paper. The method was based on the inhibitory effect of traces of Pb(II) on the redox reaction between disodium-6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphtalenesulfonic acid (artificial azo-dye "Sunset Yellow FCF", E110, in further text SY) and hydrogen peroxide, in presence of traces of Co(II) ions, in alkaline media of borate buffer. The rates of inhibited and non-inhibited reactions were followed spectrophotometricaly at λ=478.4 nm. The slope of the linear part of the absorbance-time curve was used as the measure of the reaction rate. Integral variant of the method of tangent was applied for the calculation of kinetic results. Optimal experimental conditions are set by assuming the highest sensitivity of the proposed kinetic method for the determination of Pb(II) ions. The linearity of the method was obtained in the concentration range 0.10-0.83 μg/mL of Pb(II) ions, under the following experimental conditions: $C_{SY}=4\cdot10^{-5}$ mol/L; $CH_2O_2=0.3$ mol/L; $C_{Co(II)}=12$ ng/mL; pH=9.5; t=25.0±0.1°C.The relative standard deviation for 10 replicate determinations of 0.41 μg/ml Pb(II) was 4.87 %.The proposed kinetic method could be useful for lead submicro-amounts detemination in different kind of polluted environmental or food samples, combined previously with separation techniques such as liquid or solid-phase extraction.

A NEW PALLADIUM CHELATE FOR DETERMINATION OF REDUCED SULFUR COMPOUNDS IN WATER BY FLUORESCENCE SPECTROMETRY

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This work describes the development of a new reagent for the determination of reduced sulfur compounds. A palladium-based reagent compound was prepared by reaction of tetrachloropalladate ($PdCl_4^-$) with 2-aminobenzoic acid (at a ratio of 1:2) and characterized using infrared spectroscopy and elemental (CHN) analysis. The determination of sulfide was based on the selective conversion of the palladium chelate compound to sulfide in a methanol medium, with formation of a fluorescent species. The quantitatively-formed anthranilate presented fluorescence (λ_{ex}: 245 nm; λ_{em}: 410 nm). Under the operating conditions used, the fluorescence intensity was linear in the range 2.75-55.0 μmol/L, with a detection limit (DL) of 0.13 μmol/L and repeatability (RSD) of 9.3%. An interference study revealed that low concentrations of the most commonly encountered anions (with the exception of OH^-) did not affect the determination of sulfide. The new method was successfully applied in the analysis of wastewater samples.

USE OF INFRARED AND RAMAN SPECTROSCOPY IN THE CHARACTERISATION OF SURFACE-FUNCTIONALIZED MAGNETIC NANOPARTICLES

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Magnetic nanoparticles have been recently extensively studied because of their expanding applications in biochemistry, bioseparation, tissue repair and in vivo drug delivery. The characteristics of magnetic nanoparticles can be significantly altered by tuning particular physical and physico-chemical properties, such as morphological and compositional. There is a possibility of engineering the particle surface to promote specific interactions with different targets. The key to understanding the properties and stability of magnetic nanoparticles in different environments is based on the elucidation of their surface structure and its respective chemical composition. The interaction between surfactant and nanoparticle is critical and essential for both synthesis and further applications of nanoparticles.

In this work, an in situ and ex situ approaches were followed to prepare surface-functionalized magnetic nanoparticles modified by citric and oleic acids. Such prepared nanoparticles have been suspended as a magnetic fluid sample and used as a model system for stability investigations. Different techniques were employed in the investigations including X-ray diffraction, transmission electron microscopy and in particular infrared and Raman spectroscopies, which are powerful analytical tools that may give substantial information regarding the nature of chemical bonds and the structure of interfaces. It has been shown that surface modification of nanoparticles by carboxylic acids allows the formation of a stable aqueous dispersion, moreover, the active carboxylic group on the surface of the particles can be used to immobilize other molecules and active agents.

SPECTROSCOPY OF SUBSTITUTED ARYL AMIDINES AS FLUORESCENT SENSOR FOR METAL IONS

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Pollution of the environment by toxic metals is a major problem in the last decays due to their increasing utilization in industry and agriculture. These pollutants are discharged or transported into the atmosphere and aquatic and terrestrial environments and have the potential to reach high concentrations. Large concentrations of some metal ions have been shown to cause brain damage, and are potentially lethal. Due to potential health risks associated with exposure to metals, legal constraints on the allowed abundance of metals are becoming increasingly strict.

As a result of the health threats posed by some metals, new methodologies that allow for their simple detection and quantitation are of interest. Today many fluorescent organic sensors were designed for recognition and detection of heavy and transition metal ions. These chemosensors are compounds that incorporate a binding site, a fluorophore, and a means of communication between the two. The interaction of a metal ion with an organic ligand may result in fluorescent enhancement or fluorescence quenching.

Our recent efforts have been focused largely on synthesizing novel organic fluorophores such as N-containing benzanthrone derivatives. The present work focuses on the spectral characterization of benzanthronylamidines and its complexes with transition metal ions. Obtained results testify that investigated dyes can be used as sensitive fluorescent chemosensors for transition metals: the one of them displays positive response (3-7-fold fluorescence increasing) to the presence of Ni^{2+} or Co^{2+} ions, but some synthesized amidines show negative response (quenching) in fluorescence intensity to Cr ions. Further investigations can lead to development of new method for the detection of some cations.

AS29 **Session B**

SPECTROSCOPY ANALYSIS IN RADICAL SCAVENGING ACTIVITY EXAMINATION OF GANODERMA RESINACEUM AND GANODERMA PFEIFFERI

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Bracket fungi of the genus Ganoderma are known by mycologists for the crust-like upper surfaces of their fruit bodies, which in some species, such as *G. pfeifferi* and *G. resinaceum*, have a varnished appearance. All *Ganoderma* spp. cause a white-rot, but they can degrade the woody cell walls in a number of ways, including selective delignification and simultaneous rot.

Many contemporary literature data confirmed the antioxidative potential of Ganoderma species indicating the importance and possibility of detection the radical scavenging potencies of autochthonous species in our, Balkan region using simple colorimetric assays. With regard to this aquaous extracts obtained by maceration with water of two inedible fungal species from Frushka gora Mountain were analyzed. Potential active substances are, near tested phenols (phenolic acids and flavonoids), polysaccharides.

The antioxidative activity was evaluated by using the FRAP assay (total redox potential of extracts), together with the free radical scavenging activity applying DPPH$^{\cdot}$and OH$^{\cdot}$ assay. The amount of total phenolics in the examined extracts was determined colorimetrically with the Folin – Ciocalteu reagent.

The highest DPPH$^{\cdot}$ scavenging activity was found in the extract of *G. resinaceum* (92.91% at 20 μg/ml), while the extract of *G. pfeifferi* showed 87.43% at 80 μg/ml. We were also working with synthetic antioxidants BHA and BHT, and they showed 12.35±0.64 μg/ml, 8.28±0.50 μg/ml respectively. Aqueous extracts of *G. pfeifferi* showed the highest scavenging effect on OH$^{\cdot}$ radicals (50.34%) at 0.166 μg/ml.

The antioxidative potencies correlated generally with the total phenol content (2.02-10.62 mg/g).

THE SYNTHESIS AND SPECTRAL PROPERTIES OF NOVEL MONO AND DI(THIO)-SUBSTITUTED QUINONES

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Quinones, substituted quinones, bis-quinones were synthesized during the last century and their properties extensively studied.[1-5] Their unique ability to attract electrons made them a desirable target in the fast growing fields of moleculer electronics and organic semiconductors.[6] Moreover, different ainon radicals of more complex quinonic compounds exhibit intense absorbtion in the visible or near-infrared (NIR) region. Such a property is of importance in the fields of communication devices, optical storage and organic nonlinear optical (NLO) materials. Our recent work was on the synthesis of symmetrical and non-symmetrical thioquinonyl derivatives.[4,5]

In this study we designed a synthesis of symmetrical and non-symmetrical naphtoquinonyl derivatives through which the quinone can be bridged *via* a thiolate moiety. The structures of the compounds were characterized by elemental analysis, UV-vis, FTIR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopies.

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INFLUENCES BY STANDARDIZATION AND GRINDING ON INFRARED SPECTRA OF COAL

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There are different influences on the reproducibility of infrared(IR)-spectra of brown coals when measured in transmission mode (KBr pellet technique). These are, e.g., the homogeneity and particle size of the sample, weighting, sample contamination during pellet preparation or pellet positioning in the spectrometer. Therefore a standardization of the measured spectra is obligatory. The reproducibility of the raw data of two brown coals (%transmission and absorbance) was compared to the results of 1) standard normal variate (SNV) transformation, 2) centering with baseline offset correction and 3) standardization by arithmetic mean. The arithmetic mean over the whole spectrum of the relative standard deviation (rsd) was minimized for transmission spectra from 2,7 % (raw data) to 0,6 % rsd (method 2). Absorbance spectra reveal always a higher rsd, an improvement from 6,1 % (raw data) to 1,8 % (method 3) was achieved.

In the second part of the presented work the influence of coal grinding was analyzed because coal has to be grinded to a defined particle size for IR-spectrometry. The grinding with a mortar and an ardenne vibrator were chosen for those studies and three different grinding times were examined. The unground coal was obtained by sieving the raw coal to < 80 µm. Other samples of the raw coal were grinded and subsequently sieved to < 80 µm. The absorbance spectra were standardized by arithmetic mean and used for a principal component analysis (PCA) followed by hierarchical clustering analysis (HCA). Two clusters of ground and unground coal samples were formed and finally validated by multivariate analysis of variance (MANOVA). The ground samples can be divided in two groups of shortly grounded coals and longer ground coals independently of the grinding method.

SPECTRAL CHARACTERIZATION of 2-(5-H/Me/F/Cl/NO_2-1*H*-BENZIMIDAZOL-2-YL)-BENZENE-1,4-DIOLS and SOME TRANSITION METAL COMPLEXES

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Benzimidazolyl-phenols are known as strong chelating agents. These ligands are potential N,O donors and they react easily with the metal ions to give stable chelate complexes [1-3].

In this study, 2-(5-H/Me/F/Cl/NO_2-1*H*-benzimidazol-2-yl)-benzen-1,4-diols (HL_X: X=1–5) and HL_1 complexes with $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Cu(NO_3)_2$, $Zn(NO_3)_2$ salts have been synthesized and characterized. The structures of the compounds were confirmed on the basis of elemental analysis, FT-IR, NMR, mass, UV-visible and fluorescence spectroscopy. In addition, thermogravimetric analysis (TGA), molar conductivity and magnetic moment measurements were carried out for the complexes. It is observed that all of the compounds including complexes have fluorescence characteristic. In all of the chelate complexes, the ligands are bidentate *via* the C=N nitrogen atom and the phenolate oxygen atom. All of the complexes have 1:1 M:L ratio, and they show ionic characteristic. Besides, all of the complexes contain uncoordinated nitrate ion. Zn(II) complex, $[Zn(\mathbf{L_1})(H_2O)_2]NO_3{\cdot}H_2O$, is diamagnetic and the others are paramagnetic. Coordinated and uncoordinated water molecules were determined by TGA in the complexes.

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OPTIMIZATION OF THE SIMPLE SPECTROPHOTOMETRIC METHOD FOR DETECTION OF NITRATE AND NITRITE

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Nitrite (NO_2^-) and nitrate (NO_3^-) are stable end-products of nitric oxide oxidation, and the methods based on their detection are commonly used in biological studies as a measure of NO production. The majority of the analytical methods of simultaneous nitrite and nitrate detection are expensive, time-consuming and require specialized equipment. Recently the rapid and simple spectrophotometric method of simultaneous NO_2^- and NO_3^- detection was proposed by Miranda.[1] In general the method is based on the reduction of nitrate by vanadium chloride combined with the detection of nitrite by Griess assay. In a search for the optimal, rapid, simple and cost-effective method for the estimation of nitrite and nitrate content, here we present the study of the factors affecting the kinetic and yield of nitrate reduction in the method proposed by Miranda.

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AS35 **Session B**

DETERMINATION OF VERY LOW AMOUNTS OF CHROMIUM (III) AND (VI) USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BY IN SITU FORMATION OF AN IONIC LIQUID FOLLOWED BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Liquid-liquid extraction (LLE), based on the partitioning of an analyte from one liquid phase to another immiscible liquid phase, is a traditional, widely used sample preparation technique. The conventional way of operation involves relatively high volumes of organic solvents, and so a number of recent approaches in LLE methodology have been devised to achieve a miniaturization of the scale by using only a few microliters of organic solvent (liquid liquid microextraction, LLME).

Dispersive liquid liquid microextraction (DLLME) is one of such miniaturized approaches showing particular advantages as regards simplicity, rapidity and low cost. The technique is based in the formation of a turbid solution by quickly injecting a mixture of an extraction solvent and a disperser solvent into an aqueous solution. The extraction solvent should be hydrophobic and possess a higher density than water, while the disperser solvent should be miscible with both the extraction solvent and aqueous phase. The turbid solution in this way obtained results in a large contact area between the fine extraction solvent droplets and the aqueous analyte solution and, consequently, in a decrease in the extraction time.

On the other hand, ionic liquids (ILs) possess interesting properties that make them useful in LLME techniques. Since viscosity and surface tension can easily be modified, ILs are able to form microdroplets, this fact rendering these compounds suitable for DLLME purposes. In this communication, a water-soluble IL containing a simple anionic part (1-methyl-3-octylimidazolium chloride) and an intermediate key (lithium bis(trifluoromethylsulfonyl) imide) is used. In aqueous medium a disperse phase is obtained by formation of 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl) imide. This in situ formed reagent extracts the neutral complexes that chromium ions originate with ammonium pyrrolidinedithiocarbamate (APDC). Once the mixture is centrifuged, the organic phase is injected into an electrothermal atomic absorption spectrometer for final measurement. Speciation is possible by means of a suitable selection of the experimental conditions that affect the formation of complexes between APDC and Cr(III) or Cr (VI) The authors acknowledge to CARM (Project 11796/PI/09) and to the Spanish MICINN (Project CTQ2009-08267/BQU) for financial support. Y.V.M. also acknowledges a fellowship of MICINN

PRECONCENTRATION OF SOME METAL IONS BY THREE AGAROSE BASED CHELATING ADSORBENTS IN WATER

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Agarose[1] is a highly hydrophilic matrix and its physical properties have been largely improved by crosslinking procedures[2]. The properties of three agarose based chelating ion exchanger with respectively, iminodiacetic acid (IDA)[3], dipicolyl amine (DPA)[4] and tris(2-amino ethyl) amine (TREN)[5] as functional groups are investigated with the same matrix for preconcentration[6] of Al, Cu and Mo in water. Binding capacities, adsorption rates and column recoveries of the metal ions are carefully investigated. An ICP-AES instrument is used for measurements.

The capacity of the IDA adsorbent for Cu in studied pHs was similar and is averaged to 49 (±0.5) μmol/ml, while for DPA and TREN sorbens the capacity was more variable and pH-dependent. The highest capacity of DPA adsorbent was obtained for Copper, which is averaged to 18.3 (±0.3) μmol/ml while the highest capacity of TREN adsorbent was obtained for Copper and Molybdenum which is averaged to 120 (±6) and 105 (±4) μmol/ml, respectively. Kinetic studies indicate relatively high adsorption rates of metal ions on all the sorbents, except for Al on the IDA chelators. High column recoveries were obtained for Al and Cu on the IDA adsorbent while Molybdenum, on the other hand, showed low recoveries on the IDA adsorbent. The column recoveries for DPA and TREN sorbents, were pH dependent. Copper was almost quantitatively recovered on the TREN sorbent. In general, more selectivities, slower adsorption rates and lower and more pH dependent recoveries were identified for DPA and TREN sorbents compared to that with the IDA groups.

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DEGRADATION OF ANILINE IN AQUEOUS SOLUTION UNDER UV/OZONE TREATMENT

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Advanced oxidation processes (AOPs) are now being explored for use for wastewater treatment. AOPs are defined as those technologies that utilize the hydroxyl radical (•OH) for oxidation of pollutants. The wastewater of vulcanization accelerator production contains aniline. Among various AOPs, ozone-based wastewater treatment is likely key technologies for degrading this pollutant. As known, early ozonation of wastewater containing aniline result in by-products increased the biotoxicity. The goal of this investigation is to study the by-products formed under the ozonation of aniline in aqueous solution under UV-irradiation.

References solutions of aniline were prepared from a.r. grade substance. They were irradiated with a low-pressure mercury lamp. The content of ozone in the ozone-air mix in the reactor inlet and in the outlet was measured by iodometric analysis. The concentrations of aniline and products of their oxidation was determined by optical, IR-spectroscopy, and chemical analysis. The optical absorption spectra were registered on Shimadzu UV-2450 spectrophotometer. The diffuse reflectance IR-spectra were registered by the FTIR spectrometer "System-2000" by Perkin Elmer.

The main by-products of aniline decay under treatment are amino phenols, diatomic phenols, and benzoquinones. Nitrobenzene and monatomic phenols are not detected. Diatomic phenols, that are more toxic then aniline, are formed during the treatment of the aqueous aniline solution in the first stage (< 7 hrs). When oxidized after the 7 hrs treatment diatomic phenols change into carboxylic acids. After the 13 hrs treatment diatomic phenols in measurable quantities are not detected. Specific discharge of ozone per 1 mol aniline oxidation in its reference solution after the 15 hrs of ozonation is 0.26 mol. Contamination factor of the aniline solution after the 15 hrs treatment is ~90% and does not actually depend on initial aniline concentration.

BY-PRODUCTS FORMED UNDER ELECTROCHEMICAL OXIDATION OF AQUEOUS PROPYLENE GLYCOL WASTE

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Advanced oxidation processes are currently used to treat industrial effluents. However, they have some major drawbacks if wastewater contents the large amount of inorganic chlorides. In this context, oxidative electrochemical technologies offer an alternative solution to purification of wastewater formed under oxide propylene production. The electrochemical oxidation of this wastewater contents propylene glycol and the chloride ions in basic media has been studied.

Chemical oxygen demand (COD) and concentration of the chloride ions were determined using a chemical analysis. The concentrations of propylene glycol and products of their electrochemical oxidation were determined by optical spectroscopy, IR-spectroscopy, and chemical analysis. The optical absorption spectra were registered on Shimadzu UV-2450 spectrophotometer. The diffuse reflectance IR-spectra were registered by the FTIR spectrometer “System-2000” by Perkin Elmer. The oxidation of propylene was carried out in a single compartment electrochemical cell. Stainless steel electrodes were used. The average composition of the wastewater used in the experiments was 1500 mg/dm^3 (propylene glycol), 32800 mg/dm^3 (Cl^-), 5800 mg/dm^3 (COD), and pH 11-12. The current density used was 75 mA cm^{-2}. Voltage used was 4.3 V.

The main by-products of propylene glycol decay under electrochemical oxidation are established. Mineralization of the organic waste after the 30 min treatment is ~80%. COD is ~700 mg/dm^3. The concentration of the chloride ions remains unchanged.

STEROID ANALYSIS IN WASTEWATERS BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY: THE ROLE OF THE ACQUISITION METHODS

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In this paper authors describe a GC-MS acquisition study, based on our previous work in the field [1], relating to the simultaneous analysis of representatives of diverse groups of natural and synthetic steroids, such as androsterone, ß-estradiol, transdehydroandrosterone, transandrosterone, mestranol, dihydrotestosterone, ethinylestradiol, testosterone, norethisterone, estriol, 4-androstene-3,17-dione, gestodene, levonorgestrel, etonogestrel, coprostanol, progesterone, cholesterol, medroxy-progesterone-acetate, litocholic acid, stigmasterol, cholic acid, chenodesoxycholic acid, ß-sitosterol, ursodesoxycholic acid, 3-hydroxy-7-ketocholanic acid and dehydrocholic acid, in total 26 compounds.

As novelties to the field, for the trimethylsilyl (TMS) oxime ether derivatives of steroids, at first, a multiple reaction monitoring (MRM) acquisition method has been developed, and, also for the first time, the three acquisition techniques, the full scan (FS), the multiple ion monitoring (MIM) and the currently optimized MRM ones, have been compared; all three, in parallel, under strictly the same derivatization/instrumental conditions, both from model solutions and from the wastewater samples of two Hungarian municipal wastewater treatment plants (WWTPs). Critical evaluation of the three acquisition protocols was collated on their analytical performances and validated with the same characteristics, like the six point calibration curve, the relative standard deviation percentages (RSD%) of parallel tests, the limit of quantitation (LOQ) and the instrumental limit of quantitation (ILQ) values.

Data of six point calibration (r^2 between 0.9382 and 0.9999 with avarage of 0.9938) and RSD% values (average: 7.7 RSD%) proved to be independent on the acquisition methods, while, LOQ and ILQ values furnished considerable differences. Decreasing LOQ data, (expressed in ng/L concentrations) were obtained in the FS, MIM, MRM line for all steroids and cholic acids. The same trend was determined in terms of the ILQ values. The practical utility of the optimized MRM technique was confirmed by the quantitation of the steroids contents of wastewater samples, determined by all three acquisition techniques. Results obtained confirmed the primary importance of the MRM acquisition method, even in comparison to the MIM one: avoiding the significant overestimation of the ß-estradiol (≈1000%) and ethinylestradiol (≈500%) concentrations in the wastewater samples.

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LEAD ISOTOPE RATIO ANALYSIS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY IN TREE LEAVES FROM BELGRADE, SERBIA

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The use of variations in stable lead isotope ratios has become a standard technique for characterising sources of lead contamination. In this work, concentration of lead and lead isotope ratio in leaves of common urban deciduous trees: horse chestnut (*Aesculus hippocastanum L.*) and linden (*Tilia spp.*) have been determined by inductively coupled plasma mass spectrometry (ICP-MS). The aim was to investigate the possibility of biomonitoring of airborne lead in Belgrade urban area in a multi-year period (2002-2006). Samples were collected at the beginning (May) and at the end (September) of vegetation period, from three representative urban sites in heavy traffic area of Belgrade (Karadjordjev Park, Student Park and Botanical Garden). The accuracy of the isotope ratio measurements was evaluated by analysing a certified isotopic standard NIST SRM 981. Good agreements were obtained between the lead isotope ratio measured and the certified values (relative precision for $^{206/207}$Pb isotope ratio was 0.36 %).

The concentration of lead in samples collected at the end of vegetation period was higher in comparison with samples from the beginning of the vegetation period. Also, the results indicate that the lead isotopic ratios in samples collected in May differed from those collected in September at same locations. In most cases $^{206/207}$Pb isotope ratio was lower in September in comparison with the corresponding sample collected in May. Since value of $^{206/207}$Pb isotope ratio in petrol in Europe (<1.100) is lower than average year value of $^{206/207}$Pb isotope ratio in samples collected in May (1.105-1.135), the source of lead bioaccumulation in trees leaves is most likely leaded-petrol emission from traffic in the years of investigation (2002-2006). This conclusion indicates that leaves of the examined species could be used in biomonitoring of lead as an atmospheric contaminant and for the determination of lead sources in environment.

OPTIMISATION OF A METHODOLOGY FOR THE DETERMINATION OF ENDOCRINE DISRUPTOR COMPOUNDS (EDCS) IN WATERS USING IN-TUBE SOLID PHASE MICROEXTRACTION COUPLED WITH MICELLAR DESORPTION

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Endocrine disruptor compounds (EDCs) are a class of emerging environmental contaminants that are extensively and increasingly being used in human and veterinary medicine. In recent years, there is a growing concern worldwide about their occurrence in the environment because of their adverse effects on biota and human health. After administration, they are excreted almost as unchanged compounds or their metabolites in urine and discharged into wastewater treatment plants (WWTPs). Unfortunately nowadays, WWTPs are not able to completely remove these compounds and thus important quantities are released to the aquatic environment [1]. Due to the low levels of concentration in which they are, an extraction and preconcentration step is needed prior their determination. In-tube solid phase microextraction has proved to be an appropriate method for thus aim of different types of pollutants, including EDCs [2, 3]. This preparation method uses organic solvents to extract the target analytes.

An alternative to the use of these solvents are the micellar media. The surfactants or surface-active agents are amphipathic substances that can associate to form molecular aggregates called micelles in aqueous solutions. One of the most important properties of micellar system is their high capacity to solubilize compounds from different types of matrices [4].

We proposed a new method in-tube SPME with micellar desorption for the determination of steroid hormones (estriol, norethisterone, ethynylestradiol and norgestrel) and one non-steroidal compound (bisphenol A) in environmental waters. We used capillary Supel-Q porous layer open tubular (PLOT) for this objective. We tested different parameters that affect the process: length of capillary, time of extraction, volume of sample, time of desorption, volume of desorbent, time of desorption and different types of surfactants like desorbents. Results obtained were evaluated and compared with conventional extraction by methanol. Real samples of environmental waters were investigated under optimal conditions.

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DETERMINATION OF STEROID HORMONES IN SURFACE WATERS BY ULTRA HIGH LIQUID CHROMATOGRAPHY; COMPARISON OF FUSED-CORE SORBENT AND UHPLC BEH COLUMN

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Endocrine-disrupting chemicals (EDCs) are being detected in the surface waters and interfere with the function of the endocrine systems of wildlife and humans (increasing breast cancer in women and causing reproductive abnormalities). Estrogens belong into wide group of EDCs. Owing to the low concentrations of estrogen pollutants (tens to hundreds of ng/L) in environmental samples, the analyses require highly sensitive methods combined with efficient sample preparation and preconcentration techniques [1].

Recently, new sorbent with fused-core technology was developed. Fused-Core particle has three characteristic different in comparison with conventional sorbent used for ultra high pressure chromatography (UHPLC): narrower particle size distribution, more consistent packed bed and shorter diffusion path [2].

We present comparison of new fused-core sorbent and UHPLC BEH technology, followed by determination of estrogens in surface waters by UHPLC-UV.

For optimization of analytical conditions various parameters such as type of sorbent, temperature, flow-rate and back pressure were tested. Obtained results were evaluated and compared as a function of different chromatographic parameters: time, peak shape and resolution. Ascentis Express was used for further analysis of estrogens.

Solid-phase extraction (SPE) using Oasis HLB 200mg sorbent was used for sample preparation and preconcentration of estrogens in environmental waters. SPE was optimized studying the variables that affect the extraction process: volume of sample, types and volumes of solution for clean-up and elution. The samples of environmental waters were investigated under optimal conditions.

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INVESTIGATION OF HEAVY METAL REMOVAL FROM AQUEOUS SOLUTIONS USING NATURAL AND MODIFIED ZEOLITES

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Due to their harmful effects both to humans and environment, the treatment of wastewater for heavy metals had become a major problem. Different methods can be used to remove these pollutants and precipitation is the most common one.
Adsorption techniques are also used. For this technique different types of sorbents have been used for removal of heavy metal from industrial waters [1]. In this study we have chosen natural zeolites (clinoptilolite type) because of their low cost and abundance in Romania.
The goal of the present work is to study, in static conditions, the heavy metal adsorption (Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}) from single and multicomponent synthetic aqueous solutions both with natural and pretreated forms of zeolites from Salaj and Prahova. The optimum parameters of the process were also studied. The clinoptilolite was chemical modified with the following solutions: hydrochloric acid, ammonium chloride and hexadecyltrimethylammonium bromide.
The ion-exchange process is influenced by several factors such as: ion concentration, the ratio between the zeolite and the aqueous solution, temperature, pH and the crystal structure of the zeolites [2, 3]. Natural and modified clinoptilolite used in this study was characterized by the following analytical methods XRD, FTIR, TG-DTG, SEM.
From equilibrium studies the adsorption capacity was calculated; which varies in the following order: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$. Efficiency of heavy metals removal from solutions is inverse proportion with metal concentration. For Cd^{2+} the maximum absorption capacity is 3,5 mg/g at an initial concentration of 50 mg/L and for Pb^{2+} is 21,5 mg/g for an initial concentration of 500 mg/L. Using the results obtained we can select the clinoptilolite that has the higher adsorption capacity for heavy metals.

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TRACE ELEMENT CONCENTRATIONS IN MARINE BIOTA WITH SPECIAL FOCUS ON GREY AND RINGED SEAL

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There is growing concern about health status of marine organisms that are exposed to environmental contaminants. Because of its geographic location and hydrologic properties, the Baltic Sea is continuously exposed to high anthropogenic stress. Baltic seals as the final trophic link of the marine ecosystem can be susceptible to effect of environmental pollutants, including heavy metals. This is due to (considerable) longevity as well as a long biological half-time of toxin elimination. The aim of this work is to determine and compare concentrations of heavy metals in two seal species - grey and ringed seal as well as their feed. A large collection of internal organs from grey and ringed seals were obtained from the Environmental Specimen Bank of The Swedish Museum of Natural History (Stockholm). The project is focused on seals from three areas of the Baltic Sea (northern, central and southern) and from Greenland. Seals were caught or shot between 2008 and 2010. Concentrations of Al, B, Ba, Cd, Co, Cr, Cu, Fe, Ga, Hg, Li, Mn, Mo, Ni, Pb, Se, Si, Sr, Tl, V, Zn, Ca, K, Mg, Na and REE were determined in 208 samples of liver and kidney from juvenile and adult grey and ringed seals and their feed. For the analysis inductively coupled plasma mass spectrometry (ICP-MS) were applied. On the basis of obtained results possible species and regional differences in heavy metal concentrations will be discussed.

EDXRF EVALUATION METHOD FOR HEAVY METALS IN SOME MUSHROOMS SPECIES AND SOIL

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This work is part of research project ID_624 on PNII component won by national competition which has the main aim to evaluate heavy metals up take from soils by via mushroom common species in south of Romania. It were made analyses on biological samples and soil prelevated from two forestry ecosystems of Dambovita county: Manesti and Ocnita.

Biological samples consisted in some mushrooms species: *Hypholoma fasciculare, Lactarius vellereus, Armillaria melea, Armillaria tabescens* and *Macrolepiota procera* which are growing in the forestry of Dambovita county. It were analysed the substrate samples under each exemplar. It were determinate some heavy metals as iron, manganese, copper, nickel and zinc.

For the evaluation of EDXRF results was used a certified reference sample (NIST SRM 1571- Orchard leaves). The concentrations of five heavy metals were different from a mushroom species to other and varied with the harvesting sit and soil content.

So, were determinate the iron concentrations between 400ppm at *Armillaria melea* and 812400ppm in *Macrolepiota procera harvested from* Manesti forest. Iron content of mushroom harvested from Ocnita forest had iron content between a minimum of 243ppm in *Hypholoma fasciculare* and maximum of 432ppm at *Lactarius vellereus*.

It was also calculated the bioabsorption factor by the following formula:

$$\mathrm{Bf\%} = \frac{\mathrm{Cm} \times 100}{\mathrm{Cs}}$$

were: Bf% = bioabsorption factor
Cm = metal’s concentration in mushroom
Cs = metal’s concentration in soil

The values of bioabsorption factor were different from stipe to cap, and between mushrooms species analyzed. For example bioabsorption factor was over much 100% for cap and stipe of *Hypholoma fasciculare* no matter of place of growing.

ADSORPTION OF SOME TOXIC IONS FROM AQUEOUS SOLUTIONS USING LIGNITE-BASED ACTIVATED CARBONS

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The adsorption of Hg(II), Cd(II), Pb(II), As(III) and As(V) from aqueous solutions onto activated carbon obtained from Bulgarian lignite (Chukurovo deposit) and its oxidized modifications was studied by means of the batch method. The initial carbon was oxidized using concentrated nitric acid or hydrogen peroxide. The textural parameters of the oxidized modifications were changed slightly while the chemical nature of their surfaces was most strongly affected by modification with hydrogen peroxide. Adsorption experiments were carried out by different concentrations and acidity and contact time of two hours was sufficient to attain equilibrium. The concentrations of the investigated ions were determined by flame AAS. With the increase of pH-values the removal efficiency for Hg(II), Cd(II) and Pb(II) increases substantially while for As(III) and As(V) decreases slightly.

The experimental equilibrium adsorption data were fitted to the linear Langmuir, Freundlich and Dubinin–Radushkevich models and maximum adsorption capacities were calculated. Best fit was observed by the Langmuir model, which showed correlation coefficients greater than 0.975 for all systems studied. Thus we proved that Langmuir isotherm most adequately describes the adsorption processes in the present work. Highest adsorption capacity for the studied ions was achieved using the adsorbent modified with hydrogen peroxide. For the studied activated carbons the adsorption of toxic ions increases in the order:

$$\text{As(V)} \leq \text{As(III)} < \text{Cd(II)} < \text{Pb(II)} \ll \text{Hg(II)}$$

In all cases the amount of adsorbed Hg(II) ions was considerably higher as compared with the other toxic ions hence the oxidized modifications can be used for effective removal of mercury from aqueous solutions.

Acknowledgements

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SPECIATION OF HEAVY METALS IN SEDIMENT OF SKADAR LAKE-NATIONAL PARK OF MONTENEGRO

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The aim of this work was to show a content of heavy metals (Pb, Cd, Cu, Zn, Ni, Cr, Mn, Sr and V) in different fractions of sediment of Skadar Lake and to estimate their distribution and bioavailability. Realistic environmental interpretation of sediment contamination depends on an understanding of how metals are bound to the various phases in the sediment. Sequential extraction schemes were used to determine the degree of pollution and to partition the trace elements in sediment.

The samples of the surface sediment were taken at 6 chosen locations: left and right river bank of Morača, Crnojevića River, Raduš (under the groundwater influence), Plavnica (touristic place) and Crni žar (clean, unpolluted area). There are two general methods of assessing the metal burden of sediment samples – the "total" metal content and the "potentially bioavailability" metal content. The use of strong acid digestion (HF, HCl and HNO_3) to determine 'total' metals in sediments may be misleading when assessing environmental risk as the risk may be over-estimated. The determination of potentially-available metals by sequential chemical extraction offers a more realistic estimate of actual environmental impact. The content of investigated heavy metals was deterimated by ICP-OES. 3-step sequential extraction procedure for metal fractionation was investigated to a slightly modified version of the Bureau Communautaire de Reference (BCR).

Content of Co, Mn, Ni and Sr was found in all of three fractions of sediment, Mn and Sr were found mostly in reductable fraction and content of Ni was the highest in oxidable fraction. Content of Zn and V was the same in II and III fractions, Cu, Cr and Pb mostly in III and Cd in II fraction.

The multi element measurements gave useful information to assist in the interpretation of the possible geochemical forms and sources of the trace elements in sediment.

DETERMINATION OF PESTICIDE AND PHARMACEUTICAL RESIDUES IN URBAN WASTEWATER SAMPLES

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Pesticides and pharmaceuticals are among the most important environmental pollutants because of their increasing production and extensive use. Urban wastewater is one of the most significant sources of these contaminants in the aquatic environment. Still, little is known about the loadings of pesticides and drugs from the urban wastewater systems. Previously developed liquid chromatography–tandem mass spectrometry method was validated and apply for wastewater samples. The investigated compounds include pharmaceuticals: sulfamethoxazole, trimethoprim (antibiotics); diazepam, bromazepam, lorazepam, carbamazepine (sedatives); diclofenac (analgoantipyretic); and pesticides: atrazine, simazine, propazine, linuron, monuron (herbicides); monocrotophos, dimethoate, malathion, imidacloprid, acetamiprid, tebufenozide, carbofuran, carbaryl (insecticides); carbendazim (fungicide). Sample preparation consisted of solid-phase extraction using Oasis HLB cartridges, 100 mL of the wastewater sample (pH = 6), and methanol-dichloromethane (1:1) as extraction solvent. Extracts were analyzed by ion trap mass spectrometer using electrospray ionization in the positive ion mode. Recoveries of the method validated for wastewater samples were in the range 80–118%. Repeatability of the method, expressed as the relative standard deviation, was in general lower than 20%. Matrix-matched calibration curves displayed good linearity. Low limits of detection (0.2–5 ng L^{-1}) and quantification (0.4-16 ng L^{-1}) were achieved for all investigated analytes. Validated method was successfully applied in the analysis of samples from Belgrade urban wastewater system. Pharmaceuticals, belonging to the classes of antibiotics and sedatives, were the most frequently detected contaminants.

PESTICIDE AND PHARMACEUTICAL RESIDUES IN THE DANUBE RIVER BASIN IN SERBIA

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The aim of this work was to monitor 31 pesticides and pharmaceutical residues in the Danube river basin in Serbia, and to assess their potential of reaching the ground water. A total of 70 surface and ground water samples were collected at 38 sampling sites on the Danube River and its major tributaries. They were taken in five sampling campaigns performed in summer and autumn of 2009 and winter, spring and autumn of 2010. Analyses were performed by solid-phase extraction combined with liquid chromatography electrospray tandem mass spectrometry with positive electrospray ionization. The analytes chosen for the study were pesticides and pharmaceuticals most commonly used in Serbia and two metamizole metabolites. The most significant pharmaceuticals identified in surface water samples in terms of frequency of detection, and concentration levels were trimethoprim (223 ng L^{-1}), carbamazepine (94 ng L^{-1}), 4-FAA (213 ng L^{-1}), and 4-AAA (354 ng L^{-1}). Among found pesticides the most frequent was carbendazim at relatively low concentrations (8–22 ng L^{-1}). The pesticide found at the highest concentration was atrazine (188 ng L^{-1}), which was detected at two sampling sites. It was determined that generally there was no leaching from surface to ground water, and that the bank filtration successfully eliminates pharmaceutical and pesticide residues.

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON HEAVY METALS ACCUMULATION FOR SPECIES OF *RUSSULA* GENUS

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In previous studies, macromycetes showed a high capacity to bioaccumulate heavy metals in the fruiting body. Besides species, an important factor in the accumulation of heavy metals is the substrate on which they grow, its metallic content and pH. In the present study, we analyzed six species of the genus *Russula*, macromycetes harvested from five different woods in terms of distance from anthropogenic pollution sources, vegetation and the content of heavy metals and pH. All samples were collected in July, after a rainy period, from altitudes ranging between 240 meters and 890 meters, soil pH ranging between 4.05 and 6.80. The elemental content of samples was established by Energy Dispersive X-Ray Fluorescence method (EDXRF), using ElvaX Spectrometer with a X-ray tube with Rh anode operated at 50 kV and 100 μA. The accuracy and precision of the results were evaluated by measuring a certified reference sample NIST SRM 1571 – Orchard leaves. The results show that the heavy metals concentrations decrease with increasing altitude and increase with the decreasing pH of substratum. The metals concentrations were lower in the durmast woods that in oak and beech.

FLOW-THROUGH FRACTIONATION OF ARSENIC AND ANTIMONY IN SOIL AND SLUDGE SAMPLES

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Arsenic and antimony are elements related to pollutants of the first (As) and second (Sb) classes of danger and hence are toxic even at low concentrations. However, it is known that the toxicity depends on elements forms (species) and type of their binding to sample components. Distribution of arsenic and antimony forms in soils attracts an increasing attention. Usually, information about the elements forms can be obtained using sequential extraction (fractionation) schemes. The protocol proposed by *Wenzel at al.* for the fractionation of As in soils allows one to separate non-specifically sorbed; specifically sorbed; bound to amorphous and poorly-crystalline hydroxides of Fe and Al; and bounded to crystalline hydroxides of Fe and Al fractions. The Wenzel scheme can be extended to Sb that has similar physicochemical properties. Traditionally, sequential extraction is performed in batch mode and problems of re-adsorption and phase overlapping may occur. The flow-through (dynamic) sequential extraction enables these problems to be avoided. Microcolumns of different design and rotating coiled columns are perspective systems for the flow-through fractionation.

To compare the effectiveness of different flow-through sequential extraction systems (FTES), the fractionation of As and Sb in soil and sludge samples was performed using a rotating coiled column (RCC) and a microcolumn. Results obtained using different FTES are comparable: the major portion of elements is recovered in the fraction bound to amorphous and poorly-crystalline hydroxides of Fe and Al. Dependence of elements leaching effectiveness on samples parameters (total organic carbon and carbonate contents) and FTES parameters (flow rates, mass of samples) was studied for RCC and microcolumn. The assessment of elements recovery using each approach was made.

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MULTIVARIATE STATISTICAL ANALYSIS OF DATA OBTAINED IN SURFACE WATER QUALITY MONITORING

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Surface water monitoring is a procedure used to define the water quality as well as the influence of the existing ecosystem on that quality. The data obtained in this procedure enable one to draw a series of conclusions on the analyzed water quality, to identify potential sources of water contamination, and similar. A great number of data obtained during monitoring can be most effectively processed using multivariate statistical analysis. The monitoring data presented in this paper were obtained for surface waters from a wide town-Bor area. Eight different water flows were monitored. The sampling was performed on several locations from each single water flow in the course of a year. The results of 16 chemical parameters necessary for water quality monitoring were used. Multivariate statistical analysis was applied to process the chemical analysis data obtained with statistically significant values. Statistical analysis showed a clear distinction between the characteristics of surface water for specific geographic locations.

MEASUREMENTS OF MACRONUTRIENT DEPOSITION IN AREA OF ETHANOL PRODUCTION (BRAZIL)

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Atmospheric deposition of nutrients is an integral component of biogeochemical cycling, however anthropogenic modification of this process due to increased emissions to the atmosphere can have both negative or beneficial effects. In agricultural systems these inputs can be a useful source of nutrients, however in excess they can cause acidification, impoverishment of soils and eutrophication. In this work dry and wet deposition fluxes of the macronutrients were determined at dam water in Central Region of São Paulo State, the world's largest contiguous area of sugar cane cultivation. The region has large differences in seasonal weather patterns, with distinct wet and dry periods, and well-defined sources of anthropogenic aerosols, especially agricultural biomass burning. The dam chosen for study, does not possess agricultural or antropic activities its margins. Water samples have been collected monthly since October 2010. Three sampling sites were positioned in the area of water supply to the dam and two sampling sites in the area of the spillway. Aerosols were collected by Hi-Vol sampler. A run-off water simulator system was used to evaluate inputs of ionic species by simulated run-off water. The ionic composition of macronutrients were determinated by ion chromatography. Nitrate and sulfate represented 98% of total mass of the anionic compounds found in the aerosols, with composition of respectively 54% and 44%. In the surface water, the ionic composition measured were: 9% (nitrate), 82% (sulfate), 1% (ammonium) and 2% (phosphate). At one meter of the surface the composition measured were: 18% (nitrate), 62% (sulfate), 14% (ammonium) and 1% (phosphate). Measurements made in water leaving the dam, show an increase in concentration of macronutrients species during the driest months, suggesting that atmospheric deposition is an important process to increase the availability of macronutrients in the water.

A GREEN ANALYTICAL PROCESS FOR Cd AND Ni SEQUENTIAL DETERMINATION BY HIGH-RESOLUTION CONTIUUM SOURCE FLAME ATOMIC ABSORPTION SPECTROMETRY AFTER CLOUD POINT EXTRACTION

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Cloud point extraction (CPE) is a green analytical process for separation and preconcentration of organic and inorganic analytes. For metal analysis purposes, reaction with a hydrophobic complexing agent in the presence of a non-ionic surfactant, such as Triton X-114, is required. CPE was employed for preconcentration of trace of cadmium and nickel prior to the sequential determination by high-resolution continuum source flame atomic absorption spectrometry. Cadmium and nickel reacted with di-2-pyridyl ketone salicyloylhydrazone (DPKSH) and these complexes were extracted from the aqueous phase by the Triton X-114 surfactant. Effect of pH, DPKSH and Triton X-114 concentrations were studied, and calibrations curves were made at pH 7.0, DPKSH 0.5 mmol L^{-1} and Triton X-114 2 mmol L^{-1}.The extraction was quantitative and the enrichment factor was estimated at 32.3 and 26.5 for cadmium and nickel, respectively. The proposed procedure allowed the determination of cadmium with a LOD and LOQ 0.45 and 1.5 µg L^{-1} (99.7% confidence level), respectively and a linear response was observed in the 2.5 – 50 µg L^{-1} range. The relative standard deviation was estimated at 2.1% for 40 µg L^{-1} Cd(II) (n = 8). For nickel determination, it was then obtained a LOD and LOQ 0.79 and 2.5 µg L^{-1} (99.7% confidence level), respectively and a linear response observed in the 5.0 – 120 µg L^{-1} range. The relative standard deviation was estimated at 3.5% for 100 µg L^{-1} Ni(II) (n = 8). In the whole procedure, the reagent consumption was 17 mg Triton X-114 and 2.55 mg DPKSH *per* determination. Effect of foreign species was studied and the methodology developed was applied to sequential Cd and Ni determination in environmental and biological samples. The accuracy of this method was evaluated by analysis of a standard reference material, NIST 1643e Water.

IN SITU MONITORING OF WEATHERING PROCESSES OF THE HAZARDOUS CONSTITUENTS DERIVED FROM MINING ACTIVITIES. A CASE STUDY INSIDE AND OUTSIDE A BLENDE-GALENA MINE.

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The history of mining in the Basque Country dates back over 2000 years. These mines were for a long time one of the major suppliers of iron to Great Britain and central Europe. Other ore extractions, for instance Pb, Zn, Cd or S were less profitable but not less polluting. Nowadays the number of operating mines has decreased but both, the environmental and health impact that the mining activities imply are still relevant.

In order to understand better the leaching capabilities of several hazardous chemicals, an in situ characterization of an abandoned blende (ZnS) – galena (PbS) mine was performed. To achieve that goal a portable Raman spectrometer and a handheld XRF were used. The sampling was conducted in such a way that the whole area covering the tailings and the mine itself is represented.

Sampling was carried out mainly in two steps. The first, inside the abandoned mine galleries, its roof and floor. Here, several effluorescences, host rocks, rolling rocks and even mud were analysed at different points of the galleries. The second, outside the mine along the surrounding tailings and hills, where the soil, plaints and rolling stones were measured.

Besides, liquid samples were also collected for further analysis in the laboratory by means of ion chromatography, ICP-MS and ICP-OES. These were mainly the percolating or leaching waters from inside the mine and runoff rainwater picked in various points outside.

The presence of primary and secondary minerals -outside and inside the mine- (gypsum, sphalerite, siderite, galena, smithsonite, zincite, massicot) together with the high concentrations of risky pollutants (i.e. heavy metals, mercury, sulphate) observed.

The existence of so many polluting compounds and their high concentration ranges, make it necessary to take action related with both, the human health and the environmental risk assessment, as soon as possible.

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MINIATURIZED EXTRACTION METHODS FOR TRICLOSAN IN AQUEOUS AND FISH ROE SAMPLES. BIOACCUMULATION STUDIES IN ZEBRAFISH LARVAE (DANIO RERIO)

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Triclosan is an antibacterial and antifungal agent that can be presented in the environment due to its wide use in several applications such as hygiene and personal care products or processed food and packaging. Triclosan is acutely and chronically toxic to aquatic organisms and bioaccumulates in fish tissue. The main aim of the present work is the development of adequate analytical methodologies to evaluate the accumulation capability of this chemical using zebrafish larvae as a model. To achieve this goal, miniaturized extraction methods based on the use of a vortex mixing for aqueous samples and an ultrasonic probe assisted extraction for fish roe samples for triclosan determination by GC-μECD were developed. Several solvents such as acetonitrile, hexane and ethyl acetate, and sorbents (Florisil, Florisil modified with sulphuric acid or C8) for clean-up purposes have been tested. Multivariate optimisation of the variables affecting ultrasonic extraction (ultrasound radiation amplitude, sonication time, sample temperature, and sample amount/extracting volume ratio was conduced. Finally, solvent extraction using ethyl acetate and further clean-up with Florisil modified with 10% sulphuric acid only for fish roe samples have been selected. Extraction efficiencies up to 95% and 90% and detection limits of 0.165 ng mL^{-1} and 2.7 ng g^{-1} for aqueous and fish roe samples, respectively were obtained. The optimized method was applied to bioaccumulation studies in zebrafish larvae (Danio Rerio) as alternative methodology to the OECD technical guideline 305. Bioaccumulation factors were calculated as the ratio of the compound concentration in larvae respect to that in the exposure solution at steady state (BCF_{ss}) and using a first order two-compartment (water and aquatic organism) model that describes the kinetics of the uptake and depuration processes (BCF_k). Bioaccumulation values, BCF = 2630 and 2018 at exposure concentrations of 30 $\mu g \cdot L^{-1}$ and 3 $\mu g \cdot L^{-1}$, respectively were assessed.

DETERMINATION OF METHYLXANTHINES IN THE DANUBE RIVER SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Caffeine, theophylline and theobromine are methylxanthines with various physiological effects. Caffeine is a strong stimulant of central nervous, circulatory and respiratory system while theobromine and theophylline are smooth muscle relaxants. These substances, specially caffeine, can be found in surface water mainly from unconsumed caffeinated beverages, certain foods or medications passing through the human sewage. The main goal of this study is to determine the presence od caffeine, theophylline and theobromine in the Danube river samples as an indicators of human waste. Simultaneously, applicability of a developed analytical method for determination of methylxanthines in surface water samples is examined. Samples were collected near the location of the water supply source „Ratno ostrvo“ in Novi Sad, Serbia. Analysis was performed by solid-phase extraction (SPE) followed by reversed phase high performance liquid chromatography (HPLC). The chromatography was using a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 μm particle size) at 25°C, with a mobile phase of water/THF (0.1 % THF in water, pH 8) – acetonitrile (95:5, v/v). The flow rate was 0.6 mL/min, and detection by DAD at 273 nm. Limits of detection for caffeine, theophylline and theobromine were 7, 10 and 20 μg/L, respectively. In analyzed samples the concentration of caffeine was 95 ng/L and of theobromine 7 ng/L. Theophylline was not found in any analyzed sample. The presence of caffeine and theobromine, which have no natural non-human sources, confirmed the existence of human waste in the Danube river. The data suggested that the proposed HPLC method could be used for a methylxanthines detection in a surface water samples.

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ENANTIOMERIC DETERMINATION OF ACID HERBICIDES IN APPLE JUICE SAMPLES BY DIRECT CHIRAL LIQUID CHROMATOGRAPHY ON A TEICOPLANIN STATIONARY PHASE AND ULTRAVIOLET DETECTION

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Arylphenoxypropionic and chlorophenoxy acids are selective and translocated herbicides widely used and applied as racemates for broad leaf weed control. Their residues can occur not only in raw agricultural commodities; they can also be transferred to food products, thus being a significant route to human exposure. Some of them are chiral, the (*R*)-enantiomer showing greater herbicide activity. Consequently, there is an increasing demand for developing enantioselective analytical methods to monitor and control the use and the stereochemical purity of agrochemicals for preservation and sustainability of the environment. A direct LC-UV method has been developed for simultaneous enantiomeric determination of diclofop-acid, MCPP and 2,4,5-TP, using a Chirobiotic® T column (150 x 2.1 mm, 5 μm). A mixture of methanol-0.7% triethylamine aqueous solution at pH 4 (85:15 v/v) was used as mobile phase in isocratic mode. Flow and detection wavelength were set at 0.5 mL min^{-1} and 232 nm, respectively. For chiral determination of herbicides in apple juice samples, a previous clean-up and preconcentration step on a strong cation exchanger polymeric cartridge (OASIS® MCX, 60 mg, 3 mL) was carried out. Spiked commercial samples (6 g) were diluted to 10 mL with purified water and pH was adjusted to 1. The resulting solution was filtered through a polyester membrane (0.45 μm) and percolated through the preconditioned SPE-cartridge at 2 mL min^{-1}. A washing step was carried out as follows: 2 mL of pH 1 aqueous solution, 2 mL of 50% methanol aqueous solution, and finally 2 mL of aqueous solution containing 15% methanol and 0.8% formic acid. Elution was accomplished with 1.5 of methanol containing 0.8% of formic acid. The eluate was diluted with water to 3 mL for focusing purposes before LC-UV analysis. Detection limits were between 0.015 mg kg^{-1} for (*R*)-MCPP and 0.025 mg kg^{-1} for diclofop-acid enantiomers, respectively. Good linearity ($R^2 > 0.989$, n=9) was observed in the range 0.050-1.25 mg kg^{-1} for each enantiomer. Acceptable and constant recoveries between 51% and 100% with RSDs (n=3) lower than 3.3% were achieved for enantiomer herbicide-spiked levels from 0.25 to 0.75 mg kg^{-1}. Due to co-extracted endogenous matter, which partially overlapped with MCPP enantiomers, mathematical deconvolution was the best choice as integration tool minimizing errors in peak area estimation.

In conclusion, a novel method, with detection limits around maximum residue levels permitted by European Regulations, has been developed for enantiomeric herbicide determination in a complex vegetable sample.

HEAVY METALS (COOPER AND LEAD) QUANTIFICATION IN SOILS AND VEGETABLES FROM OLD MINING AREA IN BANAT COUNTY, ROMANIA

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The work deals with the quantification of heavy metal (Pb and Cu) in soil and common used vegetables (parsley, carrot, onion and lettuce) grown in two old mining area (Ruschiţa and Moldova Veche) in Banat county, Romania. Vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals in their edible and non edible parts, than those grown in uncontaminated soil. For total heavy metals analysis, soil and plant samples were prepared according to the procedure described in literature, and heavy metals were measured using flame atomic absorption spectrophotometer with high resolution continuum source (Contra300, Germany). For each vegetable, plant concentration factor or transfer factor (PCF) was calculated and the degree of soil pollution for each metal was measured using the pollution load index (PLI) technique depending on soil metal concentrations. The daily intake of metals (DIM) and the health risk index (HRI) for the locals through the consumption of contaminated vegetables was assessed based on the food chain and the reference oral dose (RfD) for each metal. Dietary intake of heavy metals through contaminated vegetables may lead to various chronic diseases. Especially Pb and Cu toxicity causes reduction in the hemoglobin synthesis, disturbance in the functioning of kidney, joints, reproductive and cardiovascular systems and chronic damage to the central and peripheral nervous systems. Because the intake of vegetables is an important path of heavy metal toxicity to human being, is important to investigate the content and risk assessment of these heavy metals in vegetables grown in contaminated areas

WIRELESS BIOSENSOR SYSTEM FOR REAL-TIME L-LACTIC ACID MONITORING IN FISH

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In recent years, the demand for cultured fish has increased to ensure a stable food supply. In fish, the blood level of L-lactic acid is a barometer of stress. We developed a wireless biosensor system to continuously monitor L-lactic acid concentrations in fish. The biosensor comprised Pt-Ir wire (φ0.178 mm) as the working electrode and Ag/AgCl paste as the reference electrode. Lactate oxidase was immobilized on the working electrode using glutaraldehyde. The sensor output was linear and very strongly correlated with L-lactic acid levels (R=0.9959) in the range of 0.04 to 6.0 mg dl^{-1}, which covers the physiologic range of L-lactic acid levels in fish. To avoid blood coagulation and proteins coalescing on the sensor, we implanted the sensor in the fluid under the scleral surface of the eyeball (EISF). The EISF is presumed to reflect the levels of most blood components and does not include substances contained in blood that inhibit sensor measurement. There was a strong correlation (R=0.8173, n=26) between L-lactic acid concentrations in the EISF and those in the blood in the range of 3 to 13 mg dl^{-1}. Therefore, we used EISF as an alternative to blood and performed continuous *in vivo*-monitoring of the L-lactic acid concentration in fish. Wireless monitoring of L-lactic acid was performed using the sensor system in free-swimming fish in an aquarium. The sensor response was stable for over 60 h. Thus, our biosensor provided a rapid and convenient method for real-time monitoring of L-lactic acid levels in fish.

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LABEL-FREE IMMUNOSENSOR USING CARBON NANOTUBE FOR AMPEROMETRIC DETERMINATION OF OOCYTE MATURATION-INDUCING HORMONE IN FISH

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Maintaining high-quality fish eggs stably and efficiently is important for aquaculture. Environmental and physiological factors, however, might alter the fish maturation period and ovulation phase. Therefore, it is necessary to establish techniques for measuring the period of ovulation in each fish species. In the present study, we developed a label-free immunosensor system for measuring 17, 20β-dihydroxy-4-pregnen-3-one (DHP). DHP is suddenly secreted before ovulation as a maturation-inducing hormone in fish, and therefore DHP levels are an indicator for predicting ovulation. The method is based on immunologic reactions and amperometric measurement using cyclic voltammetry (CV). For biomolecular immobilization on the surface of sensing electrode, Au electrode, we used self-assembled monolayers of thiolor-sulfur compounds to fix anti-DHP immunoglobulin. In addition, we used a single-walled carbon nanotube to improve sensitivity. Using this electrode, we were able to determine the CV signal change caused by the antigen-antibody complex. The proposed immunosensor system showed a linear correlation (R=0.9827) between the anodic peak current of the CV and the DHP concentration in range from 15.6 to 50,000 pg ml^{-1}. A good specificity to DHP was also shown. The sensor system was then applied to monitor DHP of goldfish (*Carassius auratus*). Blood plasma of fish was collected every 3 hours after administering a DHP inducer. In the measurement, the anodic peak current of the CV showed distinct changes depending on DHP levels in the blood plasma. A good relationship was observed between DHP levels determined by our proposed system and the conventional method (R=0.9351).

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DETERMINATION OF HEAVY METALS IN HORSERADISH (AROMATIKA RUSTICANA) AND CARROT (DAUCUS CAROTA) FROM DIFFERENT LOCATIONS

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Regions with increased human activity are exposed to higher heavy metal pollution as well as plants which are grown in these areas. The rapid diffusion of heavy metals as environmental contaminants has necessitated their determination at trace and ultra trace levels.

Vegetable samples were collected from several locations within the following districts: Bor, Toplica and Rasina. Samples were mineralized by wet digestion and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). All statistical analyses were performed using ANOVA and two-sample *t*-test were employed to examine statistical significance of differences in the mean concentration of metals between (or among) group of families of vegetables. A probability level of $P<0.05$ was considered statistically significant.

Location of Krivelj in Bor district has the highest content of the following elements in the samples of horseradish: Cd (0.0075±0.0008 ppm), Cr (0.2738±0.0242 ppm), Cu (0.7318±0.0343 ppm), Co (0.0105±0.0007 ppm), Mn (1.116±0.086 ppm), Ni (0.7812±0.0325 ppm), Zn (2.257±0.094 ppm). Location Bor II in Bor district has the highest content of Pb (0.0829±0.0009 ppm) and Sn (0.3007±0.0058 ppm).

Location of Slatina II in Bor district has the highest content of the following elements in the samples of carrot: Cd (0.0073±0.0000 ppm), Cu (0.3282±0.0021 ppm), Mo (0.0238±0.0010 ppm), (0.0431±0.0008 ppm, Sn (0.0953±0.0076 ppm). The highest concentration of Zn in the samples of carrot is in the location of Bor II (Bor district) (1.105 ± 0.014 ppm). The highest concentration of Ni in the samples of carrot is in the location of Kupinovo (Toplica district) (0.3119±0.0388 ppm).

CHARACTERIZATION OF POTENTIALLY HAZARDOUS METALS FOR THE ENVIRONMENT IN SOFT BROWN COAL FROM THE KOVIN DEPOSIT

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Trace metals which are found in a coal deposits can be very hazardous for the environment because they are concentrated and released during coal combustion. Characterization of these metals is very complicated task due to matrix complexity, wide range of concentration levels, interferences encountered during laboratory analysis etc.

The concentrations of potentially hazardous trace elements in coal from the Kovin deposit were analysed by inductively coupled plasma - atomic emission spectrometry (ICP-AES) using EPA 200.7 standard. The complete procedure of sample preparation and analysis was done according to Meier et al. (1996).

The Kovin coal deposit is divided in two exploitation fields: western – field "A", and eastern - field "B". It is a part of the "Banat-Morava" depression, which consists of clastic sediments of Sarmatian (Middle Miocene), Pannonian and Pontian (Upper Miocene) age. These sediments transgressively and disconformly overlie metamorphic complex of the Serbo-Macedonian mass. Coal-bearing Pontian sediments are made up of loosely bounded sand and clay with three coal seams: upper (I-Ib), middle (II), and lower (III).

Forty three samples of coal and coal ash from all coal seams, from both coal fields were collected from the four borehole cores. They were analyzed on twelve potentially hazardous trace elements, namely: As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se and Th. Coal from all three seams has very high content of Cd, Cr, Ni and Pb compared to Clarke values for brown coals (using the geometric mean value; Ketris and Yudovich, 2009). Content of those elements is more than five time higher than Clarke values. The other elements (As, Co, Cu, and Se) also have increased content in all three seams. The content of Th in all seams is lower than Clarke values. Coal ash from all three seams has higher content of Cr and Ni (more than twice) compared to Clarke values for brown coal ashes (using the geometric mean value). The other analysed elements occur in concentrations similar to Clarke values.

MONITORING PRIORITY POLLUTANT METALS AND TRACE ELEMENTS IN IRISH WASTEWATERS

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In 2000 the Water Framework Directive (WFD), 2000/60/EC, was introduced and a group of chemicals, including metals and trace elements were chosen as priority pollutants according to Annex X. The levels of these priority pollutants in the environment are regulated by set environmental quality standards (EQSs) and are affected by a number of emission factors including anthropogenic activities, population equivalents, and weather. In order for these EQSs to be enforced, regular monitoring of all water bodies must be carried out, a process which is both costly and time consuming. We have developed a model defining emission levels relating to priority pollutants occurrence in the environment. This is based on information collected from local authorities, Met Eireann and pollutant levels in waste water treatment plant (WWTP) effluents.

Waste water treatment directly affects the quality of receiving water bodies and the environment. These facilities must be operating properly to ensure removal of pollutants before outflow. It is also important that sampling and extraction methods minimize any loss of sample, employing strict controls where necessary. Effluent samples have been collected from both a secondary and a tertiary WWTP over a period of 1 year, including several weeks of summer and winter high intensity sampling. Acid digestion followed by ICP-AES has been used for the analysis of these samples. These results have been incorporated into the model, allowing for predictions of pollutant levels for certain emission factors.

INVASTIGATION OF BIOTRANSFORMATION OF ZINC IN PLANTS VIA SPECIATION STUDIES

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The aim of this work was to optimize the analytical procedure enables the investigation of zinc speciation in plants exposed to the industrial contamination. For this purpose the high performance liquid chromatography (HPLC) inductively coupled plasma mass spectrometry (ICP MS) was used and the experimental conditions for various chromatography separation was optimized. The analytical scenario for speciation studies was developed toward the evaluation of the bio-mechanism which enables plants to adapt for unfavorable environmental conditions. Selected plants which are well adapted to unfavorable environmental conditions of mining dump were used in this study. Two important features of these plants are the ability to accumulate metals and to develop mechanisms protecting plants from harmful environmental influence. Therefore these plants were consider to be an attractive objects of research related to remediation processes.

In this work zinc speciation in roots and over ground parts of *Plantago lanceolata* was evaluated. Plants were grown under hydroponic conditions, than a part of floral material was treated withliquid nitrogen and used for the speciation studies, the rest was digested and used for the quantification of total amount of zinc.

Total amount of zinc in plant`s organs was measured using ICP MS after microwave assisted digestion of plants tissues. The investigation of zinc speciation was conducted using a HPLC ICP MS. The size exclusion chromatography (SEC) was used to evaluate the molecular weight of zinc-containing compounds and ion-exchange and reversed phase chromatography were used to separate various zinc species. Appropriate fractions of eluent were collected and analyzed using electrospray ionization mass spectrometry (ESI MS) in order to examine the structure of zinc compounds.

DETERMINATION OF NATURAL, FEMALE SEX SELECTED HORMONES IN ENVIRONMENTAL SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE COUPLED WITH VARIOUS TYPES OF DETECTION

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Steroid sex hormones are important biological messenger. Among the EDC's, natural and synthetic estrogens have been recognized as having the greatest endocrine-disrupting potential. Metabolites of oral contraceptives, which enter surface waters with sewage, are one of the main source of estrogens in the environment. While the amounts of ethinylestradiol (E2) and progesterone in modern contraceptives are very small, older generations of these drugs contained significantly higher amounts
of the hormones. The residues of these older generation drugs are still present in the environment and exert endocrine disrupting effects. The remaining EDC's are not regulated in any way. Taking into account the potential dangers related to these compounds, determining of its actual concentration and effects exerted by them on the environment and biota is urgently called for. Selection of the proper methods is dictated by the type of analyte, the level of its concentration, source and type of information produced by the method.

In this study the methods for the qualitative and quantitative determination of estrogenic compounds in surface waters, groundwater, wastewater and drinking water consist of the basic steps of the analytical procedure, including sample collection, preservation and storage, filtration, analyte isolation and pre-concentration (by SPE technique using different sorbents packed in columns or trapped in extraction disks) and final determination with various types of detection (HPLC/DAD/FLD/MS-MS). Selected conditions have been applied to determination of four estrogens and one progestin in several types of aqueous mixtures, including drinking water and municipal sewage in Gdansk region (Poland).

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POLYBROMINATED DIPHENYL ETHERS IN HOUSE DUST SAMPLES FIRST APPROACH

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According to many references, people spend more than 90% of the day in indoor environments. Therefore, more and more attention is being paid to indoor air quality monitoring issue. Due to the adverse health effects they may cause, SVOCs together with VOCs are among most hazardous substances present in indoor environment. They are emitted to indoor air by outdoor pollution sources (through ventilation), but mainly by large number of man-made building materials and furnishing.

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardant additives used in thermoplastics, polyurethane foam and textiles. They are compositionally similar to polybrominated biphenyls (PBBs), although their molecular configurations differ due to the presence of an ether linkage between the phenyl rings. Due to their physicochemical properties, PBDEs show high affinity to occure in dispersed phase and can settle down onto house dust particles. It has been widely commented in literature that PBDEs can easily accumulate in human tissues. High concentrations of PBDEs were reported in human blood and milk [1-2].

The extensive use of PBDEs as flame retardants in a great variety of consumer products has led to widespread contamination of the indoor environment. Taking into account growing importance and interest in issues related to indoor environment quality assessment, analysis of house dust samples will provide important analytical information on the concentration of PBDEs as well as give relevant data on human exposure to semivolatile organic compounds (SVOC) present indoor.

This study examines first attempt to develop new and complex methodology for monitoring PBDEs concentration in house dust samples. It presents results obtained by comparing different available extraction techniques; Accelerated Solvent Extraction (ASE), Soxhlet extraction and ultrasound assisted extraction at the stage of selecting the best liberating method that will enable determining PBDEs at lowest possible concentration levels.

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MEASURING BENZENE CONCNETRATION IN URBAN AIR USING PASSIVE SAMPLING

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According to the report published in 2005 by World Health Organization (WHO), presence of VOCs in atmospheric air may significantly affect its quality.

Benzene is one of the compounds, which concentration in atmospheric require systematic control. According to EU directive EU 200/69/EC concentration of benzene cannot exceed 5μg m^{-3}. This follows from the fact that benzene was listed in the Group I (Human Carcinogens) by International Agency for Research for Cancer (IARC). Long term exposure to high concentrations of benzene may increase the risk of cancer including leukemia [1].

Tri-city area is one of the most industrialized areas in Poland. There are number of large companies located here including: refinery, CHP " Wybrzeze", energy company Energa S.A and loading terminals within the port area. Tri-city is also known as a center of shipbuilding industry. Furthermore increasing vehicle traffic, as well as the number of petrol stations can be a significant source of wide range of hazardous compounds (e.g.VOCs) in the air and do affect its quality.

Accurate and reliable valuation of benzene concentration in the air requires selection of appropriate sampling technique, which enables continuous monitoring and in addition is cost effective. Therefore passive sampling technique has gained much popularity in recent years [2].

This study examines the performance of *Radiello*® diffusive passive sampler for the long-term characterization of time-weighted mean concentration of benzene. It presents the results obtained during monitoring campaign in 2007-2010 in Tricity area.

The results of research carried out demonstrate that passive sampling has turned out to be suitable for being considered as part of an emerging strategy for monitoring a wide spectrum of priority pollutants in ambient air.

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EN44 **Session B**

BIOINDICATION HEAVY METALS USING LICHENS IN THE AREA OF DONJE VLASE AND CERJE (SUOTHEASTERN SERBIA)

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Lichens are good biomonitors of air pollution because they are highly dependent on atmospheric sources for nutritiens. Besides indicating air quality by their presence or absence lichens also have been used as accumulator organisms for present heavy metals in atmosphere. Heavy metals are serious environmental problem because they are toxic, non-biodegradable, accumulate in living systems and have a long half-life in soil. In plants, through which enter the food chain, they can lead to morphological and physiological changes (high affinity for –SH group of some enzyme systems, free radical production etc.). It is shown that there is a direct correlation between the concentrations of heavy metals in lichens and air, and as such can be used as suitable biomonitors for tracking air pollution with heavy metals. In this study was determinate a concentration of heavy metals in *Evernia prunasti* and *Parmelia sulcata* lichen species from locality Donje Vlase and Cerje (region of Southeastern Serbia), in order to monitor air quality. These localities were chosen because they are in the relatively near urban areas of the City of Niš, and not under significant anthropogenic impact (motor vehicle traffic, industry etc). Collected samples of lichen were first dried then grounded, burned and demineralised with a mineral acid. The content of heavy metals in the samples was determined by inductively coupled plasma–optical emission spectroscopy (ICP–OES). The ICP-OES analysis showed that the heavy metal content in lichens from these two localities, and consequently in the air, are in usual limits for ecosystems with these kinds of anthropogenic influences.

CHLOROPLAST PIGMENTS OF GERANIUM MACRORRHIZUM L. AND DORONICUM COLUMNAE TEN. FROM THE PLACE ON A FIRE OF VIDLIČ MOUNTAIN

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In this study was determinated the content of chloroplast pigments (chlorophyll a, b, a+b, and carotenoids) in leaves of plants *Geranium macrorrhizum L.* and *Doronicum columnae Ten.* from the beech forest place on a fire of Vidlič mountain (Southeastern Serbia). As controls were taken the same kind of plants from the place that not exposed to fire. Analyses of chloroplast pigments was done in the same plants, a year later. Chloroplast pigments were determined from acetone extracts of these plants spectrophotometrically at wavelengths 662, 644 and 440 nm.

The first and second year after fire in both plant species content of chlorophyll a, chlorophyll b and chlorophyll a+b is greater at a place that not exposed to fire. Considering the physiological role of all types of chlorophyll in photosynthesis, decreased chlorophyll content, as a result of fire, even after two years can be considered as negative. Carotenoid content was higher in both plant species at fire site than at the surface without fire, a year after the fire. Two years after the fire, plant *Geranium macrorrhizum* L. from the place on a fire still has a higher content of carotenoids compared with plants outside the fire site, while *Doronicum columnae Ten.* show opposite behaviour. These differences can be attributed to the different physiology of plants, given that one belongs to the family *Geraniaceae* and the other is from the family *Asteraceae*.

TOXICITY MEASUREMENT IN IDENTIFYING AN ENVIRONMENTAL HAZARD – EXAMPLE OF THE SZCZECIN BAY (POLAND)

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Sediments can be considered as a sink for accumulation of pollutants introduced to the aqueous environment. Depending on chemical character of contaminants and structure of the sediment, these substancess can be bound in a different way to the sediments matrix and thus more easily or hardly available to aqueous organisms.

Chemical monitoring enables determination of particular substances with high precision and accuracy. Anyway it is reduced to selected chemicals considered to be pollution indicators. Such system is not able to detect presence of numerous chemicals what can have detrimental effect on the entire ecosystem. Additional restriction of chemical monitoring is difficulty to predict the influence of many chemicals presence at different concentration levels and interrelations between them.

Application of biotests as indicators of pollution level eliminates these difficulties and enables obtaining information on final threat posed by type and level of contamination, its availability and relations occurring between numerous pollutants present in the ecosystem. Battery of biotests (*Daphnia magna*, *Vibrio fischeri*, *Lepidium sativum*, *Thamnocephalus platyurus*, *Heterocypris incongruens*) has been applied to set of 200 sediment samples collected from the Szczecin Bay (northern Poland).

Localisation of sampling points characterized with elevated toxicity level and increased bioavailability indicates the Dziwna River as a pollution source in this region. Grouping of elevated toxicological indicators near the transport route suggests incidences of uncontrolled release of waste waters from the routing ships.

THE INFLUENCE OF SOIL CONTAMINATION BY HEAVY METALS ON BIOACTIVE COMPOUNDS CONCENTRATION IN WHITE CABBAGE (*BRASSICA OLERACEA* VAR *CAPITATA F ALBA*)

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White cabbage is one of the most popular cruciferous vegetables cultivated in Poland. Two properties of this plant *i.e.* the ability of pollutant bioaccumulation and high content of sulfur organic compounds were the basis of presented studies. It is known that these properties are interdependent; therefore cabbage cultivated for phytoremediation can be used for natural pesticides.

The plant material used during the studies was collected from different type of cultivation (natural-various localization in Poland and controlled). The environmental condition was characterized by measuring the metal content in soil and cabbage leaves. Harvested vegetables were analyzed as regards glucosinolates (GLS) content and composition as well as antioxidant potential of lyophilized leaves.

The heavy metals content was performed by Atomic Absorption Spectrometry and GLS by High Performance Liquid Chromatography.

Despite fairly similar climatic conditions, both GLS content and composition differed substantially for vegetables grown in different locations. Also, antioxidant activity depends on location, though not always parallel GLS content. In general, cabbages displayed the ability to accumulate metals. Those growing on more polluted soils tend to produce more GLS.

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DEVELOPMENT OF ANALITYCAL PROCEDURES FOR ORGANIC POLLUTANTS CONTROL IN SURFACE WATERS IN BULGARIA

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Chemical status of surface waters is determined through environmental quality standards, defined for 33 priority pollutants (Directive 2008/105/EC), mostly represented by hydrophobic, persistent organic compounds and pesticides. Emerging organic pollutants introduced into the environment because of humans' anthropogenic activities and characterized with higher molecular weights and polar/ionic moieties (such as pharmaceutically active compounds, endocrine disrupting chemicals, estrogens, and steroid hormones) has also generated serious concern since there is a limited knowledge for their environmental concentrations, fate, and synergistic and nontarget effects. Therefore precise and accurate analytical methods are required for reliable assessment of environmental status of surface waters and efficiency of measures for its improvement. In the present study analytical methods were developed for the determination of priority and emerging pollutants in surface waters in Bulgaria. Analytical approaches are based on solid-phase extraction as sample pretreatment procedure and liquid chromatography mass spectrometry as instrumental method. Optimal chemical conditions for quantitative separation and preconcentration of target compounds in a single step extraction procedure are defined. Method performance and matrix effects during LC MS measurements are discussed in details. Matrix matched calibration, standard addition method or method of internal standard are studied as calibration procedures. The applicability of recommended analytical procedures for different types of surface waters e.g. river, lake and sea or waste waters was tested by added/found method. The analytical recovery achieved for all compounds is greater than 86% and repeatability is up to 20%. The detection limits established ranged between 5 and 50 ng/L for alachlor, chlorfenvinphos, chlorpyrifos, diuron, isoproturon, trifluraline, acetochlor, pendimethaline, cypermethrine and is about 0,5 ng/L for estrone, 17α-etynylestradiol,17α-estradiol, 17β-estradiol, estriol and bisphenol A. The concentration ranges of measured priority pollutants in main Bulgarian rivers as well as in Black sea monitoring stations were presented. The levels of studied emerging pollutants in effluents from WWTP near Sofia and in rivers accepting untreated urban waste waters are compared and discussed.

APPLICATION OF ANALYSIS OF THE MAIN COMPONET QUALITY ASSESSMENT OF OIL AND BIODIESEL PRODUCED FROM "RICINUS COMMUNIS L" FERTILIZED WITH TREATED SEWAGE ANAEROBIC AND IRRIGATED WITH PURE WATER

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Water is a natural resource that currently is becoming increasingly scarce due to the increase in demand caused by population growth and supply reduction promoted by the pollution. The rationalization of water emerges as an alternative to minimize its scarcity, which can be done through the reuse, as well as through irrigation. The influence of the irrigation from the domestic sewage was detected through the yield of the castor bean obtained in the process of extraction with hexane, in which was obtained an oil content between 46 and 70% from 40g of seeds. It was found that productivity was higher for seeds from papaya tree fertigated with treated wastewater than those irrigated with fresh water. The data processing was performed by Principal Component Analysis, and the following variables were studied: Density at 20 º C, Viscosity at 40 ° C, acid value and their Fatty Acids biodiesels. The acquirement of the oils showed that the irrigation of papaya trees wasn't capable of alter the values of density and kinematic viscosity significantly. However, the fertigation decreased the concentration of present free fatty acids, reducing the acidity of the oil. The results showed that the biodiesel produced from fertigated and irrigated seeds presented a saturated esters rate equal to 20.07% and 6.91% respectively, and unsaturated 79.92% and 93.09% respectively. Though the presence of unsaturated fatty acids is necessary in order that occur the **fluidity** of the biodiesel, there must be an equilibrium with the saturated, to avoid the biofuel freezing in locals of cold temperature, indicating that biodiesel from fertigated castor obtained a better quality than that of biodiesel from castor irrigated with fresh water.

COMPARATIVE ANALYSIS OF DEGRADATION OF DIFFERENT GROUPS USING EMERGING CONATAMINANTES ADVANCED OXIDATION PROCESSES

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The water quality has been studied by research groups around the world, which identified a new group of persistent pollutants in environmental matrices that damage the health of animals and human beings, as important alterations in the endocrine system and breast cancer in women. These contaminants were called emerging contaminants (EC) and are found at levels of mg / L. So, detection techniques using mass spectrometry have been used to identify them, making possible to assess its presence in the environment and the effectiveness of their degradation in samples of industrial and domestic effluents. Advanced oxidation processes (AOP) has been employed aiming to degrade these pollutants. This study evaluated the percentage of degradation of two groups of EC: medicines and hormones. The experiments were conducted using UV-C, with about 50 mL of the compounds in aqueous medium at a concentration of 1 mg / L of each EC. Analysis of POA was performed by using factorial planning, studying the following variables: pH, H_2O_2, iron and time. The results showed that the process utilized was **efficient**, reaching 100% degradation of the studied medicines (aspirin, diclofenac and paracetamol) and more than 70% degradation of hormones (17 α-ethinyl estradiol and progesterone). For the medicines was found that the process UV/H2O2 is sufficient to remove them, not requiring the addition of ferrous sulfate to obtain a good removal of these substances. For the hormones was obtained a removal of 90% of 17 α-ethinylestradiol and 72.5% of progesterone. In all cases the addition of iron to the process contributed negatively to the increase of degradation, indicating that the process was sufficient to degrade UV/H_2O_2 these classes of CE.

REMOVAL OF COBALT(II) BY USING ADSORPTION ON DIETHYLENE TRIAMINE GRAFTED MACROPOROUS GLYCIDYL METHACRYLATE BASED COPOLYMER

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The aim of this study was to investigate the adsorption potential of macroporous crosslinked poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) [abbreviated PGME], grafted with diethylene triamine (deta) to produce a chelating resin (PGME-deta). Cobalt containing compounds are widely used in such industrial applications as mining, metallurgy, catalyst preparation, electronics, paint and pigments production, as well as agriculture. Co-60/58 radionuclides are also among the most critical constituents in nuclear power plant waste liquids and waste streams, concerning radiation doses to personal and environment. Acute cobalt poisoning in humans may cause bone defects, paralysis, heart damage, asthma, damage to thyroid and liver, while exposure to ionizing radiation is associated with mutagenic effects and cancer. The adsorbent was characterized by elemental analysis, mercury porosimetry, attenuated total reflectance (ATR) spectroscopy and scanning electron microscopy (SEM). Co(II) concentrations in solution were monitored by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The influence of pH, contact time, initial concentration and temperature on sorption of Co(II) by PGME-deta from aqueous solution is reported. Static batch kinetics, equilibrium and thermodynamics were analyzed. The respective data were tested with the surface reaction and diffusional kinetics models, to assess the applicability in the field of water pollution control. Adsorption isotherms were determined to evaluate the affinity of PGME-deta for Co(II), and the equilibrium data was fitted with Langmuir, Freundlich and Temkin models. The maximum experimental sorption capacity was 1.17 mmol Co(II)/g resin, at 25°C, pH 5.5 and the initial concentration of 100 mmol L^{-1}. It was demonstrated that an increase in solution temperature had a marginal effect on Co(II) sorption on PGME-deta. The negative value of ΔG^0 confirmed the spontaneous nature of the process.

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ORGANOBENTONITES AS ADSORBENTS OF COMPLEX MIXTURES OF DYES AND HEAVY METALS

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Untreated textile wastewaters often contain a great diversity of organic and inorganic pollutants (including non-biodegradable substances like dyes, additives, as well as heavy metal cations like Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, and Ni^{2+}). Finding a multipurpose adsorbent for both inorganic and organic pollutants is a great challenge. It was shown that organobentonites modified with tetraalkylammonium ions in amounts corresponding to cation exchange capacity (CEC) can be applied in such purposes [1]. In this study organobentonite obtained using domestic bentonite from mine Bogovina and hexadecyltrimethylammonium cation in amount corresponding to CEC of this clay was tested as a multifunctional adsorbent. Synthesis and characterization of the obtained adsorbent were described in previous studies [1, 2]. As a test model of wastewater the six component solution was used. The solution was prepared from three selected textile azo dyes: Acid Orange 10 (AO 10), Acid Yellow 99 (AY 99) and Reactive Black 5 (RB 5) as well as three toxic metals: Pb^{2+}, Ni^{2+} and Cd^{2+}. Initial concentration of each pollutant was 50 mg dm^{-3}. Dye concentration monitoring was performed by UV-VIS spectroscopy. It was proven that neither hypsochromic nor bathochromic shifts appeared in the spectra of dye mixture in comparison with each spectrum of single dye. It was previously confirmed that the presence of investigated metal cations does not affect either the position or the intensity of the dyes absorption bands. After adsorption experiment dye concentration of each dye in mixture was calculated using the additive property of absorbance [3]. Metal ion concentration was estimated by ICP-OES. The adsorption test showed that the adsorption affinity of adsorbents follows the trend for dyes AY 99>RB 5>AO 10 and for toxic metals: $Pb^{2+}>Ni^{2+}>Cd^{2+}$.

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DETERMINATION OF PERFLUORINADED COMPOUNDS IN SEWAGE SLUDGE AND PACKAGING SAMPLES BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY WITH QUADRUPOLE-TIME OF FLIGHT MASS SPECTROMETRY DETECTION

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Perfluorinated compounds (PFCs) have been widely used in different commercial and industrial applications such as paints, lubricants, PTFE synthesis, polishers and food packaging due to their stability and surface-tension lowering properties. The concern about PFCs has increased in the recent years because of their persistence and bioaccumulation in the environment, as well as, their toxicological effects.

In this communication, reverse-phase ultra performance liquid chromatography (UPLC) coupled to a quadrupole-time of flight mass spectrometer (Q-TOF-MS) with electrospray ionisation (ESI) operating in negative ion mode is applied to determine PFCs in samples of interest, such as food-contact materials and sewage sludge. Target analytes were perfluoroctanosulfonate (PFOS) and six perfluorocarboxilic acids (PFCAs): perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA) and perfluorododecanoic acid (PFDoA).

UPLC and ESI-Q-TOF-MS conditions were optimised in order to obtain a fast and sensitive method. The separation of the seven PFCs was achieved in 3.5 minutes, and limits of detection ranging from 0.06 to 2 ng mL^{-1} were found in liquid extracts. The linearity of the method was studied in methanol solution and in matrix extract. This study showed that the method is free from matrix-effect errors and the regression coefficients ranged from 0.997 to 0.9998.

In preliminary studies, a pop-corn bag and a sewage sludge samples were extracted with methanol using pressurised liquid extraction (PLE) and focused ultrasound solid-liquid extraction (FUSLE). PLE conditions were two cycles of 5 minutes at 100ºC, and FUSLE conditions were 10 mL of methanol and an ultrasound power of 75% for 1 min. The seven PFCs studied were found in the pop-corn packaging with levels ranging from 10 to 140 ng g^{-1}. Only three PFCs (PFHpA, PFOS and PFDA) were found in the sewage sludge sample and their levels were between 4 and 22 ng g^{-1}.

FOCUSED ULTRASOUND SOLID-LIQUID EXTRACTION OF BROMINATED FLAME RETARDANTS FROM INDOOD DUST FOLLOWED BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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A fast and simple method based on focused ultrasound solid-liquid extraction (FUSLE) followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) was developed for the determination of polybrominated diphenyl ethers (PBDEs) in indoor dust.

PBDEs have been widely used as additives for the manufacture of furniture, electrical and electronic equipment, textiles and many other consumer products because of their flame retardant properties. As they are not bonded to the plastic structure, they can migrate to the environment. Indoor dust acts as a repository of PBDEs and it is one of the main sources of human exposure.

FUSLE is a fast, low-cost and efficient extraction technique based on the application of high power focused ultrasonic waves using a micro-tip immersed directly in the extraction mixture.

First, the main FUSLE factors affecting the extraction efficiency were studied and optimised by means of a central composite design. Optimal FUSLE conditions were: 8 mL of the mixture 3:1 hexane:acetone as extraction solvent and an ultrasonic irradiation power of 65% for 20 s. Then, the influence of the number of cycles was studied and it was concluded that one cycle was enough for an exhaustive extraction.

Next, the absence of matrix effect was proved and the whole method was characterised in terms of accuracy, repeatability and sensitivity. Relative standard deviation values were lower than 10% in all cases and good recovery values, above 93%, were obtained. Finally, the method was applied to the analysis of different indoor dust samples, including a standard reference material in order to assess accuracy.

SOLID PHASE MICROEXTRACTION AS A POWERFUL TOOL TO ESTABLISH THE VOLATILE METABOLOMIC PROFILE OF *LYCOPERSICON ESCULENTUM*

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The tomato (*Lycopersicon esculentum*; Solanaceae) is one of the most widely consumed fresh vegetables in the industrialized world, used by the food industries as a raw material for the production of derived products such as purees or ketchup. Botanically, it is a berry fruit, but it is cultivated and used as a vegetable. Nutritionally, is a good source of vitamin A and C, sugars, organic acids, lipids, pigments, minerals, free amino acids, polyphenols and flavor compounds. The flavour of tomato results principally from a combination of volatile compounds for aroma and of sugars and acids for taste. Although more than 400 volatile compounds have been identified, only a limited number is essential and seems to explain really the global fresh tomato aroma profiles. Analytical methodology for determination of volatile compounds in vegetable matrices is continuously improving due to the important role of these compounds in organoleptic quality of food.

In this study the investigation of the volatile metabolites of tomatoes was carried out to identify the odorous target components responsible for the characteristic aroma of these valuable natural products. The analyses were carried out by means of headspace solid-phase microextraction (HS-SPME) as an alternative sample preparation strategy, to overcome the problems associated with conventional sampling methodologies, such as elevation costs, time-consumption, and the use of large volumes of organic solvents. The typical aroma of *tomato* can be attributed substantially to the large amounts of aldehydes and, to a lesser extent, to the content of higher alcohols and monoterpenes. The most important volatile metabolites found in tomato aroma were hexanal, cis-3-hexenal, hexanol, cis-3-hexenol, 2-isobutylthiazole, geranylacetone, 3-methylbutanal, 2-phenylethanol, 2-octenal, and 2,4-decadienal.

The results showed that the major aldehydes of *Lycopersicon esculentum* were hexanal (23.9%), (cis)-2-hexenal (8.2%), and 2,4-decadienal (3.9%). Of the Higher alcohols, the main components were 1-hexanol (10.8%), and cis-3-hexenol (4.73%), while geranilacetone (3.8%) is the most abundant sesquiterpenoid.

A SIMPLE AND FAST OPTICAL ANALYTICAL METHOD TO DETECT SIMULTANEOUSLY AFLATOXIN B AND OCHRATOXIN A

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Mycotoxins such as ochratoxin A (OTA) and aflatoxins (AFs) are secondary metabolites produced by fungi on a variety of foods such as groundnuts, nuts, dried fruit, wine, coffee, cereals. Due to the their high toxicity, the European regulations set the legal limit for both at ng/g levels, thus a simple method to detect them using the same sample procedure and the same portable instrument can be useful in order to have a cost-effective and fast measurement.

In this work, we developed a portable and fast analytical method for both AFB_1 and OTA detection, based on a fiber optic system coupled to portable and miniaturised spectrophotometer/fluorimeter.

In the case of OTA, the analysis is based on the intrinsic OTA fluorescence. Detection was carried out using an OceanOptics USB2000-FL Spectrometer at 333 nm (excitation) and 470 nm (emission). SpectraSuite software allows performing the three basic spectroscopic experiments-absorbance, reflectance, emission- as well as signal-processing functions such as electrical dark-signal correction, stray light correction, boxcar pixel smoothing and signal averaging. Using the optimized parameters like Integration Time of 1 sec, Scan Average of 50 and a Boxcar of 100 a calibration curve in methanol was constructed in the OTA concentration interval between 5 and 90 ng/mL with a detection limit of 1 ng/mL. As real sample application in order to set the analytical performance of the system, samples of white wine and soluble coffee were tested. For cleanup and extraction of the samples, two types of immunoaffinity columns (OchraTest from Vicam™ and NeoColumn from NEOGEN *Europe Ltd*.) were used with satisfactory results.

The AFB detection is based on acetylcholinesterase (AChE) inhibition by the mycotoxin and the AChE residual activity was determined using the spectrophotometric Ellman's method, as recently demonstrated by our research work. The working optimal conditions of the instrument in this case were Integration Time of 3 milliseconds, Scan Average of 80 and Boxcar of 15, and the analytical performance resulted in a linear range for AFB_1 between 80 and 240 ng/mL and a detection limit of 40 ng/mL.

In order to have the same sample treatment, Afla-OtaCLEAN™ immunoaffinity column (from LC*Tech* GmbH, Germany) in millet was used. Preliminary results will be presented.

IMPACT OF DIETARY PROTEIN SOURCES ON LIPID CONTENT AND FATTY ACIDS PROFILE OF MUSCLE AND LIVER OF JUVENILES SENEGALESE SOLES

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The use of vegetable protein sources (instead of animal ones) in diets for aquacultured fish has large sustainability and safety advantages. In this study, we aimed to assess the impact of different vegetable diets in the fat content and fatty acid profile of muscle and liver of juvenile Senegalese sole (*Solea senegalensis* Kaup, 1858). The animals considered in this study (6 groups) were fed for 92 days under controlled conditions, based on diets with different levels of vegetable protein (increasing amounts up to 75% of total protein using different mixtures of soy, wheat, corn, peas and potatoes). The fish were caught after a 24 hours fast. Samples of muscle and liver were collected and immediately frozen at -80°C. Lipid content was determined by the Folch method and fatty acid profile was analyzed by GC-FID after methylation [1].

Total lipid amount in the liver significantly increased ($p<0.05$) with the amount of vegetable protein ingested, while no differences were found between muscles ($p>0.05$) of the different groups. However, muscle of animals fed with more vegetable protein had higher relative percentages of C18:2n6 ($p<0.05$) and lower C20:5n3 (EPA) ($p<0.05$). In what concerns to the liver, an increase in the vegetable protein content of the diet results in upper levels of C16:1, C18:2n6 and C20:2n6, and a decrease in C18:0, C20:4n6 (ARA), C20:5n3 (EPA), and C22:5n3.

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A RAPID AND PRECISE METHOD FOR THE DETERMINATION OF CHLORIDE IN BREAD AND DOUGH USING A HOME-MADE ION SELECTIVE ELECTRODE

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The reduction of salt (NaCl) intake is a public health issue in many countries, because it has been identified as the main cause of several diseases such as hypertension, heart and kidney diseases, stomach cancer, osteoporosis, stroke and obesity [1]. Most of the salt in our diets is found in processed foods. Of all foodstuffs, bread has been identified as the single highest contributor to the total daily salt intake [2].

In this work, a method based on direct potentiometry was developed to replace the traditional and time-consuming method based on argentometric titration with potentiometric detection [3]. For this purpose, all-solid-state chloride ion selective electrodes (ISEs) [4] based on a tetraphenylporphyrin manganese(III) chloride complex were constructed and evaluated. The studied ISEs displayed Nernstian behavior, with average slopes of -59 mV decade^{-1}, in the concentration range from 5×10^{-5} to 5×10^{-2} M.

To evaluate the performance of the ISEs for chloride quantification, 30 samples, bread and respective dough, were analysed by both direct potentiometry and the potentiometric titration. The correlation coefficient between the results of these methods was 0.99 confirming that there is a good correlation between the obtained values. The intercept value was 1.4×10^{-4} M with confidence limits (95% level) of -4.6×10^{-4} and 7.4×10^{-4} M, and therefore was not significantly different from zero. The slope value was 0.99 with confidence limits (95 % level) of 0.95 and 1.03.

These results showed that direct potentiometry is an adequate alternative for the determination of chloride in the analysed samples.

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THERMAL AND X-RAY ANALYSIS OF DIFFERENT CHOCOLATE FORMULATIONS

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The complexity of chocolate arises from the polymorphic nature of its constituent fats that can come in several crystal forms. DSC and X-ray powder diffraction (XRPD) can be used to better understand the complexity of chocolate and assess these different crystal forms, where precise control of temperature and time are crucial. The key practical problem for the chocolate manufacturer is that the most thermodynamically stable form is not the most desirable for the consumer. Making chocolate involves ensuring that the final product is in the right crystalline form. Chocolate itself is a functional food as it contains sufficient antioxidants and flavonoids, but can bee even improved with addition of probiotic cultures**.**

Thermal analysis of 12 chocolate samples, (4 samples of dark chocolate, 4 samples of milk chocolate and 4 samples of “menaz” chocolate) with addition of different probiotic cultures (*B. Lactis*, *L. Acidophylus)* was performed on TA Instruments DSC Q 1000, differential scanning calorimeter in the aim of investigation the impact of added probiotic cultures particles on chocolate crystal forms. The cyclic scans (heat- cool-heat) in temperature range from 2ºC to 60ºC at heating/cooling rate of 3ºC/min with N_2 purge gas, were used for all DSC scans. From results obtained, temperatures of melting and crystallization (Tm and Tc) and enthalpies of melting and crystallization (ΔH_{melt} and ΔH_{cryst}) it can be concluded that there is influence of probiotic culture particles to degree of crystallinity and melting properties (T_{end}, T_{onset}, T_{peak} and T_{index} and ΔH_{melt}) of chocolate.

XRPD patterns of samples were recorded on Philips PW-1710 automated diffractometer in 2θ-range 4-50º, counting for 5s at 0.02º step. Diffraction data revealed the presence of polymorph V of cocoa butter in all chocolate samples. Milk chocolate samples showed the highest amount of amorphous phase compared to dark and “menaz” chocolate samples. Increase in crystallinity after addition of probiotic cultures was detected in all chocolate samples. This effect was most pronounced in milk chocolate samples.

THERMAL ANALYSIS OF DRIED APRICOTS

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The importance of the rubber to glass transition (Tg) and formation of the glassy state became widely appreciated in understanding and controlling of food materials.

The objective of this study was to determine optimal packaging material based on differences of dried apricot's thermal characeristics. PCA and SWOT analysis were permformed, in order to obtain statistical information regarding the quality changes of packed apricots.

Glass transition (Tg) of dried apricots *(Prunus armeniaca L.)* packed in four different packing materials, with appropriate barier properties and modified atmosphere (30% CO_2, 60% N_2), has been followed by differential scanning calorimetry (DSC). DSC scans were performed in TA Q1000 calorimeter with RCS cooling unit. All samples have been scanned in temperture range from -80ºC to 180ºC; heating rate 5º C/min in nitrogen. Water content, residue at 700ºC and total weight loss at 700ºC were determined by thermogravimetric analysis (TGA), TA Q500 analyser. Dried apricots has been packed in four packaging materials: paper-polyethylene (PAP/PE), paper-aluminium-polyethylene (PAP/AL/PE), polyester-polyethylene (PES/PE) and polyester-aluminium-polyethylene (PES/AL/PE). Thermal stability was followed in 6 month intervals during a year. Statistical analysis included PCA and SWOT.

As a consequence of aging and package conditions, some structural changes of food material has been induced through variation of glass transition temperature (Tg). Two glass transitions have been found. First glass transitions (Tg_1) was detected in temperature range of -60ºC to -18ºC and second one (Tg_2) in temperature range of -21 ºC to 34 ºC. A wide, complex endothermic peak between 50 ºC and 163 ºC was found in all samples.

According to results obtained, most stabile were samples of dried apricots packed in PES/AL/PE package, which was also confirmed by PCA and SWOT analysis.

CHARACTERIZATION OF THE GEOGRAPHICAL ORIGIN OF BEERS USING SUPPORT VECTOR MACHINES

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Beer is one of the oldest known alcoholic beverages. It is produced by the yeast fermentation of cereals germinated in water and flavoured and stabilized with hops, being this ingredient responsible of the bitter taste of this beverage. Its social and economic importance is evident. 2.5 million jobs can be accredited to the production and sale of beer in the 27 members of the European Union plus Norway, Switzerland, Croatia and Turkey. European breweries produce 427 million hectolitres of beer annually and the total revenues for the national governments are estimated approximately at 57 billion euros. There is a current trend in the European Union of establishing Protected Geographical Indication of beers. Consequently, studies on the characterization of the geographical provenance of European beers are of great interest. In this work geographical characterization of beers from Germany, Portugal and Spain has been carried out. Several chemical descriptors have been selected according to their significance on the brewing process as well as their use as quality indicators. Contents of aluminium, barium, boron, calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, strontium and zinc were determined by inductively coupled plasma optical emission spectroscopy. Ion chromatography was used to obtain the content in chloride, phosphate and sulphate. Total amino acids were determined by spectrophotometry with ninhydrine and Folin Ciocalteu reagent was used to determine total polyphenols. Other considered parameters were pH, real extract and absorbance at 430 nm. The number of input variables was reduced taking into account the results of the Kruskal-Wallis test and backward stepwise LDA, retaining the contents of iron, potassium, phosphorous, phosphate and total polyphenols as the most discriminant chemical descriptors. Once the feature reduction was carried out, SVMs were applied to construct a classification model which presented an overall classification ability of 99.3%.

SUBCUTANEOUS FAT TRIACYLGLYCEROLS PROFILE FROM IBERIAN PIGS AS A TOOL TO DIFFERENCIATE BETWEEN INTENSIVE AND EXTENSIVE FATTENING SYSTEMS

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Iberian ham is a dry-cured meat product manufactured according to traditional methods in southwest regions of Spain. Iberian pig products have an extraordinary acceptance by consumers and its demand is increasing in the last years. Nevertheless, the factor that determines the prices in the market is the fattening diet of animals being also the main factor that conditions the composition of Iberian pig adipose tissue. Several types of fattening systems can be considered. In one of them animals are reared outdoor, grazed on acorns and pasture, in an extensive system which is usually called montanera. Alternatively, animals can be fed with formulated feed in extensive or intensive systems. Ham products obtained from montanera system are much more appreciated by the consumers. The aim of this work is to analyze the triacylglycerols profile of the subcutaneous fat from Iberian pig in order to differentiate between intensive and extensive fattening systems. A total of 1773 samples of subcutaneous fat from male pure Iberian pigs were analyzed. Triacylglycerols were determined by gas chromatography using a fused silica capillary DB-17HT column (30m x 0.25 mm I.D., 0.15 μm film thickness). The oven temperature was kept at 320 ºC, and was then raised to 350 ºC at a rate of 2.0 ºC/min and held isothermally for 10 min. The injector temperature was kept at 360 ºC, while the detector temperature was 370 ºC. Hydrogen was used as carrier gas, while the make-up gas was nitrogen. The identification of seventeen triacylglycerols species was carried out by means of standards of trilinolein (LLL), triolein (OOO), tripalmitin (PPP) and tristearin (SSS) and by comparison with the relative retention times. Differentiation of the fattening systems has been performed using triacylglycerols as chemical descriptors and pattern recognition techniques like principal component analysis and linear discriminant analysis.

LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTOR IN SERIES WITH TANDEM MASS SPECTROMETRY FOR CHLOROGENIC ACIDS DETERMINATION IN CALAFATE

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HPLC-DAD-MS/MS is a potent analytical technology for characterization of functional ingredients in food. Calafate (*Berberis microphylla*) is a berry from Patagonia rich in anthocyanins (14,2 and 86,0 umol/g fresh weight), constituting one of the fruits which highest levels of this polyphenols. Other phenolic compounds, as flavonols and flavan-3-ols have also has been described in calafate, however there is no information about chlorogenic acids. These are well known phenolic secondary metabolites in plants with described high antioxidant capacity. Two factors contribute to complexity of their determination in calafate: their very similar chemical structures and the high anthocyanin concentrations in calafate berries, which interfered with UV detection of acids.

The principal aim of this work was to develop a method for chlorogenic acids idenfication and quantification in calafate fruits. With this purpose, a SPE method (MCX cartridge) and HPLC on a C-18 column were developed and optimized. Each compound was identified using HPLC-DAD-MS/MS, according their retention time, UV and MS/MS spectra in negative ionization mode. The quantification was at 320 nm and results were expressed as umol/g fresh weight.

The acids found in calafate were 3-caffeoilquinic and 4-caffeoilquinic acid (pseudomolecular ion $[M-H]^-$ at *m/z* 353 and only one product ion at *m/z* 191), showing different retention time (t_R) 11,8 and 12,7 min. Also 3,5-dicaffeoilquinic and 4,5-dicaffeoilquinic acid (pseudomolecular ion $[M-H]^-$ at *m/z* 515 and two products ions at *m/z* 353 and 191) were identified (t_R= 25,8 and 27,7 min, respectively). Fruits contain also six caffeic acid derivatives, but their identities have not been confirmed until now. The most abundant was 3-caffeoilquinic acid (0,55 ± 0,01 umol/g). The total concentration of chlorogenic acids in calafate berries was 3,12 ± 0,02 umol/g, a high level in comparison with other widely consumed fruits.

DETERMINATION OF ISOFLAVONES IN PULSES BY A MODIFIED QuEChERS METHOD AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Isoflavones are compounds naturally present in vegetables, the *Leguminosae* -and especially soy- being the most abundant sources. There are many biological activities associated with isoflavones, including a reduction in osteoporosis and the prevention of cardiovascular disease. They can also be used to attenuate the symptoms of menopause. Because of the importance of these substances, control of their levels in widely consumed foods is mandatory.

Sample treatment is a fundamental step and frequently the limiting factor when analytical methodologies are employed. QuEChERS method is based on a salting out extraction/partitioning procedure of a solid sample and dispersive solid-phase extraction (d-SPE) for cleanup. This has been used as sample treatment in the determination of pesticide residues in different kinds of samples. However, it is not easy to find references to QuEChERS method for the determination of substances naturally present.

In this work a QuEChERS method is proposed for the isolation of isoflavones (Daizdin, Glycitin, Genistin, Daidzein, Glycitein, Genistein, Formononetin, and Biochanin-A) in pulses (lentils, beans, chikpeas) with a view to obtaining clean extracts for injection into the chromatographic system. In this work, a modification of QuEChERS method was implemented: two polarity decreasing extraction steps are used and the cleanup step is avoided. Additionally, other variables were optimized.

UPLC-MS/MS was used for the separation and determination of isoflavones. A Zorbax Eclipse analytical column (4.6x50mm 1.8 μm) and acetonitrile/water in gradient regime were used, and all substances eluted within 7 min. Selective reaction monitoring (SRM) was used for quantitation. Collision energy, and cone voltage were optimized for each isoflavone. The standard addition and internal standard methods were used to avoid matrix effect. The LODs obtained were in the μg/L range.

STUDIES ON THE ANTIOXIDANT ACTIVITY OF SAGE (SALVIA OFFICINALLIS) EXTRACTS AND THE PREVENTIVE EFFECT ON LIPID PEROXIDATION OF A POLYUNSATURATED FAT ACIDS OIL

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The major diterpene, carnosic acid, present in ethanolic extract of Salvia Officinalis L. have received great attention in food science due to his antioxidant capacity. Carnosic acid is a lipophilic antioxidant that scavenges singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals, thus preventing lipid peroxidation. In the present study, the *in vitro* plants of sage were extracted with ethanol (75% v/v) and analyzed for their composition and antioxidant activity. Phenolic diterpenes (carnosic acid, carnosol, and 12-O-methylcarnosic acid) showed the highest concentrations of all the polyphenols The extracts were screened for their possible antioxidant activities with a complementary test system, namely DPPH free radical-scavenging, the IC_{50} value being 27± 1.4 mg/L. The preventive effect of the sage extracts in lipid peroxidation was evaluated according to the malondialdehyde/ high performance liquid chromatography assay in a linoleic acid and a high polyunsaturated fatty acids oil (olive oil) at 37^0C, in sodium phosphate-buffer (pH 7.0). At a concentration of 1.0 mg/ml of linoleic acid, the ehanolic extract from sage had a higher antioxidant activity 78% than α-tocopherol (68%) but less than synthetic antioxidant, buthylated hydroxytoluene (BHT, 96%). In olive oil, the extracts from sage leaves were found to exhibit a high efficacy in retardation of lipid oxidation (43%). Since the ethanolic extracts of Salvia Officinallis L exhibited excellent antioxidant activities when compared to BHT, it seems possible to keep perishable fat-containing food longer by direct addition of an extract of sage.

CHARACTERISATION AND ANTIOXIDANT ACTIVITY EVALUATION OF NIGELLA SATIVA OIL

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The seeds of *Nigella sativa* contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil.

The steam-distilled essential oil of black cumin seed (Nigella sativa L.) was investigated for its composition and antioxidant activity. GC-MS analysis of the essential oil obtained from three different samples of Nigella sativa seeds and from a fixed oil showed that the qualitative composition of the volatile compounds was almost identical. Differences were mainly restricted to the quantitative composition and antioxidative properties.

After oil analysis by GC/MS, 24 compounds were identified in the oil, representing 91.45% of the total amount. The antioxidant activity was evaluated by measuring peroxide, total carbonyl values and MDA-TBA complex of sun-flower oil at fixed time intervals. The essential oil showed strong antioxidant activity in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SQUID *LOLIGO GAHI*

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Polycyclic aromatic hydrocarbons (PAHs) constitute a diverse class of hydrophobic molecules that are ubiquitous environmental contaminants. The main health concern about PAHs is due to the fact that some have been shown to be highly carcinogenic in laboratory animals, having been also implicated in different types of human cancers [1].
PAHs are present in the marine environment and, in general, their highest concentrations are in the most urbanized costal areas, as a signature of human activity or as a result of industrial processes. As lipophilic compounds these pollutants easily cross lipid membranes and bioaccumulate in the aquatic organisms. Human beings are exposed to PAHs via inhalation, but mostly by intake of food. Like several other cephalopods, the squid species, *Loligo gahi*, is part of the traditional diet of coastal communities in southern Europe. There is a lack of data regarding the levels of PAHs present in this marine organism.
This work evaluates the content of 18 PAH compounds in squid captured in Southwest Atlantic Ocean (FAO Statistical Area 41). Samples were extracted by microwave-assisted extraction and the PAHs analysis was accomplished by liquid chromatography with fluorescence and photodiode array detection. Fresh squid samples were purchased from different local markets in Oporto region (NW Portugal), homogenised and stored at -20ºC until analysis. Naphthalene, fluorene and phenanthrene were the major PAHs detected in the squid muscle, with concentrations that ranged from 0.60 to 4.33, 0.02-0.36 and 0.72-2.03 µg/kg, respectively.

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QUANTIFICATION OF VOLATILE FATTY ACIDS IN CANARY CHEESES BY MULTI HEADSPACE SOLID-PHASE MICROEXTRACTION AND GAS-CHROMATOGRAPHY

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Cheese aroma is undoubtedly one of the most important factors involved in cheese acquisition by consumers. The majority of volatiles in cheeses are formed during ripening. Free fatty acids (FFAs) are formed during lipolysis. FFAs are present in higher contents in cheesed elaborated from crude milk than those from pasteurized milks. FFAs contribute, altogether with other volatiles, to cheese aroma. Indeed, the content and distribution profile of FFAs is a useful tool to evaluate the optimum ripening time for a cheese elaborated with crude milk.

The analytical methods usually employed to determine FFAs in cheeses require a preconcentration step prior to the analytical determination. Among them, solid-phase microextraction (SPME) is undoubtedly an important analytical tool. SPME has already been used for FFAs determination in cheeses in a number of works. However, quantitative analysis is still one of the most complex tasks when performing headspace sampling, particularly when volatiles are emitted from solid samples, like cheeses. Multiple headspace extraction (MHE) is an approach for quantification that enables to eliminate the matrix effect, but it has not frequently been applied if compared with the standard addition (SA) method or the stable isotope dilution assay (SIDA) method. Furthermore, few works have utilized the MHE approach in combination with SPME, and none of them for FFAs in cheeses.

It has been therefore studied the content of FFAs in a high number of canary cheeses elaborated with crude milk, specifically: formic acid, acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid, iso-valeric acid, n-hexanoic acid, iso-hexanoic acid, and n-heptanoic acid, using the novel MHE-SPME approach in combination with gas-chromatography.

FLUCTUATIONS IN ANTIOXIDANT CAPACITY AND POLYPHENOLIC CONTENT OF SMALL FRUITS IN STORAGE AT -20°C FOR ONE YEAR

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Fruits are excellent sources of antioxidant compounds as well as vitamins, minerals and dietary fiber, therefore, the US Department of Agriculture's healthy eating guidelines include a recommendation for consumption of at least 2.5 cups of fruits per day. Freezing at -20 °C is one of the most popular and convenient ways of storing small fruits long-term. The typical recommended storage time it is 8-12 months.

In the present study we monitored fluctuations in antioxidant capacity (Ferric Reducing/Antioxidant Power assay (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and 2,2´-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay), total phenols (TP, Folin–Ciocalteu assay), total flavonoids (TF, colorimetric assay with Al_2Cl_3) and anthocyanins (TA, pH differential method) of 10 small fruits during one year storage at -20 °C. Sweet cherries, sour cherries, strawberries, red currants, raspberries, blackberries, hawthorn, cornelian cherries, and red and white grapes were chosen for the study. The first set of fruits (sweet cherries, sour cherries, strawberries, red currants and raspberries) was frozen in June 2009, and the second set of fruits (blackberries, hawthorn, cornelian cherries, red and white grapes) was frozen in September 2009. Phytochemical content was determined at equal time intervals.

Initial TP contents in fruits ranged between 60.12-891.15 mg GAE/100g FW, TF content between 17.90-536.76 mg CE/100 g FW and TA content between 0-121.82 mg CGE/100g FW. The dynamic evolution of antioxidant capacity reflected the transient changes in phytochemical composition of analyzed fruits in storage. Fluctuations in phytochemical content observed during storage depended greatly on the type of fruit in question. Most of the analyzed fruits showed significant changes in phytochemical content and antioxidant capacity during and after one year of storage at -20°C.

VOLATILE COMPONENTS AND SUGARS AS QUALITY MARKERS IN BALSAMIC VINEGARS

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Balsamic vinegar is widely used as a dressing to flavour a wide range of food, thus playing an important role in gastronomy. It is produced traditionally (Protected Designation Origin PDO) using cooked grape musts aged in wooden barrels for at least 12 years. It seasons in progressively smaller kegs made of different types of wood until it is ready for sale. Alternatively, it can be made using grape must, vinegar and caramel with a seasoning of at least two months (Protected Geographical Origin PGO). Its chemical and organoleptic properties are determined by the raw materials used, the seasoning in wooden vessels and the technological procedures employed.

We aimed to determine and evaluate the volatile and sugar components of traditional PDO balsamic vinegar and to compare them with those of PGO balsamic vinegar.

We analyzed sugars with ionic chromatography using pulsed amperometric detection and two different columns. The monosaccharides analyzed were: glucose, fructose, xilose, arabinose, ramnose, galactose, while the di/trisaccharides analysed were: sucrose, trealose, maltose, isomaltose, maltulose, palatinose, melezitose, raffinose, erlose.

Volatile constituents were extracted with headspace-SPME (solid-phase microextraction) and carboxen-polydimethylsiloxane fiber; analysis was carried out using a wax capillary column and GC/MS detection. Several compounds were detected: aldehydes, chetones, alcohols, acids, esters, ethers, volatile phenols. Vinegar aromas were also evaluated using an electronic nose. The differences between the two types of vinegar regarding sugar profiles and volatile compounds were highlighted,.

PRESENCE OF OCHRATOXIN A IN GRAPE-DERIVED PRODUCTS

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Ochratoxin A (OTA) is a mycotoxin produced by strains of *Aspergillus* and *Penicillium* species. OTA is a nephrotoxic compound that can also shows hepatotoxic, genotoxic, teratogenic and immunosuppressive properties.

OTA persists in contaminated commodities due to its chemical stability. Although cereals and cereal-based products are considered the major source of OTA, grapes and grape-derived products, such as wine and vinegar, are considered an important source of exposure.

We evaluated the occurence of OTA in commercial samples of wine and vinegar using a method based on OIV method (MA-E-AS315-10-OCHRAT), with immunoaffinity clean-up concentration followed by HPLC with fluorescence detection. Proper pH adjustment in the initial dilution step was applied on vinegar samples.

The lowest detectable concentration was 0.02 μg/L. The EU maximum permitted level for OTA in wines and grape-must based products is 2 μg/L. No limit exists for vinegar.

OTA was detected in a relatively low percentage of analyzed wine samples. As confirmed in the literature, red wine samples were more contaminated than white. On the contrary, almost all vinegar samples were contaminated. However, all samples were below 2 μg/L. OTA was more present in balsamic vinegars than in wine vinegars.

The origin of this contamination was probably due to the poor quality raw materials used in vinegar production. OTA evaluation could help guarantee final product safety.

DETERMINATION OF BIOGENIC AMINES IN DIFFERENT TYPES OF CHEESE USING ION CHROMATOGRAPHY COUPLED WITH MS/MS DETECTION

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A new method for determination of underivatized biogenic amines in cheese based on ion exchange chromatography coupled with tandem mass spectrometric detection has been proposed. The method has been applied to the analysis of 10 biogenic amines (trimethylamine, putrescine, cadaverine, histamine, 2 - phenylethylamine, spermine, spermidine, tryptamine, agmatine and tyramine) in different types of cheese. The amines were extracted only with water without any additional derivative step or sample clean-up, which is a great advantage in terms of simplicity of sample pretreatment procedure when comparing this method to others currently existing in literature. Biogenic amines were separated using Dionex CG17 IonPac (4 x 50 mm) column, under gradient elution conditions by mixing formic acid (1.00 M) and Milli-Q water. Detection was achieved using tandem MS/MS, with the instrument set into MRM mode, ensuring high specificity.

Different linearities of response were obtained, most of them covering two or more orders of magnitude (e.g. for histamine, it was 0.1 - 10 mg/L). The detection limits in fish products ranged from 0.8 mg/kg for tyramine to around 13 mg/kg for spermidine.

This method can be used for routine determination of biogenic amines in different types of cheese in terms of regulatory and monitoring food safety issues relating to such amines, especially histamine and tyramine. It is also a useful method for evaluation of other commercial or commonly used methods that are possibly affected by the food matrix due to processing or other drawbacks arising from derivatization processes.

POTENTIOMETRIC FLUORIDE DETERMINATION IN MILK: ASSESSMENT AND VALIDATION OF A PROTOCOL TO QUANTIFY THE ELEMENT'S CHEMICAL FRACTIONS

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Fluorine is widely spread in the environment (mainly as fluoride) both in abiotic and biotic compartments. It is an essential element for animal life and it is largely used as a supplement in dental caries prevention. However a careful control of fluoride intake levels is required because its toxicity level is only slightly higher than that of the biological action. Milk is the staple food in the first years of human life and its consumption after fluoridation has been successfully promoted in many Countries for prophylaxis against caries. For these reasons some authors have dealt with the determination of natural fluoride concentration of cow's and human's milk. Attention of researchers was mainly appointed on the evaluation of the free and "diffusible" ionic inorganic fluoride and the "total" fluoride. Our contribution will propose a complete and validated (in terms of sensitivity, precision and bias) protocol for the ISE potentiometric determination of fluoride present in the following forms: i) the "free" inorganic ion; ii) the "complexed" inorganic ion, iii) the total inorganic fluoride; iv) the "casein-bonded" organic form; v) the "globulin-bonded" organic form, vi) the total organic fluorine and vii) the total element in samples of cow's milk.

RAPID DETERMINATION OF L-ASCORBIC ACID CONTENT IN FRUITS CONSUMED IN MADEIRA ISLAND, LOCALLY PRODUCED AND IMPORTED - A COMPARATIVE STUDY.

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Vitamin C, including L-ascorbic acid (L-AA) and dehydroascorbic acid (L-DHAA), is one of the most important vitamins for human nutrition, supplied by fruits and vegetables. Synthesis of collagen and hormones, immune response, iron absorption, and cell protection against free radicals are some of its crucial roles in metabolism. There are many analytical methods to measure ascorbic acid content in food, the liquid chromatography methods being the most used and reliable. [1] The objective of the present work was to determine the variability in total Vitamin C content (the sum of the contents of L-AA plus L-DHAA) of fruits grown in Madeira Island and imported ones. Passion fruits, strawberries, papayas and lemons from two different origins were analyzed using a UPLC-DAD system. L-DHAA was determined indirectly, using the subtraction method approach. [1] The chromatographic system was equipped with a Acquity HSS T3 analytical column (100 mm × 2.1 mm, 1.8 μm particle size) using a isocratic mobile phase composed of aqueous 0.1% formic acid at a flow rate of 250 μL/min and the injection volume was 2 μL. The detection wavelength for the photo-diode detector was set at 245 nm and the analytical column was kept at room temperature. Samples were extracted with a 3% metaphosporic-8 % acetic acid-1 mM EDTA solution after 24, 48, 72, 96 and 144 hours. The extracts were prepared and stored at -80ºC immediately after extraction until analysis (within 1 week). The methodology used showed a good repeatability and high sensitivity. Moreover, it proves to be an improved, simple and ultra-fast approach for determination the total content of vitamin C in various food commodities.

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IDENTIFICATION AND CHARACTERIZATION OF ANTHOCYANINS FROM SOME SMALL FRUITS BY HPLC-DAD AND MS SPECTROSCOPY

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Anthocyanins are the most important group of water-soluble pigments responsible for red, purple, blue and orange colour of fruits, vegetables and flower. They are food bioactive compounds with a double interest, one technological, due to their impact on the sensorial characteristics of food products, and the other for their health related properties throw different biological activities, one of them being their implication on cardiovascular disease risk protection.

The objective of this work was to identify anthocyanins from strawberry, raspberry, elderberry, mulberry, blackberry, bilberry, black and red currant fruits extract by combining the information acquired from diode array detector and fragmentation pattern obtained by tandem mass spectrometry. Anthocyanins extraction was carried out with acidified methanol in ultrasonic conditions 60 minutes at 25°C and 59 kHz. The separation of anthocyanins was achieved using a Dionex Ultimate 3000 apparatus equipped with photodiode array detector and a 5 μm C-18 reversed-phase column. Detection of anthocyanins was carried out at the wavelength 520 nm. Structural information required for anthocyanins assignment was provided using an advanced mass spectrometric system including a fully automated robot (NanoMate) for sample delivery by chip-nanoelectrospray ionization (nanoESI) in direct coupling to a high capacity ion trap (HCT) mass spectrometer. The identification of anthocyanins was based on mass spectral data and by comparing their retention times with those of available standards and published data. Only glycosides of the six most important anthocyanidins: cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin and very small amounts of some anthocyanindins were found in the analyzed fruits extracts.

EVALUATION OF ANTIOXIDANT ACTIVITIES OF SOME SMALL FRUITS CONTAINING ANTHOCYANINS USING ELECTROCHEMICAL AND CHEMICAL METHODS

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Anthocyanins are the broadest group of water-soluble pigments present in plants. This class of natural compounds presents a special importance in terms of high potential for their use as natural colorants due to easy incorporation in aqueous media, but also for their beneficial effects on health. Anthocyanins and other flavonoids are regarded as important nutraceuticals mainly due to their antioxidant effect, which gives them a potential role in prevention of various diseases associated with oxidative stress.

The objective of this work was to estimate the antioxidant capacity of some fruits extracts containing anthocyanins (strawberry, raspberry, elderberry, mulberry, blackberry, bilberry, black and red currant) using electrochemical techniques and classical chemical methods based on reaction between antioxidants and a chromogen compound. Anthocyanins extraction was carried out with acidified methanol in ultrasonic conditions 1 hour at room temperature and 59 kHz. Total monomeric anthocyanins content (TMA) in the fruits extracts was quantified by a pH differential method and the results were expressed as cyanidin-3-glucoside equivalents. The total phenolic content (TP) was determined spectrophotometrically by Folin-Ciocalteu method and the results were expressed as gallic acid equivalents. Cyclic voltammetry was used for the evaluation of overall reducing capacity of extracts. Electrochemical experiments were carried out using using a three-electrode electrochemical cell equipped with a working electrode (glassy carbon), a platinum wire auxiliary electrode and Ag/AgCl, KCl sat. reference electrode. Also, evaluation of antioxidant activities of fruits extracts was performed by using FRAP (ferric reducing /antioxidant capacity), ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulphonate]) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. The oxidation potentials were correlated with the antioxidant activities evaluated by the chemical methods. Also, the results regarding antioxidant capacities of the extracts were correlated with their anthocyanins and total phenolics content.

EFFECTS OF DIFFERENT DRYING TREATMENTS ON PHENOLIC ACIDS IN THYMES *(Thymus vulgaris)*

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In Europe thyme *(Thymus vulgaris)* is a well known and daily used herb. But nowadays in the food industry it has more and more significance as natural antioxidant. Beside the mean volatile components such as thymol and carvacrol, the non-volatile phenolic components has important role as antioxidants. The aim of this work is to determine the non-volatile phenolic acids and the effects of the different drying methods on these components.

In this research two chemotypes of the thymes (thymol type and non-thymol type thymes) and three treatments were compared, namely fresh, naturally dried and oven-dried at 40 ºC. Based on the literature[1] the following phenolic acids were chosen as analytes: p-coumaric acid, caffeic acid, ferulic acid, syringinic acid, sinapic acid, rozmarinic acid and chlorogenic acid.

Aqueous extracts of the samples were analyzed using an HPLC-ESI-(QQQ)MS/MS coupled system. In order to investigate the ratio of the conjugated and non-conjugated phenolic acids portions of the extracts were subjected to with alkaline hydrolysis. Consequently, results of non-hydrolyzed extracts represent the amounts of free phenolic acids, while concentrations measured in the hydrolyzed extracts indicate the total amount of conjugated and free phenolic acids.

The results showed that in the non-hydrolyzed extract of both type of thymes rosmarinic acid was the major component, but caffeic, ferulic, chlorogenic and syringinic acid could also be found. In the hydrolyzed samples ferulic acid was the major analyte, and p-coumaric, caffeic and syringinic acids were also determined. The drying experiments showed that the in the naturally dried samples the free phenolic acid concentration was 14-32% higher than in the samples dried at 40 ºC. Moreover, the results showed that in the thymol type samples the concentration of free phenolic acids were 10-50% higher than in the non-thymol type thyme samples.

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DETECTORS EMPLOYED IN HIGH TEMPERATURE LIQUID CHROMATOGRAPHY FOR THE ANALYSIS OF FOODSTUFF

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High Temperature Liquid Chromatography (HTLC) has emerged as a new chromatographic technique where the mobile phase is heated up to get several advantages such as the shortening in the retention times, the reduction in the mobile phase polarity and viscosity with the possibility to avoid organic solvents and to work with 100 % water as the mobile phase (green chemistry). Consequently, solvent gradients can be substituted by temperature gradients. For a range of new hyphenation techniques, this is a real breakthrough because in spite of the possibility of use only water as mobile phase, hot mobile phases improve the nebulization process, which is a preliminary step of many detection modes such as Evaporative Light Scattering Detector (ELSD), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)…

Our studies have been related with the search of the best detector to hyphenate with HTLC. The first one tested was an ELSD for the determination of sugars (monosaccharides and disaccharides) in different dairy products, sweets, isotonic beverage, beer… with a Hypercarb column, the second one was a Refraction Index (RI) detector for the determination of alcohols in alcoholic beverages and the third one was an ICP-AES detector which was appropriate for both alcohols and sugars. Moreover this detector has the capability to determine metals in the same injection. The results were satisfactory in virtually all the cases. Moreover, an UV-vis spectrophotometer has been tested with HTLC for the determination of vitamins in vitamin complexes.

COMPARISON OF DIFFERENT ORANGES, MANDARINS AND CITRUS HYBRIDS CULTIVARS ON THE BASIS OF THEIR ASCORBIC ACID, SUGARS AND TOTAL PHENOLIC CONTENT

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Spain is the largest producer of oranges and tangerines in Europe. In this study ascorbic acid, sugars and total phenol content has been determined in eighteen different cultivars of sweet oranges (*Citrus sinensis*) belonging to the Navel, Common and Blood groups; some late-season clementines (*Citrus clementina)* and *Citrus* hybrids. For doing this research, two HPLC methods have been optimized, one to determine ascorbic acid using a Zirchrom SAX column and a photodiode arrayed detector and for the sugars determination (e.i fructose, glucose and sucrose), a Hamilton RCX column with a refractive index detector have been used. Total phenol determination was obtained using the Folin & Ciocalteau method. All the samples were collected at the optimum maturation stage and cultivated in the same experimental orchard. The results have shown that the ascorbic acid concentration varies in high degree among varieties from 19 up to 104 mg/100ml. In relation to sugars, significant differences in sucrose content has been found between oranges and tangerines, except for Ricolate orange which presents the highest sucrose content, reaching levels up to 10,4 g/100ml, whereas the rest of the orange juices analysed show contents nearly 5 g/100ml. It has also been observed that the sucrose/glucose ratio allows to differentiate between orange and tangerine juice. Finally, the assessment of the total phenolic compounds shows not significant differences between oranges and tangerines. As was expected the blood type sample Sanguinelli orange is the one with the highest concentration, 82.2 mg galic acid/100ml. From the data obtained it is possible to establish a classification of the samples in function of their similarities or differences shown taking into account the ascorbic acid, total phenolic and sucrose, fructose and glucose content.

IMPROVED ANALYSIS OF FAME ISOMERS BY NEW IONIC LIQUID -BASED CAPILLARY COLUMNS

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Analyses of fatty acid methyl esters (FAMEs) are continuing to gain importance as more research is focusing on their biomedical impacts. This includes the analysis of saturated and polyunsaturated FAMEs along with the positional geometric (cis and trans) FAME isomers.

Traditionally, FAME analyses have been performed using silicone polymer or polyethylene glycol based stationary phases that yield typical elution patterns. Analysts performing the task of analyzing the fatty acid composition of food have a wide variety of capillary column selectivities available for resolving the fatty acids as FAMEs depending upon the information they require from their analyses. Nonpolar methyl silicone columns provide a boiling point separation of the FAME isomers with limited resolution of polyunsaturated isomers. Polar polyethylene glycol columns resolve the isomers by degree of unsaturation with minimal overlap of the carbon chain lengths. The highly polar cyanosilicone columns will resolve cis and trans isomers along with possibly providing positional geometric isomer separations depending upon the column type. Although cyanosilicone columns are the current standard for the separation of geometric FAME isomers there are still some limitations especially in the separation of both mono- and polyunsaturated FAMEs and conjugated linoleic acids (CLA).

New stationary GC phases based on ionic liquids have been developed in recent years. A phase with a 1,9-di(3-vinyl-imidazolium) nonane bis(trifluoromethyl) sulfonyl imidate was capable of providing separation of selected geometric and positional isomers of the FAMEs 18:1, 18:2 and 18:3. [1]

In this report we present separations of several artificial and original multi-unsaturated fatty acid (MUFA) mixtures on a new more polar GC column based on ionic liquids. The results will be compared with those obtained from traditional cyanosilicone columns.

References:
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MONITORING OF NITRATE AND NITRITE IN FOODS FOR INFANTS AND YOUNG CHILDREN IN VOJVODINA (SERBIA)

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The purpose of this article was to present the results of the monitoring of nitrate and nitrite content in foods for infants and young children marketed in Vojvodina, Serbia. Following limit concentrations have been established in Serbian law: 200 mg/kg for nitrates and 0.2 mg/kg for nitrites, except the foods based on fruit and vegetables, with the limit concentration for nitrites 2 mg/kg.
Nitrite and nitrate levels were determined in 60 collected samples (27 of domestic origin and 33 imported), classified as cereal products, cereal-based with added fruit and/or vegetables, fruit and/or vegetable purees and fruit juices. Analysis was performed using adapted Griess method, on the basis of detecting the nitrite anion initially, followed by reduction of sample to ensure that all the nitrate is converted to nitrite (Cu/Cd column, conversion efficiencies approaching 100 %), and repeating the nitrite analysis. Nitrate content was calculated from the difference between nitrate plus nitrite (expressed as nitrite) and nitrite content.
Overall nitrate content ranged from 8.4 mg/kg to 200.2 mg/kg and the mean was 55.5 mg/kg. The highest contents of nitrates were found in group of cereal-based products with fruit and/or vegetables (mean 81.6 mg/kg), and the lowest in fruit juices (mean 28.2 mg/kg). Nitrates, as such, are not dangerous for human health, but they can be converted to nitrites. Nitrites were detected in 68 % of samples. In group of cereal products, 77 % samples had nitrite content above legal limit (range 0.7-1.8 mg/kg, mean 1.1 mg/kg; levels apply to the products as proposed ready for consumption). In products from other groups nitrite contents were in admissible limits (range 0.3-1.8 mg/kg, mean 1.0 mg/kg). Nitrites in food are a health problem because their presence may lead to methaemoglobinemia, particularly hazardous for infants, and to the formation of carcinogenic nitrosoamines.

SURVEY ON THE CONTENT OF TOXIC METALS OF DIETARY SUPPLEMENTS IN VOJVODINA (SERBIA)

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The objective of this study was to evaluate the results of the monitoring of metal contamination levels in dietary supplements available on market of Vojvodina (Serbia), manufactured by both domestic and foreign manufacturers, in years 2009-2010. 172 samples of different dosage forms, such as tablets, pastiles, and hard and soft capsules, were classified as products containing vitamins and/or minerals (65 samples) and medicinal herb-based products (107 samples). The scope of their intended use was very broad.

Metal concentrations were determined by atomic absoption spectrometry (AAS): flame AAS for Pb and Cd, and cold vapor technique for Hg. Obtained results were compared with maximum allowable levels (MAL): Pb 3 mg/kg, Cd 3 mg/kg for algae supplements and 1 mg/kg for other supplements, Hg 0.1 mg/kg.

Overall, 29 samples (16.9 %) had at least one of the analysed metals above limit of quantification (5 in group of products containing vitamins and/or minerals, and 24 in group of medicinal herb-based products). The most frequently detected element was Cd (in 22 samples), then Pb (12 samples), and Hg (2 samples). The following concentration ranges were obtained (in mg/kg) for: Pb 0.1-90.0; Cd 0.02-0.73; Hg 0.01-2.4. Several analysed products had metal levels above MAL. One mineral-based product of domestic origin contained 90.0 mg/kg of Pb, and recommended dose of 1 tablet daily would result with intake of Pb of 1.1 μg/kg bw/day from this source only. According to WHO (2011) intake of 1.2 μg/kg bw/day is associated with a population increase in systolic blood pressure of 1 mmHg. One imported medicinal herb-based product contained 2.4 mg/kg of Hg, which presents 2400 % of MAL. Such products could present a significant additional source of metals in the human diet, and therefore could be harmful for human health.

THE CHARACTERIZATION OF SOME COMMERCIAL ROMANIA FRUIT JUICES USING MASS SPECTROMETRY METHODS (IRMS, GC/MS AND ICP-MS)

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Authenticity has probably always been a major concern of many consumers and it is still gaining more and more importance. Isotope ratio mass spectrometry (IRMS) and ICP-MS are promising tools for origin assignation of food, thus ^{13}C, ^{18}O, ^{2}H and trace element measurements are intensively used in forensic study to prove product authenticity. These applications have been particularly useful in food quality control, because it allows the detection of added sugar and water in commercial fruit juices and also in tracing the geographical origin of food. Other main objective in food analysis is determination of pesticide in fruit juices samples is usually hampered by interfering compounds present in the complex matrix. Thus, the challenge for analysts is to maximize recovery of the analytes and minimize the accompanying interferences by use of appropriate extraction and clean-up procedures. In this paper, we described a rapid method to analyzing of pesticide traces of several commercial fruit juices using GC/MS and as well as measurement of isotopic ratio for C, O, H and trace metals. For traces pesticides determination by GC/MS, extraction was done by liquid-liquid technique. The choice of solvent is critical for the success of the selective extraction of target compounds. Pesticides have been identified based on mass spectrum obtained in EI mode. The method was validate on a number of four compound selected based of frequent in use. The procedure was rapid, simple and inexpensive. Five samples of commercial fruit juices from Romania market have been analyzed, and compounds were identified and quantified based on chromatogram area taken on the larges ion in mass spectrum. The results are discussed in connection to fruit variety and origin.

ETHYL PROPIOLATE AS A POST-COLUMN DERIVATIZATION REAGENT FOR THIOLS: DEVELOPMENT OF A GREEN LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF GLUTATHIONE IN VEGETABLES

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The present study reports the development, validation and application of a new green liquid chromatographic method for the determination of glutathione (GSH) in vegetable samples. In this work we introduce – for the first time – ethyl propiolate (EP) as an advantageous post-column derivatization reagent for thiolic compounds. GSH (t_R = 6.60 min) and N-acetylcysteine (NAC, internal standard) (t_R = 11.80 min) were separated efficiently from matrix endogenous compounds by using a 100 % aqueous mobile phase (0.1 % v/v CH_3COOH in 1 mmol L^{-1} EDTA, Q_V = 0.5 mL min^{-1}) and a Prevail® reversed phase column that offers the advantage of stable packing material in aqueous mobile phases. The parameters of the post-column reaction (pH, amount concentration of the reagent, flow rates, length of the reaction coil and temperature) were studied. The linear determination range for GSH was 1 – 200 μmol L^{-1} and the LOD was 0.1 μmol L^{-1} (S/N = 3). Total endogenous GSH was determined in broccoli, potato, asparagus and Brussels sprouts using the standards addition approach. The accuracy was evaluated with recovery experiments and ranged between 91 and 110 %.

COMPARISON OF UPLC/TUV AND GC/MS IN ANALYSIS OF PAHs IN GRILLED MEAT

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The aim of this study was to explore the applicability of Ultra Performance Liquid Chromatography (UPLC) for determination of Polycyclic Aromatic Hydrocarbons (PAHs) in grilled meat, and to compare it with existing, routinely established Gas Chromatography Mass Spectrometry (GC/MS) method. Samples of grilled meat used for this study were sausages made from pork neck and they were further treated according to standard AOAC method. Determination of PAHs was performed on Waters Acquity UPLC system with TUV detection at 254 nm with Pinnacle DB PAH column (100×2.1 mm, 1.9 µm) as stationary phase**.** Running of prepared samples was done in gradient elution, with acetonitrile starting from 55 up to 100%, within 12 minutes. GC/MS analysis was performed on Agilent Gass Chromatograph 6890N with capillary column HP-5MS 30m × 0.25mm i.d. × 0.25µm and Agilent Mass Selective Detector 5973N. Standard PAH mixture PM-831 as well as four deuterated PAH standards were used for identification and quantification.
The range in differences between methods was from -59 to +24% calculated as the difference in UPLC value relative to the GC/MS value for all investigated compounds. A significant advantage of UPLC over GC/MS is the time required for analysis, while the detection limits for GC/MS were generally lower comparing to UPLC/TUV. The greatest deviation was observed for pyrene and chrysene ranging from -59% to -49%, respectively. In contrast, the best correlation was obtained for acenaphtene, fluorene and benzo *[a]* pyrene i.e. + 7%, 24% and 25% respectively. These results are in accordance with previously published data which compare HPLC/FDL and GC/MS. However, this study should be based by statistics with multiple samples and should include greater interlaboratory comparison, to obtain more valuable conclusions.

Key words: PAHs, UPLC, GC, benzo [a] pyrene, grilled and smoked meat

DETERMINATION OF OCHRATOXIN A AND 2,4-DICHLOROPHENOXYACETIC ACID IN FOOD BY ENZYME IMMUNOASSAY USING ENHANCED CHEMILUMINESCENCE REACTION CATALYZED BY SOYBEAN PEROXIDASE

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The monitoring of environmental samples for mycotoxins and pesticides is considered to be a matter of great importance. Direct competitive enzyme-linked immunosorbent assays (ELISAs) for ochratoxin A (OTA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were developed. To increase the sensitivity the novel potent enhanced chemiluminescence reaction (ECR) was used. The ECR was based on luminol oxidation, where anionic soybean peroxidase and 3-(10’-phenothiazinyl)-propane-1-sulfonate were applied as a label and enhancer, respectively. It was shown that the values of the lower detection limit, IC_{50} and the working range for OTA and 2,4-D were 0.02, 0.08, 0.02-0.30 ng/mL and 1.5, 64, 6.5-545 ng/mL, respectively. Using the chemiluminescent ELISA for determination of OTA the recovery from 3 spiked samples produced by extracting soy beans and containing different OTA concentrations (0.07, 0.1 and 0.15 ng/mL) was estimated. The recovery values were ranged from 72 to 125%. The recovery from 3 spiked samples of Vietnamese oranges containing different 2,4-D concentrations (10, 100 and 300 ng/mL) was 92-104%. Using the chemiluminescent ELISA the concentration of 2,4-D was determined in real samples of the oranges.

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APPLICATION OF GAS CHROMATOGRAPHY/TANDEM QUADRUPOLE MASS SPECTROMETRY TO THE MULTI-RESIDUE ANALYSIS OF PESTICIDES IN GRAPES

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A new method was developed for the simultaneous determination of 45 pesticide residues in grapes by gas chromatography coupled with a triple quadrupole mass analyzer (GC-MS/MS), mainly using the multi reaction monitoring (MRM) mode. A single extraction of 10 g of the sample with acetonitrile followed by liquid-liquid partition formed by the addition of 4 g of MgSO4 and 1 g of NaCl was applied in sample preparation. The cleanup of the extracts was carried out by applying dispersive solid-phase extraction (D-SPE) with primary secondary amine sorbent (PSA). The analysis time was 30 min. The recovery data were obtained by spiking blank samples at two concentration levels (10 and 100 µg/kg), at five replicates, yielding recoveries in the range of 70–120%. The precision values expressed as a relative standard deviation (%RSD) were lower than 20% for the intraday precision. The linearity was studied in the range of 10–500 µg/kg and determination coefficients (R^2) were higher than 0.99. The limits of detection (LODs) and limits of quantification (LOQs) were established as 5 and 10 µg/kg, respectively. The developed method was validated in a matrix with high sugar content, and it was applied successfully to identify and quantify pesticide residues in real samples such as grapes.

DETERMINATION OF BIOGENIC AMINES IN FISH SAMPLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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An accurate, rapid and sensitive high-performance liquid chromatographic (HPLC) method for the determination of dansyl derivative of 5 biogenic amines was developed. Histamine and other biogenic amines formation in row, salted and cold smoked rainbow trout, as well as essential oils treated fish samples, as a function of storage and temperature was studied. The method involved the derivatisation with dansyl chloride followed by liquid chromatography gradient elution analysis. Putrescine (PUT), cadaverine (CAD), histamine (HIM) ,spermidine (SPD) and spermine (SPM) are extracted from fish with hydrochloric acid 0.1M. The amines are reacted with dansyl chloride at alkaline pH, and placed in an automatic injector. The chromatographic system was equipped with a reversed-phase C-18 column and a DAD detector. A mobile phase of acetonitrile/phosphate buffer was used. The developed HPLC method showed a good linearity, sensitivity and repeatability. The average repeatability of the method ranged between 3.5% and 5.3%. Calibration curves showed very good linearity (R^2 from 0.9994 to 0.9999). Recovery test ranged from 86.4% to 99.2% while detection limit was less than 0.1 µg/ml (1mg/kg) for PUT, CAD and HIM, and less than 0.3 µg/ml (3mg/kg) for SPD and SPM. The levels of biogenic amines in freshly prepared samples ranged from 1 to 20 µg/ml (mg/kg), which is 10 times less than recommended regulatory acceptable level of 200 mg/kg. Biogenic amines in salted rainbow trout during the storage at two temperatures (4ºC and 20ºC) were also investigated. No significant differences between storage times were found. The levels of putrescine, cadaverine and histamine in fresh fish increased as decomposition of rainbow trout progressed at both 4ºC and 20ºC**.** Biogenic amines did not change throughout storage in salted and cold smoked rainbow throat, as well as essential oils treated fish samples. Putrescine was the main biogenic amine formed, followed by cadaverine and histamine.

Keywords: Biogenic amines; Dansyl chloride; HPLC; trout

ICP-AES ANALYSIS OF SOME OLIGOELEMENTS IN FRUIT WINES

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Fruit wines in Serbia have a reputation as a source of good health, immunity and blood count. They contain a low percentage of alcohol and are consumed as aperitifs. They are considered to contain hemoglobin precursors as well as oligoelements which are necessary for good health. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) provides a rapid and precise means of monitoring elements simultaneously for minor- and trace- levels. The ICP-AES technique is widely regarded as the most versatile analytical technique in the chemistry laboratory.

Our aim-goal was to determine a content of oligoelements (iron, zinc, copper, chrome, manganese) in fruit wines, as well as in domestic red wines, using ICP-AES spectrometry, and to compare results obtained. We analyzed 4 types of fruit wines (blackberry, raspberry, cherry, apple) and red wine of various producers, a total of 30 samples. Samples were prepared in two ways: by dilution with water to obtain 3% (v/v) ethanol, or microwave sample digestion. It was not determined statistically significant difference between these two methods of determination. Domestic fruit wines are a good source of oligoelements, as well as some grape wines from the market. The advantage can be given fruit wines because of the lower ethanol content, making them suitable for the general population.

DETERMINATION OF CARBON AND NITROGEN STABLE ISOTOPE RATIOS OF SERBIAN HONEY BY EA-IRMS

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Carbon and nitrogen stable isotope ratios ($\delta^{13}C_{honey}$, $\delta^{13}C_{protein}$, $\delta^{15}N$) were determined in 380 samples of Serbian honey in order to investigate their authenticity, botanical and geographical origin. Samples of six floral types (five monofloral honeys – sunflower, rapeseed, acacia, golden rod, linden and multifloral honey) were collected directly from beekeepers. The stable carbon and nitrogen isotope ratios were measured in honeys and in the protein fractions extracted from these honeys that originated from thirteen different regions of Serbia. Samples were prepared in accordance to Association of Official Analytical Chemists' (AOAC) method 998.12 and analyzed by Elemental Analyzer (Flash EA 1112HT) which was connected to an Isotope Ratio Mass Spectrometer (ThermoFinnigan DELTA V Advantage). Results for $\delta^{13}C_{honey}$ were between -27.81‰ and -22.21‰, for $\delta^{13}C_{protein}$ were between -27.07‰ and -23.02‰ and for $\delta^{15}N$ were between 1.19‰ and 6.93‰. On the basis of the results obtained, authenticity, botanical and geographical origin of Serbian honey were discussed. The obtained data together with basic physico-chemical and other specific parameters can be used for systematic characterization and formation of the data-base of honey produced in Serbia.

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ANYLYSIS AND IDENTIFICATION OF AMPHETAMINE TYPE STIMULANT (ATS) BY A PORTABLE FT-IR AND DSC (DIFFERENTIAL SCANNING CALORIMETRY)

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The fight of synthetic illicit drug (ATS) traffic is in continuous update and one of the most recent initiatives is the monitoring of the ATS precursors in order to prevent the ATS manufacturing. A contribution to this fight is furnished by the European project DIRAC (funded by the European Community's Seventh Framework Programme), which aims at developing a new advanced sensor for detecting ATS drugs and their precursors.

In parallel of this project, other analytical techniques for detection and identification of ATS compounds and precursors were explored. For this purpose portable FT-IR, DSC and GC-MS have been compared in order to identify their limits and complementarity.

The set of compounds used for this study comprises three principal ATS compounds, MDMA (3,4-methylendioxymethylamphetamine), Methamphetamine and Amphetamine and the corresponding precursors: ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, BMK (benzylmethylketone), safrole, isosafrole, piperonal, PMK (piperonylmethylketone).

FTIR analysis was undertaken using a Nicolet iS5 apparatus (Thermo Fisher) with a ATR (attenuated total reflectance) system using ZnSe diamond. Thermal analysis was tackled with a DSC1 Star System (Mettler Toledo) with a working temperature range between -60 to 650 °C.

The results indicate that the two techniques are complementary to identify the listed compounds, both in a standard pure form and mixed with other compounds, as it usually common in trafficked tablets. At the same time DSC is a very powerful technique for distinguish between isomer forms as it is the case of ephedrine's group compounds. Data treatments of IR spectra permit to classify the listed compounds in groups as stimulant, entactogen, hallucinogen, depending their chemical structure which is a useful tool in presence of an unknown compound.

THE EUROPEAN PROJECT BONAS, «BOMB FACTORY DETECTION BY NETWORKS OF ADVANCED SENSORS»

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The aim of BONAS (BOmb factory detection by Networks of Advanced Sensors) is to design, develop and test a novel wireless sensors network for increasing citizen protection and homeland security against terrorist attacks, in particular against the threat posed by IED devices. This network will support the "factory's location", allowing an early threat thwart. A feasibility study will assess the usefulness and potential advantages that the BONAS intends also to investigate and prepare the potential future deployment of key sensors aboard a flying platform with a view towards increasing the BONAS network detection capabilities. The wireless sensor network will feature a variety of sensing devices (in-situ and remote), that will jointly provide broad chemical spread and low false alarm rates through an expert system management of the data collected. In particular, BONAS will develop: Lidar/Dial system; QEPAS sensor; SERS sensor; an Immunosensor.

BONAS includes a multidisciplinary team of 14 European research groups from six European countries (Estonia, France, Finland, Germany, Italy, Portugal, Sweden, Switzerland, United Kingdom) and includes one public research centre (ENEA, Italy) coordinating the project, universities, private research centres, enterprises and industrial organizations and one end-user. All the partners have previous experience and activity in the field of specific local and remote sensors development and with experience on Security projects. The wireless sensor network will feature a variety of sensing devices (in-situ and remote), that will jointly provide broad chemical spread and low false alarm rates through an expert system management of the data collected.

The BONAS project, which planned duration is 42 months. has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 261685.

A PROFILING PERSPECTIVE OF RESIDUAL SOLVENT OCCLUDED IN COCAINE SAMPLES FOR LAW ENFORCEMENT PURPOSES

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Law enforcement authorities often require evidence to link drug dealers and users or information on local distribution networks. In this sense, solvent impurity profiling provides data amenable to comparisons between different illicit drug seizures for detecting similarity or identity. The resulting information may be used as objective information useful to decrypt the drug traffic organisation and distribution pattern for law enforcement purposes (intelligence) or used in a more traditional manner as evidence in court for demonstrating that two seizures are composed of the same illicit products.

In this work, the intra- and inter- variability of the residual solvents (RS) composition was studied in cocaine seizure in order to determine the potential information of the analytical method compared with the profiling of the main alkaloids. The main cutting agents were also analysed in order to evaluate their influence in the RS profile.

The cocaine seizures and the main cutting agents were analysed by Headspace Solid-Phase Microextraction (HS-SPME) coupled to Gas Chromatography Mass Spectrometry (GC-MS), using a carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber.

The intra- and inter- variability were evaluated by measuring the Pearson correlation. 14 variables were selected which are mostly present in the RS profile. It is possible to differentiate the sample coming from the same or the different seizures thanks to the chromatogram observation and the statistical analysis.

STRATEGIES AND APPROACHES FOR ILLICIT DRUG INVESTIGATION USING CHIRAL ANALYSIS

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One of the most abused drugs in recent years are amphetamine and its analogues. These drugs are substances which have a potent central nervous system stimulating effects. These effects are diverse and are classified into the three main classes: emphatogenic, hallucinogenic and psychoanaleptic. Their pharmacological activities are different depending on the enanciomeric composition.

Enantiomeric analysis of illicit drug is important process for a number of scientific disciplines including forensic science laboratories and pharmaceutical industry.

Enantiomeric characterization and composition determination of amphetamines are significant issues in forensic area. Because enantiomer ratio of appropriate drug is closely related with the species of precursors and reagents used for the synthesis, this knowledge can provide useful information concerning the origins and synthetic methods used for illicit manufacture. This information is important, since it can be utilized for regulation of the precursors, investigation of the manufacturing sources, and resultant prevention of abuse.

The knowledge about enantiomeric composition of amphetamines will also become important for forensics, to distinguish between consumption of amphetamines as a pharmaceutical drug or as a abused drug

Enantiomeric composition of drugs is also important in pharmaceutical industry because enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects.

To obtain this information, analytical techniques using chiral analysis are required. The most commonly used are chromatography techniques: gas chromatography, liquid chromatography, high pressure liquid chromatography and thin layer chromatography.

Here we will discuss chiral analytical strategies and methods for the identification of synthetic routes, purity of products when applied to illicit drugs and in particular amphetamine type drugs as model compounds

ID01 **Session B**

DETERMINATION OF POYCYCLIC AROMATIC HYDROCARBONS (PAH) IN COMPLEX MATRICES OF PLASTIC SAMPLES WITH COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND MS DETECTION (GCxGC(qMS))

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PAH are known to be absorbable by skin contact [1] and they occur in consumer products as contaminants from extender oils and soot. Since there are no specific regulations for PAH in these products, their levels should be as low as reasonably achievable (ALARA) to avoid health risks [2].
We analysed many commodities and toys for their PAH contents according to ZEK 01.2-08 [3]. This document implies risk analysis, categorisation, test and evaluation for the 16 compounds listed by EPA. Due to complex matrices (for example in acrylnitril butadiene styrene materials (ABS)), analyte identification especially for PAH with molecular mass fragments between m/z 166 and m/z 228 is difficult even in cases with a additional purification step on a silica phase. To separate the PAH from matrix comprehensive two-dimensional gas chromatography/mass spectrometry (GC x GCMS) was used. The GC x GCMS analysis was performed using a rapid scanning quadrupole GCMS (GCMS-QP2010 Ultra, Shimadzu (100 scan/s, 20000 amu/s).Using in the first dimension a Zebron TM ZB-5HT 30 m x 0.25 mm I.D., 0.25 µm connected to a VF-17µs 1 m x 0.1 mm x 0.1 µm the separation of the analytes from matrix signals was achieved in the second dimension (polar) which was not possible using one dimenionsal GC (both clean-up step with silica gel).
Fluoranthene and pyrene could be qualified as main PAH-contaminants. The extracted ion contour plot of the mass m/z 202 (fluoranthene) as an example shows, that overlaying polymeric matrix of analogue mass can be separated easily in the second dimension.
As a conclusion, comprehensive two-dimensional gas chromatography is suitable not only to separate complex analyte mixtures, but also matrix interferences from target compounds in complex matrices. The analysis of different polymeric matrices can be performed to categorize
matrix interference and to quali- and quantificate the PAH contaminants in consumer products.

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FULLY AUTOMATED SEQUENTIAL INJECTION-BASED IN-TUBE SORPTIVE MICROEXTRACTION AS A FRONT-END TO GAS CHROMATOGRAPHY-TANDED MASS SPECTROMETRY FOR DETERMINATION OF PRIORITY ENVIRONMENTAL POLLUTANTS

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This work describes a novel, simple, and affordable analytical setup for automated polydimethylsiloxane (PDMS)-based sorptive microextraction of organic contaminants as a front-end to gas chromatography-tandem mass spectrometry (GC-MS/MS).

The analytical procedure involves the exploitation of a short single-strand PDMS tube (2 mm ID, 0.5 mm wall thickness, 50 mm length) integrated within a Sequential Injection (SI) network for automated aspiration of the aqueous sample and delivery through the PDMS tube by programmable flow. The analytes are then extracted into the PDMS followed by elution with a metered volume of organic solvent, which is whereupon quantitatively transferred into the programmed temperature vaporization injector (PTV) of GC. Diffusional resistance to mass transfer was overcome by effecting the overall concentration and stripping steps at a single PDMS tubing interface. Four different organic solvents of varied polarity, yet with elevated vapour pressure, were investigated as eluents in the SI-intube PDMS assembly, *namely,* ethyl acetate, methyl butyl ether, hexane and chloroform. Chloroform was proven the most suitable solvent for expedient elution and fast evaporation into PTV.

The proof-of-concept of the novel hyphenated system was demonstrated for the determination of organochlorine pesticides (OCPs), *namely,* heptachlor, dieldrin, endrin, endrosulfan, *p,p´*-dichlorodiphenyldichloroethane (DDD), *p,p´*-dichlorodiphenyltrichloro-ethane (DDT), dichloro-ethylenedichlorodiphenyl (DDE) and endrin ketone in environmental waters at concentration levels far below the maximum allowed concentrations as endorsed by the current EU Water Framework Directive 2008/105/EC.

AMONIUM CONDUCTIMETRIC DETERMINATION BY A MULTISYRINGE FLOW INJECTION SYSTEM COUPLED WITH A GAS DIFFUSION CELL

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A multisyringe flow injection system (MSFIA) coupled to a gas-diffusion cell has been developed to determine ammonium. A multisyringe burette equipped with some additional three-way solenoid valves has allowed the use of various reagent or samples with further propelling into a gas diffusion cell. Different designs of diffusion cells, with two and three channels and dissimilar contact surface, have been tested in order to enhance the sensitivity. Two hydrophobic membranes have also used with the same purpose. The detector used was a homemade conductimetric cell.

The proposed MSFIA system has been compared with other FIA and SIA techniques based on the same principle, and has also been validated by applying the method to determine ammonium in environmental samples.

ADAPTATION OF A FLOW INJECTION ANALYSER CONDUCTOMETRIC COUPLED SYSTEM FOR THE FIELD ANALYSIS OF VERY LOW LEVELS OF FREE CO_2 IN MARINE WATERS

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A Flow Injection Analysis (FIA) conductometric coupled system has been adapted for the field analysis of very low levels of free CO_2 in natural waters and used in a number of field campaigns for marine water monitoring.

In order to make the system suitable and easy usable for on-line in field measurements aboard of oceanographic ships, the single components of the FIA analyser have been compacted into a robust and easy to use system.

All the measurement operations, including the calibration step, have been automated.

The gaseous CO_2 presents in the sample is separated by diffusion using a non wetting gas porous membrane (PTFE), and the permeating gas is incorporated into a stream deionised water and measured by means of a micro-conductometric detector.

The calibration of the system is carried out by using standard solutions of potassium bicarbonate at different concentration.

The detection limit of the method is 8.64 $\mu molCO_2/kg$.

Both accuracy and repeatability, evaluated by replicate measurements of a certified reference solution (Dickson, batch 92, 2008), are <5%.

The method also allows to perform the direct inorganic carbon speciation in water, without the knowledge of the pH value of the sample and with no need to know the value of the thermodynamic constants of carbonate system in natural waters.

The system allows to analyse both actual or acidified samples in order to determine the free CO_2 or the DIC (Dissolved Inorganic carbon) contents, respectively.

Results concerning performance qualification of the system and its application in experimental surveys, are reported and discussed.

EFFECT OF PRESSURE ON THE SOLUBILITY OF DIMETHYL β-CYCLODEXTRIN IN WATER AT 298.2 K

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Solubility of several inorganic and organic compounds in water or organic solvents has been measured at high pressure up to 400 MPa in our laboratory using the high-pressure vessels designed by one of us [1]. In this work, solubility of dimethyl β-cyclodextrin(CD) in water was measured at pressures in the range of 0.10-200 MPa.

An excess of dimethyl β-CD and water and a Teflon ball were put into a pressure vessel with an outlet valve. The sample mixture was pressed with a hydraulic pump and the vessel was shaken on a seesaw in a water bath at 298.2 ± 0.1 K for a week. After shaking for a long time, the sample liquid was slowly taken out from the vessel keeping the high pressure. The sample was weighed before and after drying off at 381.2 K to determine the concentration.

Reference

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EXPERIENCES OF HIGH THROUGHPUT ANALYTICS FOR POLYOLEFINS

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With the ever increasing competitive nature of many industrial sectors, focus has turned towards high throughput experimentation as a method to highlight areas of research which have the highest chance of success, before moving these experiments to a larger, more costly scale.

The aims for high throughput experimentation can range from the simplest assessment of a yes/no answer up to a more defined set of parameters. In order to obtain the most value from these experiments typical analytical methods can be applied. At the same time it should always be kept in consideration that it is only worth performing the number of experiments that the analytics can handle, in which case rapid analytics may be required.

Here we discuss the Rapid Analytics suite, developed by Freeslate (formerly a part of Symyx), supplied with their, commercially available, Parallel Pressurised Reactor (PPR) system and how they have been implemented and developed to assist in our daily research. Focussing on polyethylene and polypropylene catalyst development the Rapid Analytics were supplied with a GPC, FTIR and DSC alongside a software suite for operational control and results management.

The benefits, compromises, performance and possibilities of the Rapid Analytics Suite are highlighted including the mutual benefit of open discussions with the creators and suppliers, Freeslate.

A NEW POLARIMETER WITH NO MOVING PARTS

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The present work describes the construction of a new precise and robust polarimeter, with no moving parts. The instrument employs a new principle of polarimetric measurement, which uses the ratio between the intensities of the ordinary and extraordinary beams produced by a calcite crystal polarizer to access the angle of rotation. The radiation source is a low cost diode laser modulated at 1.0 KHz to allow for the use of lock-in amplification. The instrument uses two twin detectors to obtain the ratio between the ordinary and extraordinary beams (fE / fO). The laser beam and a polarizing film are positioned at 45º. The polarized beam is directed to the calcite crystal passing through a measurement cell. When a non-optically active substance is present in the cell the ratio of the two beams is equal to 1, corresponding to a rotation of 0º. In the presence of an optically active substance the ratio is different from 1. It may be higher or lower. The ratio of the two beams corresponds to the cotangent of the displaced angle (difference of 45º). The evaluation of the prototype was carried out using aqueous sucrose and fructose solutions in a cell with an optical path of 10 cm. The repeatability expressed by the standard deviation of 35 measurements of two solutions containing 2% and 40% (p/v) of sucrose were 0.006º and 0.01°, respectively. A predicted by Biot`s Law, the instrument produces results with excellent linearity (r2 = 0.9996 for sucrose solutions in the range of 2 to 40%) and provides for fast measurements (~5 s) even with highly absorbing solutions. These characteristics and the absence of moving parts allow proposing the use of the new polarimeter for on/in-line process monitoring.

FAST AND EFFICIENT DETECTION OF PHOSPHOPEPTIDES IN MALDI-MS USING TRANSITION METAL CONTAINING SOL-GELS

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Phosphorylation is the most common post-translational modification on cellular proteins which can be detected efficiently using various mass spectrometric techniques. Ionization efficiency of targeted analyte is one of the most important parameter for their successful detection in mass spectrometry. However, the ionization efficiencies of phoshoproteins are very low in mass spectrometric conditions. Additionally, amounts of nonphosphorylated proteins are always greater than the amounts of phosphorylated proteins in complex mixture of cellular proteins. Therefore, performing characterization and detection for phosphoproteins using various analytical techniques requires challenging approaches. To overcome these problems, phosphopeptide specific enrichment methods are developed and carried out prior to mass spectrometric analysis in this field. Metal oxide affinity chromatography (MOAC) is commonly used as an enrichment technique which is based on specific interaction between metal oxide and phosphorylated peptides or proteins.

In this study, different metal containing sol-gels were developed individually as highly selective materials for phosphopeptide enrichment and they were used for the enhancement in detection limits of phosphopeptides analysis using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). Titanium(IV) isopropoxide, tantalum(V) ethoxide and zirconium(IV) ethoxide precursors were used to synthesize different types of metal containing sol-gel materials and the porosity of the sol-gel materials was increased by chemical incorporation of poly(ethylene glycol) (PEG) into sol-gel structures. In the studies, it was observed that the parameters including pH, temperature and different solvent conditions were highly effective during adsorption, washing and elution steps for successful phosphopeptide enrichment. Furthermore, the efficiencies of metal containing sol-gels for phosphopeptide enrichment methods were tested according to variable porosity, which is provided by chemical integration of PEG into sol-gel materials. Consequently, the metal containing sol-gel materials were developed using quite fast and cheap sol-gel synthesis approaches and alternative phosphopeptide enrichment methods were successfully developed in this study.

DETERMINATION OF 5-NITROIMIDAZOLES AND THEIR HYDROXYMETABOLITES IN PLASMA AND EGG SAMPLES WITH LC-MS/MS TECHNIQUE

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Nitroimidazoles are veterinary drugs with antibiotic and anticoccidial activity. They have been used in veterinary medicine for the treatment and prevention of certain bacterial and protozoal infections in poultry and swine. Because of their potential genotoxic, carcinogenic and mutagenic properties, they have been banned from use in food producing animals in many countries.

These compounds are imidazole heterocycles with a nitrogen group incorporated in the structure. The nitroimidazoles dimetridazole (DMZ, 1,2-dimthyl-5-nitroimidazole) metronidazole (MNZ, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole), ronidazole (RNZ, 1-methyl-2-(carbamoylmethyl)-5-nitroimidazole) and ipronidazole (IPZ, 2-isoprophyl-1-methyl-5-nitroimidazole) are rapidly metabolized into hydroxylated compounds. DMZ and RNZ into HMMNI (2-hydroxymethyl-1-methyl-5-nitroimidazole) IPZ into IPZOH (1-methyl-2-(2-hydroxyisopropyl)-5-nitroimidazole) and MNZ into MNZOH (1-(-2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole).

EU’s Community Reference Laboratories (EU-RL) have published Guidance Paper on the recommended concentrations that all National Reference Laboratories (NRL) should be able to detect and confirm the presence of residues. The recommended concentration for the nitroimidazoles (except IPZ and IPZOH) is 3 ppb in all matrices.

An analytical method was developed and validated at the level of recommended concentration to determine 5-nitroimidazoles in plasma and egg samples. The sample treatment consists an extraction by SPE on mixed-mode strong cation-exchange column.

Samples were analysed with a LC-MS/MS using multiple reaction monitoring in electrospray ionization (ESI) and positive ion mode.

DIRECT CALIBRATION OF LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY WITH SOLID CERTIFIED REFEREMCE MATERIALS – POSSIBILITIES AND LIMITATIONS

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Laser ablation inductively coupled plasma mass spectrometry (LA ICP MS) is considered as a very attractive technique mainly because it allows to perform direct, multi elements analysis of any solids. LA ICP MS is widely used not only to determine the content of major, minor and trace elements but also to evaluate their distribution over the surface as well as in sub-surface domains, with high spatial resolution.

The main advantageous of LA ICP MS lies in its micro-destructive feature, it allows direct micro-sampling of solids by laser ablation accompanied by very good sensitivity of ICP MS. It has been successfully used for various applications, although the lack of suitable matrix-matched solid standards is still recognized as its severe limitation for the accurate quantitative analysis.

In respect of metal samples, several certified reference materials (CRM) are available, however they composition was certified for the bulk material. The aim of this work was to examine the homogeneity of the selected CRMs with the resolution of micrometer scale and to evaluate the certified values together with their uncertainties towards its possible usefulness for the direct calibration of the LA ICP MS. The spectroscopic CRMs in form of disc (40 mm x 20 mm) or rods (6 mm x 100 mm), designed for optical emission (OES) and X-ray fluorescence (XRF) spectrometry, produced in Institute for Non-ferrous Materials (Gliwice, Poland) were examined. All elements listed in the certificates of copper, lead and their alloys CRM's were monitored during LA ICP MS measurements. The accuracy of the results were evaluated by using cross calibrations, means that the special algorithm was build, which allows to evaluate the results of one versus another CRMs. Moreover, the homogeneity of the elements distribution were examined and compared to the uncertainties of the reference values. Thus it was possible to evaluate whether the usefulness of selected CRM's designed for OES and XRF could extend for the calibration of LA ICP MS.

MALDI MASS SPECTROMETRY OF ALUMINIUM–FLUOROQUINOLONE COMPLEXES

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Maldi mass spectrometry has been applied to the study of complex species formed between aluminum(III) ion and some fluoroquinolone: fleroxacin, moxifloxacin and ciprofloxacin. At metal to ligand concentration ratio 2:1 to 1:3 (C_{Al} = 2.5 x 10^{-5} mol/L) in the pH range 3.0 to 6.0 polynuclear complexes were detected and mononuclear species,too. MALDI mass spectra were recorded on BRUKER Ultraflextreme TOF/TOF instrument with Nd Yag laser and Opretaive system Bruker Daltonics flexAnalysis. The instrument operating in reflection positive ion mode. Identified dimer species by MALDI for fleroxacin are at m/z 1894.77 $[2\ Al^{3+}+5L^{-}]^{+}$, for moxifloxacin are at m/z 2054.36 $[2Al^{3+}+5L^{-}]^{+}$ and for ciprofloxacin are at 1703.88 $[2Al^{3+}+5L^{-}]^{+\cdot}$ Mononuclear species AlL^{2+}, AlL_2^{+}, AlL_3^{+} are also identified by electrospray ionization mass spectrometry with ion trap analyser.

MS05 **Session B**

TANDEM MASS SPECTROMETRY OF ALUMINUM –FLUROQUINOLONE COMPLEXES

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The complexation equlibria between Al(III)-ion and fluoroquinolones: ciprofloxacin, moxifloxacin and fleroxacin were investigated by ESI MS/MS spectrometry. Metal to ligand concentration ratio 2:1 to 1:3 has been used ($C_{Al} = 2.7 \times 10^{-5}$ mol/L) in the pH range 3.0 to 6.5. The positive ion mode was selected with m/z between 50 to 1300. The obtained data indicate the formation of the complexes: AlL^{2+}, AlL_2^{+}, AlL_3^{+} at m/z =203.60, m/z =763.23, m/z=1170.58 for fleroxacin+Al complex respectivelly, m/z = 216.81, m/z= 827.31, m/z = 1266.88 for moxifloxacin + Al complex and m/z=249.05, m/z=687.75, m/z=1040.00 for ciprofloxacin+aluminium complex, respectivelly.The several MS/MS scan type-product, precusor and neutral loss were performed and in this way the structural characterisation of the complexes species were done.

DESORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY FOR TRACE ANALYSIS OF CYCLOPHOSPHAMIDE ON SURFACES IN HEALTH CARE SETTING

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The use of cytostatic drugs (CDs) for the treatment of cancer is an increasingly common practice in hospitals. Occupational exposure to antineoplastic agents has been recognised as a potential health hazard since the 1970s, when Falck et al. showed an association between contact with antineoplastic drugs and increased urinary mutagenicity. [22] Moreover many publications have demonstrated that contamination of CDs is still present on work surfaces after the ending of cleaning procedures.[23, 24]

Among cytotoxic drugs handled in hospital care settings, we have chosen cyclophosphamide (IARC, group 1) as an indicator for occupational exposure because of its widespread use.

With the aim to establish exposure levels of hospital personnel preparing and administering CDs, here we present an innovative and rapid screening method for cyclophosphamide based on the use of the desorption electrospray ionization (DESI[25]) interface coupled with a linear ion trap (LIT).

This pioneering technique is carried out by directing charged droplets, produced from a pneumatically-assisted electrospray, onto a solid samples at atmospheric conditions. Ions are produced through the interaction of the charged droplets and the analytes. The resulting mass spectra is similar to ESI one. Therefore we have selected this interface for the direct analysis of passive sampler.

Since workplace contamination is often due to aerosolisation of CDs powder or liquid, direct contact with contaminated materials and wrong cleaning procedures, we have used a PTFE-printed glass slides, as passive sampler, laid on the surface to test. After the sampling period (the work shift or the decontamination operations) the cyclophosphamide collector is directly processed with the DESI-LIT without any further treatment.

The direct analysis of surfaces has a great potential value and represents a completely new approach to evaluate the occupational exposure to CDs. Furthermore, it can provide reliable qualitative information on the presence/absence of cyclophosphamide and offer rapid, accurate, eco-friendly and low-cost analyses.

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TANDEM LIQUID CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY VALIDATED METHOD TO QUANTIFY PROPRANOLOL IN HUMAN PLASMA

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Hypertension is one of the emerging medical problems. Considering increased number of people suffering from hypertension, the use of antihypertensive medications such as propranolol, has increased as well, and methods for propranolol quantification have come in focus. A rapid, sensitive and accurate liquid chromatography–tandem ion trap mass spectrometry (LC/ITMS) assay for the determination of propranolol in human plasma was developed applying metoprolol as internal standard. Sample preparation was performed in the manner to minimize matrix affect, extracting analyte and internal standard from aliquots of human plasma after protein precipitation using acetonitrile. After centrifugation, supernatants were filtered through membrane filters, and like this analyzed by LC/ITMS system. Chromatographic separation was performed on a C18 column. Acetonitrile/water mixture of solvents was used for isocratic elution, and formic acid was added to improve protonation of products of interest. Conditions in positive electrospray ion source were optimized to obtain good analytical signals of ions of interest. The column effluent was monitored by positive ion SRM scan mode in ion trap mass spectrometer. Transitions 260>183 and 260>116 were obtained by 30% of normalized collision energy in collision induced dissociation of isolated protonated propranolol at m/z 260. A low limit of quantitation of propranolol in plasma was obtained (0.23 ppb) and wide linear dynamic range was established (from 0.23 ppb to 148 ppm). The absolute recovery of propranolol (93.25%) and internal standard (97.92%) from spiked plasma samples was consistent and reproducible. The intra- and inter-run assay precision was within ±10%. The method described here is rapid, sensitive, and suitable for the determination of propranolol in human plasma.

MS08 **Session B**

THE TREE EXUDATE CITRIC ACID AND ITS ABILITY TO CREATE COMPLEXES WITH CADMIUM(II)

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Exudation of low-molecular-weight organic acids (LMWOAs) as citric acid (CA) by plants serves, among others, as a protection against intoxication by toxic metals such as cadmium [1]. By this way cadmium as relatively soluble metal ion becomes immobile [2]. The ability of cadmium to create relatively stable complexes with a variety of LMWOAs was proved by electrochemistry [3, 4] as well as by ESI-MS [5].

In the ESI mass spectrum are shown cadmium complexes with CA, which can be divided into three groups; note that all listed *m/z* values refer to the light isotopes (^{1}H, ^{12}C, ^{35}Cl, and ^{110}Cd): (i) dicationic complexes with neutral CA: $[Cd(CA)_2]^{2+}$ (*m/z* 247) and $[Cd(CA)_3]^{2+}$ (*m/z* 342); (ii) monocationic complexes with deprotonated CA (CA-H): $[Cd(CA\text{-}H)(H_2O)]^{+}$ (*m/z* 319), $[Cd(CA)(CA\text{-}H)]^{+}$ (*m/z* 493) and $[Cd(CA)_2(CA\text{-}H)]^{+}$ (*m/z* 685); (iii) monocationic complexes with neutral CA and Cl^- originated from the metal salt precursor: $[CdCl(CA)]^{+}$ (*m/z* 337). The high complexation ability of CA is also demonstrated by the presence of CA complexes with potassium and sodium which were present as minor contaminants.

The most abundant Cd/CA species is a monocation which includes cadmium(II) with one deprotonated and one neutral CA ligand, i.e. $[Cd(CA)(CA\text{-}H)]^{+}$.

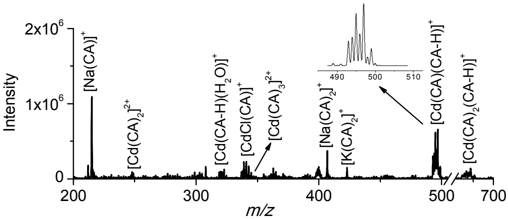

Figure 1 The positive mode ESI mass spectrum of $CdCl_2$ ($5 \cdot 10^{-5}$ mol L^{-1}) and citric acid ($5 \cdot 10^{-4}$ mol L^{-1}) in pure water as a solvent.

Acknowledgement

This work was supported by the European Research Council (AdG HORIZOMS), and Ministry of Education, Youth and Sports of the Czech Republic (MSM 6046070901).

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THE FUNGICIDE TEBUCONAZOLE COMPLEXES IN FOREST SOIL SOLUTION AFTER LIMING

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Tebuconazole (racemic 1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)-pentan-3-ol) is a systemic fungicide widely used against foliar and soil-born pathogens. Its presence in soil may cause changes in cations sorption onto soil matrix and by this way may change their (bio)availability. The soil amendment with basic rocks as a nutrition balance tool for acidified soils provides the increase of soil pH and the sorption of basic cations. That is why such competitions have to be studied.

The electrospray ionization mass spectrometry (ESI-MS) is able to detect various complexes without sample pretreatment [1, 2] in the manner to calculate their stoichiometry and the assessment of their stability upon ESI MS/MS measurements.

For this purpose the soil solution obtained from acidified, depleted and subsequently limed forest Spodosol was used. In the ESI mass spectrum (Figure 1) are shown dicationic calcium and magnesium complexes with tebuconazole (**1**). The stoichiometry of these calcium complexes can be generally described as $[\mathrm{Ca}(\mathbf{1})_x]^{2+}$, where x = 2 - 5. The stoichiometry of magnesium complexes can be described by the general formula $[\mathrm{Mg}(\mathbf{1})_y]^{2+}$, where y = 3 - 5.

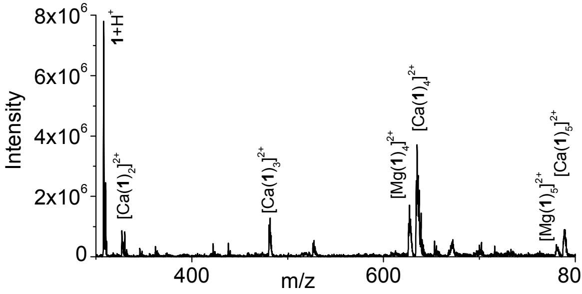

Figure 1 The positive mode ESI mass spectrum of tebuconazole ($2.5\cdot10^{-5}$ mol L^{-1}) in forest soil solution/methanol (1/2 v/v) as a solvent.

Acknowledgement
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HIGH-RESOLUTION ACCURATE MASS MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETRY UTILIZED TO FACILITATE METABOLITE IDENTIFICATION

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The structural elucidation of metabolites by LCMS remains challenging in many cases. Some metabolic pathways yield modifications that are labile in the ion source. This can reduce the protonated molecular ion's intensity so that confidence in its assignment diminishes. Some analytes yield a small number of intense fragments so their spectra lack the richness required to propose a structure with certainty. To meet these challenges, the number or complexity of experiments is often increased. Performing successive product ion, precursor ion, or neutral loss experiments under different conditions adds to the scientist's workload. Elaborate data-dependent MSn protocols can tax the duty cycle of an instrument, and are difficult to design so that all of the appropriate data are captured. The preferable solution is to gain more information from fewer, simpler experiments. Herein, we describe how multi-reflecting time-of-flight mass spectrometry (MR-TOFMS) enriches the structurally relevant information of a mass spectrum. MR-TOFMS technology can exhibit massindependent and acquisition rate-independent resolving power greater than 50,000 and sub-ppm mass accuracy. The instrument's novel design, including gridless mirrors and periodic ion beam refocusing affords this resolution at no cost to sensitivity. These factors, together with high dynamic range detection, allow lowabundance fragment ions to be extracted from complex backgrounds, and assigned with certainty. This is demonstrated by identifying metabolites of a complex multi-component pharmaceutical product in urine, without any sample treatment other than dilution, using a fast ultra high pressure liquid chromatographic method that resulted in significant coelution.

CHARACTERIZATION OF POLY(ETHYLENE GLYCOL) INTERMEDIATES, END PRODUCTS AND DEGRADATION PRODUCTS BY PROTON TRANSFER REACTION Q-TOF MASS SPECTROMETRY AND ^{1}H-NMR SPECTROSCOPY

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Several electrospray mass spectrometry (ESI-MS) techniques have during the past years been described to enable the characterization work of large poly(ethylene glycol)s (PEGs) and PEGylated molecules. The proton transfer reaction ESI-MS method utilize amines to charge reduce the electrospray envelope of PEGs, hence PEG molecules are aminated instead of protonated. This method simplifies the mass spectrum of large PEGs, and enables the interpretation of charge state of the observable envelopes. Hence, deconvolution of the MS data can be performed and relative molecular masses of the individual chain lengths of the PEGs can be calculated. However, as the poly-dispersity of PEGs may vary from batch to batch and from sample to sample it was of interest to examine if the method could distinguish between these kinds of different material. Therefore sample materials of each intermediate obtained at five synthetic steps during synthesis of a 40 kDa PEG molecule were collected. These four intermediates, starting material and the target molecule were examined by 1H-NMR spectroscopy and ESI-MS using a proton stripping base. The study revealed that the charge-stripping ESI-MS method can differentiate between even small changes of a molecule not present in the same sample material. In addition, the structure of a degradation product of one of the unstable intermediate could be given based on the PTR ESI-MS data.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY DETERMINATION OF BENZO[A]PYRENE: INVESTIGATION OF THE EFFECTS OF THE ELECTRICAL PARAMETERS OF THE TRIPLE QUADRUPOLE ANALYZER

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Benzo[a]pyrene (BaP) was recently classified as carcinogenic for humans by the International Agency for Research on Cancer (IARC) [1], so analytical methods characterised by increased sensitivity are necessary. In order to reach the requested sensitivity all aspects of the analytical instrumentations must be optimised. Among them, the electrical parameters of the mass spectrometry detector can be very important.

In this study a central composite design, obtained from a two-level fractional fattorial design and a star design, was used to optimise the BaP mass spectrometry signal with respect to the modificable electrical parameters of the triple quadrupole. The final aim was the enhancement of the sensitivity of the high performance liquid chromatography method that employs the mass spectrometer as detector, operanting in selected reaction monitoring (SRM). The three most intense mass spectrometry transitions were monitored. The investigated electrical parameters were: nebulised current (NC), declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE) and collision cell exit potential (CXP).

The regression models were used to simulate the experimental responses and to search the optimal settings of the electrical parameters of the spectrometer. The grid search algorithm and the desiderability functions were used to determine the best conditions.

Starting from the initial conditions optimised by the instrumental autotuning procedure, it was possible to obtain more than a 20% increase of the BaP mass spectrometry signal intensity.

The same increased was observed in the sensitivity of the HPLC-MS/MS method for the determination of BaP in food samples.

[1] IARC, Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures, IARC Monographs on the Evaluation of Carcinogenic Risks to Human, International Agency for Research on Cancer, Lyon (2010).

IDENTIFICATION OF EARLY STEP UV/H_2O_2 DEGRADATION INTERMEDIATES OF ANTRAQUINONE DYE REACTIVE BLUE 19 BY DIRECT INTRODUCTION ELECTROSPRAY IONISATION MASS SPECTROMETRY

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Degradation of antraquinone dye Reactive Blue 19 (RB19) in aqueous solution was carried out in presence of hydrogen peroxide in batch mode UV photoreactor with ten low pressure mercury lamps (253.7 nm). Aliquots were taken after 0, 2, 4, 6, 10 and 60 min of irradiation and analyzed by electrospray ionization mass spectrometry in negative mode. Three characteristic peaks were observed in the –ESI/MS spectra of RB19; two single charged, sodium adduct at m/z 602.9 $[M\text{-}2H+Na]^-$ with low abundance, and $[M\text{-}H\text{-}H_2SO_4]^-$ at m/z 483.1, which is base peak in the spectrum, and one double charged ion $[M\text{-}2H]^{2-}$ at m/z 289.9. Possible fragmentation pathway of RB19 was proposed, considering multistage mass spectra (MS^n) obtained with ion-trap analyzer. In samples corresponding to 2 and 4 min of irradiation several mono-hydroxylated intermediates, with higher m/z values than that of parent molecule (m/z 499.0, 618.9) were detected, while sample corresponding to 6 min of irradiation was shown formation of di-hydroxylated intermediate (m/z 514.9). All those intermediates are colored, which indicated that still retaining chromophoric group, and formed by OH radical addition on the aromatic part of RB19 dye. According to MS^n spectra of mono- and di-hydroxylated intermediates, hypothesis about possible positions of OH radical attack on dye molecule was formulated. One more initial step degradation intermediate was identified as 1,4-diamino-9,10-anthraquinone-2-sulfonic acid corresponding to m/z 317.1, which is not observed during the fragmentation of RB19. The above mentioned intermediates were not detected in sample after 10 and 60 min of irradiation which indicated theirs further degradation and probably formation of low molecular weight products which need other techniques for detection.

DEGRADATION OF HERBICIDE CLOMAZONE BY UV/H_2O_2 PROCESS

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In this study degradation of widely used herbicide clomazone under UV light in presence of H_2O_2, was investigated. 250 mL of aqueous solution with 50 mg dm^{-3} initial clomazone concentration and 25 mM initial peroxide concentration was exposed to irradiation of ten low pressure mercury lamps (253.7 nm) in period of 60 min. Changes of clomazone initial concentration during the treatment were determined by high-performance liquid chromatography with diode array detection (HPLC-DAD. Also, analysis were performed by direct introduction electrospray ionisation-mass spectrometry in positive mode (+ESI/MS) in order to detect some degradation products. During the treatment intensity of peaks at m/z 240 ($[M+H]^+$) and at m/z 262.1 ($[M+Na]^+$) constantly decreases and after 20 min they were disappeared. On the other side, intensity of peak at m/z 125.2, which corresponds to loss of the -$C_5H_8NO_2$ (m/z 114), increases. It can be conclude that this ion is probably one of clomazone degradation products. Any degradation products were not detected after 60 min of irradiation which indicated that herbicide clomazone was successfully degraded using UV/H_2O_2 process.

DIRECT DERIVATIZATION AND RAPID GC-MS SCREENING OF NERVE AGENT MARKERS IN AQUEOUS SAMPLES

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In order to obtain rapid screening and identification methods based on derivatisation of alkylphosphonic acids, the degradation products of organophosphorus nerve agents, and analysis with gas chromatography mass spectrometry have been developed. The screening and identification method involves a rapid direct derivatization of aqueous samples using highly fluorinated phenyldiazomethane reagents [*e.g.* 1-(diazomethyl)-3,5-bis(trifluoromethyl)benzene]. The derivatives of APAs were screened by GC-MS negative ion chemical ionization and identified by electron ionization mode. Further structure information was obtained with nitrogen containing phenyldiazomethane reagents. The conditions for the derivatization were optimized using statistical experimental design and multivariate data analysis. Method robustness was evaluated using aqueous samples from an official OPCW Proficiency Test and all APAs present in the sample were conclusively identified. Detection limits for rapid screening using SIM NICI was 10 ppb, and for identification using full scan EI was 100 ppb.

DIRECT ANALYSIS OF UV-LIGHT STABILIZERS IN POLYMERIC MATERIALS BY MASS SPECTROMETRY

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Because of their unique properties and the wide range of applications, polyolefines belong to the most important materials. For the extension of the lifetime of polymers, various types of stabilizers are needed to protect the material from environmental impacts. To ensure long term stabilization, hindered amine light stabilizers (HALS) are the most common type of UV-stabilizers employed in polyolefines. HALS are compounds of higher molecular weight which can interfere with the formation of reactive radicals caused by UV-radiation. Contrary to antioxidants, light stabilizers will be regenerated within a cyclic reaction process, so that they are not consumed.

Analytical methods published so far are not yet fully suited for quantitative routine analysis of HALS. Pyrolysis-GC/MS, HPLC-UV and photometric methods after dissolution and liquid/liquid extraction have been developed, but cannot distinguish between different HALS in all cases.

In this work we the identification of three monomeric and two oligomeric HALS without any chromatography is demonstrated. The samples are introduced into a Q-TOF-mass spectrometer by flow injection using electro spray ionization (ESI). Many HALS are present as oligomers containing several different substructures and show single-charged to triple-charged ions. This causes complex mass spectra, but the main signals can be precisely assigned to the molecular formula of the monomeric and also of oligomeric HALS. Thereby, a tool for quick quality control of UV-stabilizers in plastic materials and their identification is available.

COMPARATIVE ANALYSIS OF RHAMNOLIPIDS PRODUCED BY *PSEUDOMONAS AERUGINOSA* NCAIM (P) B 001380 ON DIFFERENT CARBON SOURCES BY HPLC-ESI-MS

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Rhamnolipids are microbial secondary metabolites with tensioactive properties and numerous advantages over the chemical surfactants. Conditions for improved production of rhamnolipids has been reported for a few microbial genera. This study represents analysis of effect of carbon sources on composition of rhamnolipid mixture produces by a novel strain *P. aeruginosa* NCAIM (P) B 001380 isolated from high alkaline mineral cutting oil. Strain was grown on PPGAS medium supplemnented with different carbon sources (2%) incluiding waste matter (fryer sunflower oil and sunflower oil mill effluent). Isolated rhamnolipid mixtures were analyzed by HPLC ESI-MS. Results showed that retention times in condition of gradient elution with formic acid and acetonitrile, depend on lipidic component of rhamnolipid, not only on molecular weight. In all, or almost all, rhamnolipid mixtures were present mono-rhamno-di-lipidic congeners: Rha-C8-C8, Rha-C8-C10/Rha-C10-C8, Rha-C10-C10:1/Rha-C10:1-C10, Rha-C8-C12/Rha-C10-C10, Rha-C10-C12:1/Rha-C12:1-C10, Rha-C10-C12/Rha-C12-C10, Rha-C10-C14/Rha-C14-C10/Rha-C12-C12, Rha-C10-C14:1/Rha-C14:1-C10/Rha-C12-C12:1/Rha-C12:1-C12 and Rha-C10-C10-CH3 and di-rhamno-di-lipidic congeners: Rha-Rha-C8-C10 (all sources except frying sunflower oil), Rha-Rha-C10-C10, Rha-Rha-C10-C12:1/Rha-Rha-C12:1-C10, Rha-Rha-C10-C12/Rha-Rha-C12-C10, Rha-Rha-C10-C14:1/Rha-Rha-C14:1-C10/Rha-Rha-C12-C12:1/Rha-Rha-C12:1-C12, Rha-Rha-C12-C12 and Rha-Rha-C10-C10-CH3. Some rhamnolipidic congeners were detected only sporadically. Mono-rhamno-mono-lipidic congener Rha-C10 was detected on glucose and kerosene and Rha-C14:2 on sunflower oil mill effluent, whereas observed di-rhamno-di-lipidic congeners were: Rha-Rha-C10 (sunflower oil mill effluent and glucose), Rha-Rha-C8-C8 (sunflower mill effluent and glucose with addition of kerosene), Rha-Rha-C10-C10:1/Rha-Rha-C10:1-C10 and Rha-Rha-C14-C16/Rha-Rha-C16-C14 (frying sunflower oil and sunflower mill effluent), and Rha-Rha-C14-C14 (frying sunflower oil). This comparative analysis showed that carbon source had a considerable effect on composition of rhamnolipid mixtures. Differences in rhamnolipid profiles were reflected on mono- and di-rhamno-mono-lipidic and di-rhamno-di-lipidic congeners.

PHOSPHOPEPTIDE ENRICHMENT USING AMINE-FUNCTIONALIZED SOL-GELS FOR MALDI-TOF-MS

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Mass spectrometry is one of the most important techniques for the characterization of phosphoproteins having crucial roles in cellular processes. However, phoshopeptides have low ionization efficiency and also phosphorylation has substoichiometric nature in the cell. Many different sorbents were used to enrich and eliminate the matrix effected the phosphopeptides analysis so far, but still more effort should be spent to obtain more reliable mass spectrometric data for the phosphopeptides.

In this study N'[3-(Trimethoxysilyl)-propyl]-diethylenetriamine (TPDA) and tetramethylorthosilicate (TMOS) were used as precursors for synthesizing amine functionalized sol-gels for the specific separation and enrichment of phosphopeptides. Digested peptides of β-casein were used to examine the affectivity of the sol-gels to the phosphopeptides. Adsorption of phosphopeptides onto the amine functionalized sol-gels was performed at pH=4.5 and acetonitrile/water/TFA (1:1:0.001, v/v) mixture was used for elution at pH=1.5. All mass spectra were acquired using Voyager-DE Pro MALDI-TOF-MS (Applied Biosystems, USA) equipped with 337-nm pulsed nitrogen laser in 2,5-dihydroxybenzoic acid MALDI matrix.

It is well known that amine groups can be easily protonated at pHs lower than 7.0. However, phosphate groups are mainly negatively charged in a wide pH range because of the high acidity of phosphoric acid groups. Here, it was determined that the phosphopeptides in a complex peptide mixture from the tryptic digestion of β-casein were effectively adsorbed onto the surface of the amine-functionalized sol-gels at pHs close to neutral conditions. Phosphopeptides were eluted from the surface of sol-gel providing acidic conditions (pH=1.5). Because, conversion of the phosphate groups to the phosphoric acid groups can be performed adjusting the pH lower than 2.0. Thus, the interactions between phosphate and amine groups are dissipated. As a consequence, it was observed that the signals of nonphosphorylated species have been depleted as well as the signals of the phosphopeptides have been increased in MALDI mass spectra.

ANALYSIS OF MEMBRANE AND HYDROPHILIC PROTEINS SIMULTANEOUSLY DERIVED FROM THE MOUSE BRAIN USING CLOUD-POINT EXTRACTION IN COMBINATION WITH BOTTOM-UP MASS SPECTROMETRY APPROACHES

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Membrane proteins (MPs) are of particular interest in proteomic studies due to their great therapeutic potential and they are considered to be of utmost importance as drug targets. Alterations of MPs in the tissue from the central nervous system could lead to various neurodegenerative disorders. However, studies of MPs are complicated due to their heterogeneity, general low abundance and very hydrophobic nature.

In this study, a temperature induced phase fractionation known as cloud-point extraction (CPE) was used to simultaneously extract, concentrate and fractionate hydrophobic MPs and hydrophilic proteins from mouse brain tissue. Two mass spectrometry based bottom-up proteomic approaches were used to comprehensively identify the extracted proteins. In the first "shotgun" based bottom-up approach, the proteins were tryptically digested on 3kDa spin filters and then analyzed with reversed phase nanoliquid chromatography (RP-nanoLC) in combination with electrospray ionization (ESI) tandem mass spectrometry (MS/MS). In the second approach, the extracted intact proteins were first separated by one-dimensional (1D) gel electrophoresis and then in-gel digested with trypsin and analyzed with nanoLC-MS/MS. The quantitative reproducibility of the CPE method was evaluated with the widely used iTRAQ 4-plex labeling approach.

In total, 1825 proteins were unambiguously identified and the percentage of membrane proteins was 26% which is at the reported genome expression levels of 20-30%. The protein overlap between the two approaches was high. The majority (77%) of the identifications in the first approach was also found by the second method. The protein overlap between the CPE extracted hydrophilic and hydrophobic proteins fractions was rather small (16-23 %) for both methods. A quantitative evaluation of the CPE with iTRAQ labelling and nanoLC-ESI-MS/MS analysis gave iTRAQ ratios at the expected levels and an overall variation of the entire method to be 17-31%. Thus, the entire brain tissue analysis method with CPE, protein delipidation/precipitation and nanoLC-MS/MS analysis yields highly reproducible results. Furthermore, the CPE provide a representative insight in the proteomic expression levels in brain tissue and can readily be used for large comparative proteomic studies of clinical samples and animal models.

TWO DIMENSIONAL TLC-MS ANALYSIS TO SEARCH NATURAL DRUG LEADS

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Two-dimensional TLC is a trdational method for detection of materials in complex mixtures, and TLC-MS is useful as a powerful tool for chemical screening of new natural products or a conventional detector of synthetic products. In our investigation on bioactive substances from natural products, we planned to use 2D TLC analyses for detection of a small amount of bioactive substances in the lipid-rich extracts. In this research the2D TLC-MS analyses have been applied for search of novel polyketide-like compounds from cultured dinoflagellate cells.

Marine dinoflagellates of the genus *Amphidinium* are well-known as a producer of unique cytotoxic metabolites. We have isolated lots of cytotoxic macrolides, amphidinolides and iriomoteolides, from the marine dinoflagellate *Amphidinium* species. Several macrolides are expected for anticancer drug leads. Because contents of these macrolides are very low (< 1% from the dry cells), we have developed the method for analysis of these macrolides in the dinoflagellate extracts. However a large amount of free fatty acids included in the extracts prevents qualitative and quantitative analyses of the macrolides by HPLC and 1D TLC. Therefore, we examined the sovent system for 2D TLC analyses to detect the macrolides in the extract.

Samples were prepared from the solvent extraction of the dried dinoflagellate cells, and 1 mg of formulation was spoted. The first solvent system is hexane-acetone, and the second is hexane-ethyl acetate including diethylamine. Diethylamine is very effective to prevent development of large quantities of fatty acid included in the dinoflagellate extracts. TLC-MS measurement was performed using ESI-TOF mass spectrometry equipped with DART ion source instead of ESI ion source. Sample on TLC plate was set to the platform of MS, and was ionized by spray of hot herium gas in atmosphere pressure, and MS data was obtained by TOF detectorFrom DART-MS measurements of these observed spots, it was revealed that these corresponded amphidinolides H and X. This slide is DART-MS spectrum of amphidinium on TLC plate. Pseudomolecular and dehydration ions were observed.

In this symposium, we describe the detail experiments of 2D TLC-MS analyses of dinoflagellate extracts. The 2D TLC-MS analysis of the crude extracts is useful for screening of new natural products.

MALDI-TOF CHARACTERISATION OF COLLAGEN

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Collagens, one of the most abundant on the Earth, are family of proteins which constitute the basis of connective tissue (extracellular matrix) in multicellular organisms. This study was preformed to analyse structure and morphology of collagen. We used collagen type II from bovine Achilles tendon which was digested with collagenase from *Clostridium histolycium*. In further work, digested collagen has been analysed on MALDI-TOF (matrix-assisted laser desorption/ionisation time-of-flight) mass spectrometry (MS) in order to find peptide's fragments that are characteristic for collagen. Also, we tried to establish MALDI quantitative method for this protein. Morphology has been studied by transmission electron microscopy (TEM) and atomic force microscopy (AFM). With these methods we studied surface morphology, shape and length of fragments from collagenase-treated collagen.

HIGH ACCURACY ANALYSIS OF CUMENE OXIDATION PRODUCTS

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The most widespread Cumene method for phenol and acetone production via Cumene Hydroperoxide (CHP) providing about 10 million MT of phenol annally require reliable and precise methods for production site operation control to improve it efficiency and safety. Cumene oxidation process is carried out in a system of successive reactors, where CHP concentration gradually increase up to 25 – 30% to provide the process best selectivity. It reqwires high accuracy analysis of cumene oxidation products with standard relative deviation not exceeded 0.3%, preferably better, for tight control of oxidation system. Common methods used for analytical control of the process are GC and iodometric titration do not provide fast and precise enough analysis for the process control.

Near Infrared (NIR) spectroscopic analysis applied to online analysis of cumene oxidation products in real time. The method provides spectral noice limited reproducibility of 0.05% or better and 0.3% reference method limited accuracy for CHP concentration in technical Cumene oxidation product. Further accuracy improvement require more precise titration. The problem is that titration results are depended on a number of factors due to concurrent reactions occurred such as oxidation iodide-ion by oxygen in air and interaction reactants with compounds presented in the sample. Investigation of these processes influence on results and accuracy of analysis is the aim of this work.

A number of factors influence on titration results was investigated and optimum conditions for analysis were found. Standard relative deviation for technique developed is 0.3%.

The factors influencing on CHP analysis are: oxygen present in atmosphere and analytic solutions, ambient light, the compounds presented in the sample such as dimethylbenzene alcohol (DMBA) and acetophenone (AP), dicumylperoxide (DCP). The most influencing factor is oxygen. The analysis sensitivity to other factors determined too. Limits for influencing factors to provide the desired accuracy are determined.

IN-LINE MONITORING OF CHEMICAL ANALYSIS PROCESSES USING WIRELESS SENSOR NETWORKS

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Main features, applications, and future trends of Wireless Sensor Networks (WSN) in in-line chemical analysis are dealt with in this work. Basically, WSNs are formed by many small devices –called sensors or motes- that are able to obtain information of the surrounding area using transducers, and transmit it by means of suitable wireless communication systems to the so-called sink node. After the appropriate information handling, it is stored on a database, be it local or through other communication systems (e.g., Internet) regardless of the physical channel (Ethernet, WiFi, 3G technology, etc.). This database is located on a remote server and will be accessible for its on-line evaluation or later analysis.

In the last years the number of papers related to WSNs has increased substantially, although only a few of them describe applications in the field of chemical analysis. We will discuss those relevant contributions, which aim at reaching an even higher level of automation in analytical chemistry.

One of the most significant advantages when using WSNs in Analytical Chemistry is the possibility of monitoring a given chemical parameter that varies with both time and position. This provides invaluable information, for example, to check the evolution of a toxic spill. Additionally, WSNs are straightforward with fault tolerance. The existence of a great deal of sensors (widely distributed) allows for detecting and filtering anomalous values, if necessary. Furthermore, the presence of many routers –one for each sensor node- permits to find alternative routes in case of malfunctions.

Therefore, WSNs are highly promising tools in advanced analytical chemistry. Nevertheless, more emphasis should be put in developing new transducers, since size, price, and maintenance are very important aspects in the success of these devices. In this sense, key research challenges to be addressed include the development of low-cost, low-power sensors, miniaturized fluidic transport systems, antibio-fouling sensor surfaces, sensor calibration, reliable and robust system packaging, as well as associated energy delivery systems.

PROCESS ANALYTICAL TECHNOLOGY: CHEMOMETRIC SOLUTIONS FOR PHARMA INDUSTRY

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Growing quality standards is a pronounced trend in today's pharmaceutical production. The necessity to assure intended product quality during manufacturing is claimed by the Process Analytical Technology (PAT) initiative. We consider three generic PAT solutions, which cover various production stages, from incoming to outcoming inspection.

The first solution is a routine testing of substances in warehouses when Near Infrared (NIR) spectra are acquired by a fiber optic probe. This technique is an especially challenging when measurements are carried out through closed polyethylene (PE) bags and due to the changes in the position of the probe. We present [1] a two stage approach that recognizes the perfect substances reliably. We suggest applying trichotomy recognition: accepted, rejected, or extra measurements required.

The second solution is a new method of the drug release profiles prediction during a running coating process. The in-line NIR spectra acquired during a process through an immersion probe reflect the coating thickness that is inherently connected with the drug release. Pellets sampled at process time points from the different coating batches were subjected to the dissolution. The drug release kinetics has a sigmoid form with a pronounced induction period. The autocatalytic model has been successfully applied to the profiles. Combination of the hard modeling with the PLS regression enabled the profiles prediction [2].

The third solution concerns the finished products' testing. Since a NIR spectrum reflects both the chemical composition and physical structure, NIR spectroscopy combined with multivariate modeling techniques is very effective in solving this problem [3]. Addresses issues are the batch-to-batch variability, proper choice of spectral region, classification methods.

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2. Analyst, in print DOI:10.1039/C0AN01033B
2. TrAC, 29 (8), 781-938 (2010)

RADIOANALYTICAL METHODS FOR URANIUM AND 226Ra DETERMINATION IN WATER AND SOIL SAMPLES

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The determination of low activity concentration of uranium and radium isotopes in environmental samples could be performed by using alpha spectrometry in combination with radiochemical separation. This procedure usually requires demanding radio-chemical procedures before qualitative and quantitative analysis. Each step in the chemical separation process can involve losses of the analyte. The chemical recovery could be determined by using appropriate yield tracers such as 232U and 133Ba. For the preparation of the thin sources for high resolution α-spectrometry electrodeposition and/or microcoprecipitation are the most often used methods.

Several analytical techniques can be applied for determination of uranium and radium isotopes in waters and soils. The two largest categories of methods are radiometric and non-radiometric methods. Radiometric methods are used as routine procedures combined with selective solvent extraction. They give rapid information on total uranium and radium activity concentration, and their isotopic composition. Radiometric techniques need longer sample preparation than non-radiometric, but they give more reliable information about isotopic ratios of interest.

Samples of water after filtering and spiking with a known amount of 232U or 133Ba tracer (depending on radionuclides to be analyzed) should be chemically treated. On the prepared samples mikrocoprecipitation could be applied (determination of uranium isotopes needs previous separating using UTEVA resin).

Samples of soil should be ashed to exclude any organic matter, and after spiking with a known amount of already mentioned tracers, the adequate chemical procedure should be applied. The final phase of the procedure is almost identical as for water samples.

Source prepared after the radiochemical treatment by electrodeposition and/or microcoprecipitation should be measured by means of semiconductor Si detector with surface barrier (or PIPS detector), for several days due to low specific activity of the initial sample. After complex spectral analysis the unknown activity could be obtained.

Poster presentations
Session C

Chromatography	CH01-CH40
Environmental Analysis– Part II	EN57-EN110
Hyphenated Techniques	HT01-HT05
QA/QC in Analytical Laboratories	MQ01-MQ05
Nanoanalytics	NA01-NA09
Analytics in Pharmacy and Medicine	PM02-PM45
Chemical Separations	SC01-SC06
Sample Preparation	SP01-SP36

RETENTION BEHAVIOUR AND LIPOPHILICITY OF SOME N-(4-PHENYLMONOSUBSTITUTED) CYANOACETAMIDE DERIVATIVES

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The chromatographic behaviour of N-(4-phenylmonosubstituted) cyanoacetamides was investigated using reversed phase thin-layer chromatography. The chemicallly bonded RP-C_{18} was used as stacionary phase and the mixture of water and protic solvents: methanol, ethanol and aprotic solvents: acetone, tetrahydrofuran were used as the mobile phase. Changing the amount of organic modifier (φ) in the mobile phase was calculated retention parameter, R_M of cyanoacetamides. Chromatographic retention constants R_M^0 was determined extrapolating the obtained R_M values to 0% of organic modifier concentration by using equation:

$$R_M = R_M^0 + m\,\varphi$$

The calculated retention constants, R_M^0, represent the most applied chromatographic lipophilic parameter. Obtained R_M^0 values are different for each investigated cyanoacetamides and depend on chemical stuctures.

Hansch's substituent parameter, π, were calculated as measure lipophilicity of tested acetamides. This parameter is convenient, since it determines the influence of certain substituents on the overall lipophilicity of a investigated molecule. Values of π measure the free energy change caused by particular substituent and to relate to biological activity. At first, the value of partition coefficient, *log P,* was determined and from applicated equation was calculated Hansch's substituent parameter, π.

$$\pi = \log P_R - \log P_H$$

Chromatography retention constants R_M^0 (RP TLC) were correlated with obtained Hansch's parameter, π, separately for substituents with negative and with positive π values. Good linear relationships in both of case represented that chromatographic retention constants may be successfully used to express lipophilicity and predict biological activity of investigated compounds.

Acknowledgement

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DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COUPLED TO LIQUID CHROMATOGRAPHY FOR THIAMINE DETERMINATION IN FOODS

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Dispersive liquid-liquid microextraction (DLLME) coupled to liquid chromatography (LC) with fluorimetric detection was evaluated for the preconcentration and determination of thiamine (vitamin B_1). Derivatization was performed by chemical oxidation of thiamine with $5x10^{-5}$ M ferricyanide at pH 13 to form fluorescent thiochrome (TC). For DLLME, 0.5 mL of acetonitrile (disperser solvent) containing 90 µL of tetrachloroethane (extraction solvent) was rapidly injected into 10 mL of sample solution containing the derivatized TC and 20% (w/v) sodium chloride, thereby forming a cloudy solution. Phase separation was carried out by centrifugation, and a volume of 20 µL of the sedimented phase was submitted to LC. The mobile phase was a mixture of a 90% (v/v) 10 mM KH_2PO_4 (pH 7) solution and 10% (v/v) acetonitrile at 1 mL min^{-1}. An amide-based stationary phase involving a ligand with amide groups and the endcapping of trimethylsilyl was used. Specificity, linearity, precision, recovery and sensitivity were satisfactory. Calibration graphs were performed by the standard additions method. The enrichment factors and the detection limits were calculated. The esters thiamine monophosphate and thiamine pyrophosphate can also be determined by submitting the sample to an enzymatic treatment. The method was applied to the determination of thiamine in different foods such as beer, brewer´s yeast, honey and baby foods including infant formulas, fermented milk, cereals and purees. The reliability of the procedure was checked by analyzing a certified reference material, pig's liver (CRM 487).

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DETERMINATION OF FREE AND TOTAL ELLAGIC ACID CONTENT IN BERRIES GROWN IN SERBIA

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Berry fruits are most often the source of different phytonutrients. Among them, ellagitannins and ellagic acid are well known antioxidants that play an important role in human nutrition. Their bioactivity is related with other biological properties, including antioxidant, anticancer, anti-atherosclerotic, anti-inflammatory, antihepatotoxic, antibacterial and anti-HIV replication activities. Accurate quantification of ellagitannnins and ellagic acid conjugates in berries is very important. Although Serbia is one of the world's largest producers and exporters of raspberries, there is a lack of systematic control of the phytonutrients. Here we present a simple and rapid procedure for determination of free and total ellagic acid in raspberries, developed in our lab. Several procedures were followed in order to optimize conditions. Amount of free ellagic acid was determined in methanol and acetone extracts. Total EA concentration was determined after acid hydrolysis with 4 M HCl in both extracts, and results were compared. Quantification was done using High-Performance Liquid Chromatography (HPLC) based on isocratic elution on C18 column with UV (λ = 254 nm) and MS detection. Content of mono- and disaccharides in water extract was analyzed by Ion-exchange Chromatography (IC) using CarboPac PA-100 column.

A FAST SCREENING GC-METHOD FOR A WIDE RANGE OF VOCs OF DIFFERENT POLARITY

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In the present work, we propose the use of a polar GC column such as DB624 for the simultaneous and fast separation of both polar and non-polar volatile organic compounds (VOCs), including compounds as diverse as short chain carboxylic acids, carbonyls, glycols and aromatic VOCs. The developed analytical method facilitates the screening of chemical emissions from building and other consumer products, commonly used in indoor environments. Analysis of such complex chemical mixtures, VOCs with wide range of polarities, traditionally requires separation techniques using different GC columns as one or two step procedure..

The proposed analytical method has been applied to evaluate the emissions of VOCs from commercially available linoleum and paint samples. For this purpose, linoleum pieces and paint samples were placed inside 0.020 m^3 environmental chambers for a period of 28 days. The chamber was supplied with clean air and it was working in dynamic mode (air exchange rate of 0.5 h^{-1}), to mimic the air exchange in a common indoor environment. Relative humidity and temperature in the chamber were set to 50% and 23 °C.

Aliquots of 2 L were actively pumped through cartridges containing Tenax sorbent at around 100 mL/min after 1, 3, 7, 14 and 28 days. Retained VOCs were subsequently thermally desorbed, separated by the proposed chromatographic method and identified by means of mass spectrometry operating in the scan mode (30-530 amu at an acquisition speed of 5000 amu/s). The whole TD-GC-MS procedure was carried out in less than 40 minutes, being the VOCs separated in 14 min.

SIMULTANEOUS DETERMINATION OF CYANIDE AND HEXAVALENT CHROMIUM IN MIXED PLATING RINSE WASTEWATERS BY ION CHROMATOGRAPHY WITH SUPPRESSED CONDUCTIVITY DETECTION

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The high toxicities of CN^- and Cr(VI) for living organisms are well known. Whereas Cr(VI) can be toxic and carcinogenic, Cr(III) may be considered as an essential trace element for the proper functioning of living organisms [1]. The large scale presence of cyanides in the environment is attributed to the human activities as cyanide compounds are extensively used in industries. Simultaneous determination of CN^- and Cr(VI), thus, in plating rinse wastewater has crucial importance for employees and environment. The synthetic solution was prepared according to procedure described by N. Sapari and friends [2].

The synthetic solution, firstly, was passed through a column (1x10 cm) containing strongly acidic cation exchange resin at 1 mL min^{-1} flow rate to remove cations from the solution. The pH of 50,0 mL solution collected from column was adjusted to 12 by addition of 0,1 mL 10 M NaOH. Because HCN has low dissociation constant (pK=9.2), we derivatized CN^- to CNO^- (pK=3.66) by addition of Chloramine-T (10%, 50 µL) to both standart and synthetic solutions[3]. Then, solutions kept in a water bath approximately 10-15 min at 80°C. After the reaction was completed, solutions were cooled until room temperature. Then, each solution was filtered with 0.20 µm PES filter. 10 µL of filtered solutions were injected to Dionex ICS-3000 ion chromatography system. The optimized chromatographic conditions: the eluent gradient was 0-10 min 10 mM NaOH isocratic, 10.1-20.0 min gradient from 10 to 30 mM NaOH, 20.1-25.0 min 10 mM NaOH isocratic; suppressor current was 19 mA; column temperature was 30°C, and flow rate was 0.25 mL min^{-1}. We, in addition, used Dionex AS20 anion exchange column (2.0x250 mm) and AG20 as guard column. Correlation constants were higher than 0.999 for both ions. Moreover, the method LOD were 1.47 µg/L and 2.47 µg/L, recoveries were 98% and 91% for CN^- and Cr(VI), respectively. The method was also applied to a real sample of electroplating wastewater. Our study provides easy, sensitive, reproducible, and simultaneous a method for determination of CN^- and Cr(VI) in the electroplating wastewaters.

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SEPARATION OF UROBILINOGENIC CHLOROPHYLL CATABOLITES ISOMERS ON DIFFERENT STATIONARY PHASES

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Isomer analysis is of interest in the tetrapyrrole chemistry. Tetrapyrrole chiral molecules with several chiral centres should be separable on reverse phase (RP) stationary phases. However, achieving the separation may be difficult as the on-column separation speed must match or be faster then the rate of isomer interconversion. Often, this requirement can be satisfied by using different temperature conditions and by improving selectivity via use of different stationary phases. In this study the separation of urobilinogenic chlorophyll catabolites isomers, within a conventional LC temperature range on different RP stationary phases is presented. Analysis of retention data rendered thermodynamic information on the mechanism of retention and selectivity.

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR PRIMARY VOLATILE AROMA COMPOUNDS IN WINES WITH SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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Gas-chromatography coupled to mass spectrometry (GC-MS) is widely used for the determination of the varietal volatile aroma compounds in wines. The aim of this work was the development of a GC-MS method for the quantitative determination of 21 primary volatiles in white wines using solid-phase microextraction (SPME) to isolate/preconcentrate the target species. The efficiency of five different SPME materials was investigated using both direct immersion and headspace analysis modes. The best results were obtained with a StableFlex fiber coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) coating in the direct immersion mode. The effect of different instrumental and chemical parameters was studied using an experimental design approach and optimisation of the significant factors was performed by a Simplex procedure. The method was validated in terms of linearity, recovery and reproducibility at six concentration levels. Determination of the glycosidically-bound aroma compounds was performed after solid-phase extraction isolation, using Isolute ENV+ cartridges, enzymatic hydrolysis and subsequent analysis of the aglycons with SPME.

STEREOSELECTIVE GAS CHROMATOGRAPHIC SEPARATION OF CARBOXYLIC ACIDS FORMED DURING *N*-ALKANE METABOLISM IN ANAEROBIC BACTERIA

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n-Alkanes are the main constituents of crude oil and various petroleum products. Their biodegradation in anoxic natural environments such as contaminated aquifers or petroleum reservoirs plays an important role in the global carbon cycle. During the last decades several denitrifying and sulphate-reducing bacteria capable of utilising *n*-alkanes as sole source of carbon and energy have been isolated and characterised. A pathway for the complete oxidation of *n*-hexane via substituted diacids and branched carboxylic acids to carbon dioxide has been suggested. However, the stereochemical course of the enzymatic reactions involved is still unknown. Therefore, we have investigated the stereochemistry of key metabolites of *n*-alkane metabolism by means of GC and GC-MS. For the assignment of configuration, synthetic authentic standards with defined stereochemistry were compared with methylated und underivatized acids extracted of *n*-hexane-utilising bacteria. In the case of succinic acid derivatives with two stereocentres no suitable enantioselective GC column was found that enabled the separation of all four stereoisomers. However, micro-scale derivatization to succinimides using pure enantiomers of chiral aromatic amines allowed the resolution even on non-enantioselective columns. Scope and limitations of these approaches with respect to general strategies for the stereochemical assessment of carboxylic acids occurring in microbial metabolism will be discussed.

SIMULTANEOUS DETERMINATION OF MELAMINE AND ITS HYDROLYSIS PRODUCTS BY HILIC-MS/MS

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The aim of this study was the simultaneous separation and determination of melamine and its hydrolysis products (ammeline, ammelide and cyanuric acid) by hydrophilic interaction liquid chromatography – tandem mass spectrometry (HILIC-MS/MS). The mobile phase, the pH of ammonium formate buffer, the dilution solvents of standard and sample solutions in vials, the stability of the standard solutions and the column equilibration time were optimized on TSKgel Amide-80 in order to separate ammelide-melamine (isobaric compounds) and simultaneously determine all the compounds which are ionized in negative and positive ESI in two different chromatographic segments. Melamine and ammeline were determineted on positive and cyanuric acid and ammelide on negative electrospray ionization experiments. Ammonium formate buffer, pH 4 / acetonitrile was used as a mobile phase for the separation. The retention mechanisms of the four analytes on TSKgel Amide-80 and aminopropyl-column (Hypersil APS-2) were studied, as well. For the aminopropyl column, melamine retention seems to follow Electostatic Repulsion Hydrophilic Interaction Chromatography (ERLIC). ERLIC is a different mode of chromatography in which an ion-exchange column is eluted with a predominantly organic mobile phase. Under ERLIC conditions, melamine and functional groups from the stationary phase present the same charge. The successful separation and the simultaneous determination of melamine and its hydrolysis products was achieved at aminopropyl- and at TSKgel Amide-80 (using 5/95 ammonium formate-formic acid pH 4/ACN as the mobile phase and 10/90, respectively). The method was successfully applied to infant formula.

SIMULTANEOUS DETERMINATION OF SEVEN ARTIFICIAL SWEETENERS IN WASTE WATER BY HILIC-MS/MS

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Sweeteners are divided in two main groups: nutritive and non-nutritive compounds. Nutritive sweeteners are carbohydrates such as glucose, fructose and maltose. Non-nutritive sweeteners belong to various chemical classes and they are usually known as artificial sweeteners. The food industry is heavily promoting its artificially-sweetened products (frequently called ‘‘diet’’ or ‘‘light’’). Low-calorie food products and beverages can help in the treatment of obesity, the maintenance of body weight and management of diabetes. However, due to increased consumption, sweeteners are widely distriduted in the aquatic enviroment and are characterized as emerging pollutants. Herein, the separation of seven artificial sweeteners, namely aspartame, alitame, acesulfame K, saccharine, sodium cyclamate, sucralose and neohesperidin under Hydrophilic Interaction Chromatography (HILIC) conditions is presented. The ionization of the compounds was achieved by negative electrospray ((-) ESI). Two silica-based columns were compared, XBridge HILIC and Kinetex HILIC. For the HILIC optimization, the effect of the following parameters was studied: the mobile phase, the pH and the ionic strength of the ammonium formate buffer, the stability of the analytes and the temperature of the separation on Kinetex HILIC. The optimized mobile phase was 5mM ammonium formate buffer pH 3.5 / methanol / acetonitrile (15/10/75 v/v), with a flow rate of 100 μL/min at 50 ^{o}C. The asymmetry factor of the peaks is lower on Kinetex HILIC column (fused-core silica) than that on Xbridge (porous silica). The retention mechanism of all analytes on silica columns seems to be partition to the water layer as well as hydrogen bonding. The method was validated in waste water samples.

FLAVONOLS AND PHENOLIC ACIDS CONTENT IN GARCINIA CAMBOGIA

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Garcinia cambogia, also known as Malabar tamarind, is a plant native to Southeast Asia. The dried rind has been extensively used for centuries throughout Southeast Asia as a food preservative, flavoring agent and carminative. Garcinia species are employed in traditional medicine for treatment of hepatitis, laryngitis and mouth infections. G. cambogia extracts are commonly marketed as dietary supplement for weight management.

In the present work, a method involving ethanolic extraction, HPLC–RP-C18 column chromatography with DAD detection is developed for determining the level of quercetin derivatives (e.g., quercitrin, hyperoside, rutin, rhamnetin, keampherol and miricetine) and derivatives of benzoic and cinamic acids (gallic, caffeic, chlorogenic, ferulic, p-coumaric and p-hydroxybenzoic acids) in Garcinia Cambogia and dietary supplements. The influence of extraction method (water-bath extraction and ultrasonic extraction) was tested.

The HPLC system equipped with auto sampler SIL-20AC HT and photodiode multi-wavelenght detector (SPD-M20A Prominence Diode Array Detector), SHIMADZU (Kyoto, Japan) was applied. Phenolic acids were detected by photodiode array detector at three wavelengths: 254, 295 and 325 nm, using a 45-min program, and 360 nm for flavonols at 50-min gradient program. Analyses were carried out on Discovery RP-C_{18} column (5 μm particle size, 150×4,6 mm, SUPELCO), maintained at 30°C. Mobile phase for phenolic acids was A: 2% acetic acid whereas phase B was methanol and for flavonols was A: isopropanol-water (95:5 v/v), B: isopropanol-water-THF (50:40:10 v/v). Every one of solvents and standards were of HPLC grade. The rate-flow was 1ml/min in both program.

The total amount of studied flavonols and phenolic acids was compared with total content of polyphenols using Folin-Ciocalteua’s method. The developed method was validated for specificity, repeatability, recovery and accuracy.

CHEMICAL ANALYSIS OF STEAM CRACKING TAR

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Steam cracking tar (SCT) is a by-product of thermochemical decomposition of gaseous hydrocarbons at elevated temperatures in the absence of oxygen. For the most part SCT is used in the production of carbon black. However, due to ongoing worldwide increase in ethylene capacities there is a substantial interest in converting SCT into more valuable commercial products. Therefore, in order to correctly design SCT processing schemes, its chemical composition must be accurately known. The naphthalene and sulfur content, as well as the amount and composition of distillation residue and light fraction should be defined.

In this work, chromatographic, spectroscopic, thermogravimetric and elemental analysis techniques were employed in the analysis of SCT samples from several ethylene plants.

The amount of distillation residue in the samples is 30-35% according to TG. Sample weight loss occurs in two steps: within 70-310 °C and 310-600 °C, corresponding to the light fraction and the distillation residue, respectively.

Close to 20 volatile components of SCT were quantitatively characterized by GC using acetophenone as an internal standard. The procedure adopted in this work does not require preliminary removal of distillation residue.

To obtain more detailed information, the original SCT samples were divided into several fractions. More than 40 components of the light fraction were additionally identified by GC-MS owing to background noise reduction and chromatographic signal enhancement. Identity of naphthalene and biphenyl, the principal components of SCT, was ascertained by ^{13}C NMR.

The fractionation allowed us to discern a number of components previously not found in SCT: isomers of dicyclopentadiene, 1-methyl-1,2-propadienylbenzene, 1-ethenyl-3-methylene-cyclopentene and others. Molecular fragments containing four and five aromatic rings were identified in the distillation residue by Py-GC-MS at 1000 °C. Distillation residue was found to be similar in its nature to synthetic petroleum resin.

Generally, the chemical composition of the SCT samples studied is as follows:

Component	% wt.
naphthalene	3.0-13.0
biphenyl	0.5-9.0
1,1-diphenylethane	*ca.* 5.0
indene	0.5-4.0
1-methylnaphthalene	1.0-2.5
2-methylnaphthalene	1.0-3.0
1-methyl-2-cyclopropene-1-yl-benzene	0.5-2.0
2-methylindene	0.5-2.0
1,4-dihydronaphthalene	0.5-1.5
phenanthrene and anthracene	1.0-1,5
4-methyl-1,1′-biphenyl	*ca.* 1.0
2-, 1-ethylnaphtalene	*ca.* 1.0

SEPARATION AND RETENTION BEHAVIOR OF NEWLY SYNTHESIZED ANTICONVULSANT SUCCINIMIDE DERIVATIVES IN RP TLC

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Succinimide derivatives exhibit a pronounced anticonvulsant activity, combined with some antimuscarinic effects. The succinimide moiety provides a pharmacophore for potential antiepileptic agents. Potential new drugs from the group of succinimide derivatives seem to be structurally guaranteed. Investigation of retention behaviour in RP TLC is important for drug candidates at an early phase of drug development, because retention constants determined in RP TLC system is related to the lipophilicity. Lipophilicity is one of the physicochemical properties which influence a drug's permeation ability as well as its distribution properties.

In this paper retention behaviour was performed on precoated RP-18W/UV254 plates (Macherey-Nagel GmbH and Co., Düren, Germany). The following mobile phases were used: A: water – dioxan; B: water – acetone; C: water – acetonitrile. Conditions for best separations were determined. Effect of lipofilicity on separation of newly synthesized anticonvulsant succinimide was discussed. Effect of chemical structure on separation was discussed, too.

Investigation of retention behaviour, separation and determination of lipophilicity of drug candidate by use of the TLC method is simple and inexpensive.

EFFECT OF MOBILE PHASE ACIDITY ON SEPARATION OF S-TRIAZINES HOMOLOGOUS SERIES IN RP TLC

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The most important triazines are symmetric triazines (s-triazines) that have a six – member heterocycle with symmetrically located nitrogen atoms. s-Triazine names and their principal properties are primarily determined by the substituent in position 2; this is most often –Cl (the commercial name ending in – azine). Positions 4 and 6 are usually occupied by substituted amino groups and exert substantially smaller effects on the properties. s-Triazines are compounds with pharmaceutical, chemosterilant, and industrial importance. They are widely used as herbicides in agriculture and industry and some of the compounds also have fungicidal properties.

Effect of mobile phase acidity on separation of homologous series of nine s-triazines was investigated with mobile phase: acetone – dilute formic acid (pH = 1.6 – 4.6). Best separation was obtained with mobile phase: acetone – dilute formic acid (pH = 4.6). s-Triazines are ionizable compounds and their retentions depends on mobile phase pH. Thus we investigate the influence of mobile phase pH on the retention of s-triazine derivatives and determine ionisation constants, pK_a, These values were correlated with ionization constants determined with different computer software.

COMPARISON OF TWO DIFFERENT GC-MS METHODS FOR ANALYSIS OF ESSENTIAL OIL COMPOSITION

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Identification of essential oil components based solely on their chromatographic data and mass spectra is not reliable method of their determination, since essential oils represent very complex mixtures of sometimes highly similar isomeric compounds with similar MS. Retention time locking (RTL) approach, which uses matching of spectral data with the locked retention times in a corresponding compound database can also be used in such analyses instead of the more time-consuming calculation of Kovats indices (KI). In this study, two different chromatographic methods, one of wich uses RTL (and a freely available Flavor2 compound database), and other which relies mainly on spectral matching and KI calculation, were compared and tested for determination of essential oil components of oregano (*Origanum heracleoticum* L.) and common thyme (*Thymus vulgaris*). Influence of different run times of these methods, temperature gradient, as well as the effect of oil concentration on the quantitative analysis was also examined.

The obtained results show that concentration of the injected essential oil plays a very important role in accurate determination of the compounds. Retention time locking and use of Flavor2 database can be used to simplify the analysis of essential oils composition, but due to a small number of entries in the database (around 400) and shift of retention times in the case of most abundant compounds, its usage is limited mainly to screening of less complex compound mixtures. It can be concluded that calculation of Kovats indices still represents an important step in identification of similar compounds in highly complex natural products such as essential oils.

HERBAL DRUG *FAGOPYRI HERBA* AS A SOURCE OF BIOACTIVE PHENOLIC COMPOUNDS

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Due to a high content of rutin (2-10%), *Fagopyri herba* has been shown to be efficient in the treatment of vascular diseases. As such, it can be considered as a source of bioactive compounds for functional food formulations.

The effects of ethanol water ratio and temperature on the extraction efficiency and phenolic composition of *Fagopyri herba* extracts were investigated. Extracts were obtained by maceration with water and ethanol/water mixtures, with the ratio of raw materials to ethanol solution of 1:50, for 24 h at room temperature and subsequent extraction in an ultrasonic bath for 10 min. These extracts were compared with corresponding extracts which were previously brought to boiling. Identification of the phenolic compounds in extracts was carried out by LC-DAD-ESI-MS/MS, and quantification of identified compounds was carried out by rapid resolution reverse phase HPLC/DAD method.

According to obtained results, extraction with boiling ethanol/water (80:20) has been shown to be the most efficient for achieving the highest yield of rutin (4.99%). Comparing the results of rutin yield obtained by different extraction procedures, it has been concluded that temperature exhibited the highest influence on water extraction.

SEPARATION OF DERIVATIZATED AMINO ACID ENANTIOMERS USING COLUMN-SWITCHING CHROMATOGRAPHY TECHNIQUE

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It is known that free D-amino acids are contained in highest living organisms including mammals. The physiological role of D-amino acids has been studied for only few substances. Developing of stereoselective methods for amino acid analysis leads to further understanding of sophisticated biochemical processes. Two-dimensional "heart-cutting" chromatography is the most powerful method for the high-selective and high-sensitive determination of targeted amino acids in complicated matrixes. The first step separation on the reversed-phase column is followed by enantioseparation on the chiral phase.

Column-switching chromatographic system for stereoselective amino acid (AA) analysis is proposed in this work. Before separation amino acids were derivatized with *o*-phthalic aldehyde (OPA) in combination with various non-chiral thiols (R-SH). Further the derivatives were resolved on the first reversed phase column. Chiral stationary phase Nautilus-E (250x4.6 mm, 5 μm, BCM, Russia) with eremomycin chemically bonded to silica was choosen as the second column for enantioseparation.

O-phthalic aldehyde is a suitable reagent for amino acid analysis. It allows to use several commercial solutions for the first step separation. OPA also provides flexibility in chromatographic properties by changing SH-reagent. Serine, aspartic and glutamic acids were chosen as most important for diagnostic goals, according to the literature. The chromatographic behavior of OPA derivatives with 1-mercaptopropionic acid (MPA), ethanethiol (ET) and 2-(dimethylamino)ethanethiol (CAPT) was investigated.

The effect of the mobile phase on the resolution of amino acids enantiomers on chiral column was studied by varying the mobile phase composition (type and percentage of organic modifiers, pH and ionic strength of the buffer solution). It was determined that acetonitrile provides a higher selectivity than methanol in amino acid diastereoisomers separation, and the concentration of buffer solution affects on selectivity weakly.

Good separation of serine (R_S= 0.88) and aspartic acid enantiomers (R_S= 0.80) was obtained after derivatization with ethanethiol. Base-line separation of asparagic acid enantiomers (R_S=1.15) and partial separation of glutamic acid enantiomers (R_S= 0.71) was achieved after derivatization with 2-(dimethylamino)ethanethiol. Full separation of D,L-serine was observed after derivatization with 1-mercaptopropionic acid, however the efficiency was unsatisfactory (N=450 – 550 t.p. per meter).

Thus, the performed researches allowed to develop the method for determination of D-serine and D-aspartic acid in blood and urine using OPA/ET or OPA/CAPT as derivitazing reagents. The linearity of calibration curves of OPA/ET derivatives of amino acids in the range 0.08-20 mg/l was determined ($R \geq 0.999$) for fluorescence and diode-array detection. The detection limits were found between 2.0×10^{-3}-2.5×10^{-2} mg/L for fluorescence detection.

NAPHTHALENE-2,3-DICARBOXALDEHYDE PRE-COLUMN DERIVATIZATION OF BIOGENIC MONO- AND DIAMINES AND HPLC DETERMINATION IN CHEESE WITH FLUORIMETRIC DETECTION

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A new fluorescence- HPLC method was developed for the simultaneous determination of eight biogenic monoamines (histamine, methylamine, tyramine, ethylamine, propylamine, tryptamine, 2-phenylethylamine, isoamylamine) and two biogenic diamines (putrescine, cadaverine), in the presence of heptylamine, as internal standard. The amines were pre-column derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) using cyanide ions as a nucleophile. The influence of the derivatization reaction conditions on the output of the reaction was investigated. The derivatives were separated on an Inertsil ODS-3 column (250 x 4 mm i.d., 5 μm) using a binary eluting system, flowing under gradient conditions, and detected fluorimetrically at excitation and emission wavelengths of 424 and 494 nm, respectively. Limits of detection between 0.002 – 0.85 ng injected on-column (10-μL loop) were achieved. The proposed method was successfully applied to the determination of biogenic mono- and diamines in Greek cheeses, with satisfactory recoveries.

FLUORESCENCE-LC ANALYSIS OF BIOGENIC MONOAMINES IN MILK BY PRE-CHROMATOGRAPHIC DERIVATIZATION WITH NAPHTHALENE-2,3-DICARBOXALDEHYDE

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A novel, simple, sensitive and selective HPLC method with fluorescence detection is described for the simultaneous determination of six biogenic monoamines, in the presence of hexylamine as internal standard, after pre-column derivatization with naphthalene-2,3-dicarboxaldehyde/cyanide ions. The derivatization reaction conditions, including the pH-value and the concentration of the derivatization buffer, the reaction time, the NDA and the cyanide concentrations, were investigated. The isoindoles formed were separated on an Inertsil ODS-3 column (250 x 4 mm i.d., 5 μm), using a mobile phase of methanol/water (80:20, *v/v*) at a flow rate of 1 mL min^{-1}. The isocratic elution program resulted in baseline separation of the analytes in less than 30 min. The limits of detection were in pg level with a sample volume of 10 μL. The whole procedure was evaluated and fully validated for the determination of biogenic amines in milk samples.

DETERMINATION OF ASCORBIC AND DEHYDROASCORBIC ACID IN CHEWING TABLETS WITH VITAMIN C

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It is proposed a new method for determination of ascorbic (AA) and dehydroascorbic acid (DHAA) in chewable Vitamin C tablets. The determination is performed by highly efficient liquid chromatography (HPLC), where AA is determined directly and DHAA indirectly. Total AA is determined after the reduction of DHAA to AA with 10 mM dithiothreitol. DHHA is obtained by subtracting the total non oxidized AA, which is determined directly, from the total AA which is determined after the reduction. Since AA is very unstable in neutral and basic environment, the sample preparation is performed in 5% phosphoric acid. There are certain conditions that are optimal for the determination of solubility (solubility of time, acidity). The proposed method is validated and applied for the determination of AA and DHAA in chewable tablets, stored at room temperature and accelerated aging conditions (30 ° C ± 2 ° C/65% RH ± 5% and 40 ° C ± 2 ° C / 75% RH ± 5%). Oxidation and degradation products do not occur at room temperature, but they are noticed in accelerated aging tablets.

CHARACTERIZATION OF SOLID RESIDUE OF BEFF TALLOW BIODIESEL AND INFLUENCE ON FUEL QUALITY

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Biodiesel production has increased over the last decade because of the benefits associated with this fuel, including renewability, domestic feedstock, lower toxicity, and biodegradability. However, the performance of biodiesel in cold weather conditions is worse than diesel because of crystallization of insoluble, accelerating the plugging of fuel filters and injectors of the vehicle engine. From 2008, the use of beef tallow as a feedstock for biodiesel production in Brazil has increased in significance, representing the second largest source of biodiesel, after soybeans. Studies have been conducted on beef tallow biodiesel, mostly related to the properties of thermal and oxidative stability. However, few studies has described the nature of the solid residue formed and its influence on product quality. The induction agents have been suggested to be saturated monoglycerides. This study monitored the levels of mono-, di- and triglycerides, the oxidation stability and the cold filter plugging point (CFPP) in beef tallow biodiesel samples from two commercial producers in Brazil for a period of twelve months. Samples were analyzed by comparative techniques of GC-FID, HPLC-MS-IT-TOF and TG to verify the nature of the residue, using monopalmitin and monostearin as reference standards. The formation of precipitate reduced the levels of monoglycerides in the beef tallow biodiesel. GC-FID and LC-MS-IT-TOF results confirmed the nature of the residue as saturated monoglycerides, predominantly monostearin and monopalmitin as the second major component of the precipitate. Moreover the TG analysis of the residue indicated similar thermal decomposition of the reference standards. In addition, the residue did not affect the oxidation stability of beef tallow biodiesel and the CFPP characteristic to blends up B50. However, the presence of iron reduced significantly the oxidation stability of biodiesel. These results will aid in the establishment of limits for monoglycerides in biodiesel by regulatory agencies and biodiesel producers.

SELECTIVE ANALYSIS OF POLYAROMATIC HYDROCARBONS AND PHENOLS

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Due to strong polarity of the amino-phase sorbent, it adsorbs polar compound, for example, phenol homologues better than aromatic compounds. Aromatic fraction can be desorbed with the help of the solvent with low polarity, e.g. dichloromethane; phenolic fraction with the help of the mixture of dichloromethane and acetonitrile (as rather polar). Desorption efficiency of aromatic compounds (benzene and naphthalene) and phenolic compounds (o-cresol, p-m-cresol, 2,6-xylene un 2,4-2,5-xylenes) from amino-phase sorbent with dichloromethane and with the mixture of dichloromethane and acetonitrile (1:1 v/v) was studied.

Desorption efficiency of benzene and naphthalene with dichloromethane reaches 91.9% and 94.6%, respectively. On the contrary, the phenolic fraction practically does not desorb with dichloromethane; desorption efficiency of o-cresol, p-m-cresol, 2,6-xylene and 2,4-2,5-xylenes remains in the interval 2.6–6.9%. Absolutely opposite results were obtained during further desorption of compounds with the mixture of dichloromethane and acetonitrile. Only 5.9% of benzene and 2.9% of naphthalene adsorbed, which makes desorption efficiency of benzene and naphthalene with dichloromethane and the mixture of dichloromethane and acetonitrile, in sum, 97.9% and 97.9%, respectively. Desorption efficiency of the phenolic compounds with the mixture of dichloromethane and acetonitrile is in the interval 94.4–97.2%, with the summed up desorption efficiency of the investigated phenolic compounds ranging from 98.1% to102.7%.

In order to analyse phenolic compounds, phenol derivatization is needed. Optimal derivatization conditions were investigated. Cross comparison of parameters reveals that optimal derivatization conditions for phenols with BSTFA are 40 min of the derivatization time and solution temperature of 50 °C because using a lower temperature and shorter time does not complete derivatization, while heightening temperature and time practically does not change the derivatization efficiency.

An optimal ratio between dichloromethane and acetonitrile was investigated in order to achieve a maximally complete desorption of both aromatic and phenolic compounds from amino-phase sorbent.

MULTIRESIDUAL ANALYTICAL METHOD FOR SIMULTANOUS DETERMINATION OF SELECTED PHARMACEUTICALS AND PESTICIDES IN SURFACE AND GROUND WATER

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A sensitive multiresidual method was developed for quantification and confirmation of twelve pharmaceuticals from different therapeutic classes (antibiotics – β-lactams, sulfonamides and macrolides; benzodiazepines; antiepileptics and analgoantipyretics), two metabolites, as well as thirteen pesticides belonging to various chemical classes (organophosphates, neonicotinoides, carbamates, diacylhydrazines, benzimidazoles, triazines and phenylureas) in surface and ground waters, in a single analytical step. The proposed method includes solid-phase extraction, as the sample preparation method, followed by liquid chromatography–tandem mass spectrometry analysis of the obtained extracts. The optimization of analytical parameters affecting the recovery of the analytes, as volume and pH-value of the water sample, and composition and volume of the elution solvent was conducted. The confirmation of the residues detected in real samples was performed by repeated analysis of the positive sample extracts with additional transitions to that used for quantification. The majority of analytes was extracted with good recoveries (71–121%), and low limits of quantification (3–185 ng l^{-1}). The method was applied to 14 surface and 11 ground water samples collected in Serbia, and traces of carbamazepine, metamizole metabolites (4-AAA, 4-FAA), trimethoprim, sulfametoxazole, lorazepam and azithromycin, as well as carbendazim were detected.

CLUSTER ANALYSIS OF FACTORS GOVERNING THE RETENTION IN MICROEMULSION LIQUID CHROMATOGRAPHIC SYSTEMS

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The objective of this work was the evaluation of the factors that might affect the retention behavior of pramipexole and its five impurities under microemulsion liquid chromatographic (MELC) conditions, as well as the elucidation of the similarities and dissimilarities between the retention characteristics of employed systems by means of cluster analysis (CA). MELC provides three simultaneous partition systems: first system comprises microemulsion droplets/bulk of the eluent; second, microemulsion droplets/stationary phase and the third, bulk of the eluent/stationary phase. For that reason the analytes' retentions are affected by many factors. CA, as a method which divides data into meaningful and useful clusters, could be very versatile for the evaluation of the stated factors. In the presented study butyl-acetate was used as the internal phase, *n*-butanol as co-surfactant, while sodium-dodecyl sulfate and Brij®35 were surfactants. For the evaluation of stationary phase hydrophobicity C4, C8 and C18 (all Hypersil GOLD 150x4.6 mm, 5μm columns) were used. Experimental matrix was defined by the full factorial 2^4 design. On the retention data hierarchical cluster analysis was applied to identify homogeneous groups of variables based on selected characteristics. Key issue in cluster analysis is determination of the clusters' number. Hierarchical procedures examine tabular or graphical output to identify the gaps that define logical clusters. In this study complete linkage amalgamation rule with Euclidean distance measure was used to quantify the similarity. Branches length in the obtained dendrograms indicates the differences between retention factors of analyzed substances, while the similarities are indicated by linkage pattern of the clusters. Impurities BI-II751xx, 2-aminobenzothiazole and BI-II786BS had the same retention behavior on all analyzed stationary phases and their retention was influenced mainly by the mobile phase characteristics. For the impurity BI-II820BS, BI-II546CL and pramipexole retentions were affected simultaneously by stationary phase hydrophobicity and mobile phase composition.

A QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIP STUDY OF ADAMANTANE DERIVATIVES OF 4-AMINO-7-CHLOROQUINOLINE

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The chromatographic behaviour of adamantane-derived 4-amino-7-chloroquinoline series of potent antimalarials, was examined by reversed-phase thin-layer chromatography in order to correlate their structure with retention. All examined compounds possess three common components that participate in chromatographic interactions: 4-amino-7-chloroquinoline aromatic part, adamantane, and alkylamino part. The presence of highly polarized, π electronic quinoline ring offers a possibility for dipolar interactions between the molecules and with both, mobile and stationary phase. Based of respective retentions, the lipophilicity ($\log K_{ow}$) of the investigated compounds was determined by simultaneous chromatographing with standard solutes.

Retention parameter, R_M^0, demonstrating partition of the compounds between non-polar stationary phase and water, together with $\log K_{ow}$ values, were used to conduct a quantitative structure–retention (property) relationship (QSRR, QSPR) study. Principal component analysis (PCA) followed by partial least squares (PLS) have been used to select descriptors that best describe the behaviour of the investigated compounds in chromatographic systems and to quantify their influence so enabling the prediction of behaviour of congeners. The application of PCA allowed the classification of analytes with respect to their structural characteristics. The obtained models are statistically significant, and the descriptors included are of similar nature and significance. The models indicate the importance of nonpolar properties of the solutes and their ability for hydrophobic interactions, as well as the importance of proton donating abilities, hydrophilic and π-interactions pointing out the possible separation mechanism in the observed chromatographic systems.

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PURIFIED AND CHEMICALLY-MODIFIED CARBON NANOTUBES AS STATIONARY PHASES FOR GAS CHROMATOGRAPHIC SEPARATIONS

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Carbon nanotubes (CNTs) are an attractive alternative to conventional GC stationary phases because their large aspect ratio provides high external surface area, and their nonporous nature leads to fast mass transport [1]. Different classes of compounds have been analyzed on CNT-based gas-solid chromatographic columns [1,2], with better performance than traditional carbon sorbents [2]. The influence of CNTs impurities content on their retention behaviour has been assessed and, in particular, metals proved to powerfully affect CNTs surface properties [3], while amorphous carbon was shown to be the main cause of peak tailing [4].

Interestingly, the prospect to modulate the interaction solute-stationary phase has been lately investigated on functionalized Multi-Walled CNTs. These have been tested for the analysis of alkanes [3] and alchools [5], with higher performance than non-modified MWCNTs.

In this work, amino-terminating chains derivatized MWCNTs (MWCNTs-$CONH_2$) and HCl-purified MWCNTs have been evaluated as column packing sorbents for GC-FID analysis of compounds showing diverse polarity and volatility, namely esthers and chlorinated aromatics. Glass columns (2 mm i.d., 25 cm lenght) have been used, with a N_2 flow rate of 12 mL min^{-1}.

Markedly different performance was seen on non-modified and MWCNTs-$CONH_2$. In particular, these last were shown to be high selective sorbents, allowing good chromatographic profiles at relatively low temperatures (<120°C), for both esthers and aromatics. Test mixtures of analytes were successfully analyzed, with good resolution. Contrariwise, only partial separations were possible on MWCNTs, and high temperatures (up to 250°C) were required for desorption. Results indicate that functionalization is a key factor to obtain optimal solute-sorbent interaction.

With the aim to improve time analysis and separation efficiency, experiments to prepare a capillary column functionalized on the inner surface with oxidated CNTs are ongoing.

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PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF TWO MEDICINAL *CYNARA* PLANTS (ARTICHOKE AND CARDOON) FROM MADEIRA ISLAND AND TWO ARTICHOKE-BASED FOOD SUPPLEMENTS: QUALITATIVE AND QUANTITATIVE HPLC-DAD-ESI-MSN ANALYSIS.

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Cynara cardunculus var. *scolymus* (artichoke) and *Cynara cardunculus* var. *ferocissima* (cardoon) are two Asteraceae plants used in the traditional medicine of Madeira Archipelago for digestive purposes and to lower blood cholesterol.

The present worked aimed to compare the phenolic composition and quantify the main compounds of these two plants and two artichoke-based dietary supplements (juice and dragées).

The alcoholic extract of the endemic plant, *Cynara cardunculus* var. *ferocissima,* cardoon, was basically composed of caffeoylquinic acid isomers. The same compounds were observed in artichoke extract, where there was a larger number and variety of other phenolic compounds.

Variations in qualitative and quantitative composition of the three artichoke based products were extensive, with only 3 components being common to all 3 products. Mono-*O*-diglycosilated flavonoids (luteolin, apigenin and quercetin) were found in the artichoke products but not in cardoon.

1,3-diCQA (cynarin), usually considered the main responsible for the biological properties of artichoke was found in amounts ranging from 11 to 990 mg per daily dose in most samples but was not detected in the analyzed commercial dragées.

The antioxidant assays results reflected the highly diversified composition of the artichoke-based products, showing a high radical scavenger and reducing capacities, while the extract from cardoon was a poor antioxidant.

MULTI-WALLED CARBON NANOTUBES AS SOLID-PHASE EXTRACTION ADSORBENTS FOR THE SPECIATION OF COBALAMINS IN SEAFOODS BY LIQUID CHROMATOGRAPHY

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Solid phase extraction (SPE) is a recognized, efficient tool for sample preconcentration. Multiwalled carbon nanotubes (MWCNTs) have been evaluated as adsorbents for miniaturized SPE coupled to liquid chromatography (LC) for the speciation of four cobalamins representing various forms of vitamin B12, namely, cyanocobalamin (CN-Cbl), hydroxocobalamin (OH-Cbl), methylcobalamin (Me-Cbl) and 5'-deoxyadenosylcobalamin (Ado-Cbl). The preconcentration on the MWCNTs was based on the retention of analytes by introducing the sample on-line into the minicolumn system, dimethylsulfoxide (DMSO) being then used to elute the retained vitamins. The minicolumn was packed with 13 mg of MWCNTs and activated by passing 30 mL of water and 30 mL of 10 mM phosphate buffer (pH 5) solution at a flow-rate of 1 mL min-1. A volume of 10 mL of sample was pumped through the column at 1 mL min-1. Then, the column was inverted and cobalamins were eluted in the opposite direction by pumping 400 µL of DMSO through the bottom of the column at 100 µL min-1. The first 100 µL fraction was discarded, and the following 300 µL were collected for injection in the LC. To avoid cross contamination, the column was washed
between samples using 600 µL of DMSO and 3 mL of phosphate buffer solution. Aliquots of 20 µL were injected into the LC. A diode-array device was used for detection and good resolution was obtained with gradient elution using a mobile phase of 10 mM KH_2PO_4 (pH 7) – acetonitrile at 1 mL min-1. An amide-based stationary phase involving a ligand with amide groups and the endcapping of trimethylsilyl was used. The elution order was OH-Cbl (7.3 min), CN-Cbl (11.4), Ado-Cbl (12.9) and Me-Cbl (20.1). Specificity was demonstrated by the retention characteristics and UV spectra.

Linearity, precision, recovery and sensitivity were satisfactory. Calibration graphs were obtained by the standard additions method. Detection limits ranged from 0.35 to 30 ng mL-1. The method was applied to the determination of cobalamins in seafoods such as mussels, clams and cockles, both fresh and canned, which were extracted from the sample with a buffer solution using an ultrasonic probe. The only species found was CN-Cbl. The reliability of the procedure was checked by analyzing a certified reference material.

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ANALYSIS OF FOOD ADDITIVES IN BEVERAGES BY ION CHROMATOGRAPHY

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Since many food additives are introduced to a food product during the manufacturing process and/or food is contaminated through packing, etc., the determination of food additives is an analytical problem of great interest. Ion chromatography is now a routine technique for the analysis of food products and beverages of complex composition due to its versatility, sensitivity and rapidity.

In the present study, several stewed fruit, fruit juices, soft drinks and wines of different manufacturers were studied. The most commonly used acidity regulators in foods such as citric, tartaric, phosphoric, malonic and sorbic acids were analysed by portable ion chromatography equipment Shimadzu PIA-1000 (Japan) with Shim-pack IC-A1S (4.6 × 100 mm) column and conductometric detector. Recently, this single-column analyzer has been successfully applied for the determination of inorganic anions in water. Analytical conditions for the analysis of mentioned above food additives in beverages have been previously optimized.

The approach given allows to detect the organic acids even in excess of chloride, nitrate and sulfate ions. The method enjoyed a wide linear range, good recoveries and high sensitivity. The results showed that the method was simple, accurate and can be used for analysis of food additives in beverages.

DEVELOPMENT OF GAS DETECTION IN THE ANAEROBIC DIGESTION OF WASTE

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The process of anaerobic digestion of municipal solid waste allows a biogas with a high energy value, the main components of biogas are the H_2, CH_4, CO_2 and H_2S. In this research work is sought to obtain the optimal conditions for a Gas Chromatograph (GC-2010 Shimadzu) that allowed detection of all the different components of biogas in the shortest time possible. The gases analyzed were the H_2, CH_4, CO_2, O_2, N_2 and H_2S. The first five components were analyzed from a thermal conductivity detector (TCD) and the column used was a Supelco Carboxen 1010 Plot and for the SH_2 was used a Supelco Supel-Q Plot column and the detector used was flame photometric (FPD). The calibration of equipment used different patterns with different composition. The result was a method that allowed the joint detection of all components of biogas in a time of 7 minutes.

BIOGAS COMPOSITION FROM SINGLE AND TWO PHASE SYSTEMS OF BIOMETHANIZATION

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Biogas obtained from the anaerobic digestion of sorted organic fraction of municipal solid waste under thermophilic conditions is a valuable renewable fuel. Methane and carbon dioxide are the main components, but biogas also contains contaminants such as hydrogen sulphide. This study sought to determine the concentration of the different components of biogas from anaerobic degradation. We carried out a study of the composition of biogas in a single phase and another in a separate phase. The results were that both single-stage reactor as the methanogenic reactor yielded a rich biogas CH_4, with yields exceeding 60% and with a production of sulfide that sometimes exceeded the 5000 ppm. While the results of the acidogenic reactor, showed a biogas rich in H_4 and CO_2 and a production of methane and sulfide negligible.

ENVIRONMENTAL FACTORS AFFECTING THE CORROSION BEHAVIOUR OF STAINLESS STEEL IN ALUMINIUM SMELTER: INFLUENCE OF FLUORIDE CONCENTRATION AND pH VALUE

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Iron and stainless steel have been largely used as construction materials in processing industry. In the aluminium smelter, one of the main environmental problems is air emissions which include fluorides. Environmental concerns are also a reason for corrosion control. From an industrial point of view, presence of fluorides is an important issue limiting the service life of the equipment used in processing industry. The fluoride concentration in aluminium smelter “Aluminij” d.d. Mostar was determined by potentiometry and obtained values were below the maximum allowed concentration (5 mg m^{-3}). These results were base for setting up the experimental conditions under which corrosion of stainless steel was studied. The influence of fluoride concentration (0.01 i 0.1 mol L^{-1}) and pH value of solution (4.5, 5.5; 6.5) on electrochemical behaviour of stainless steel 304 was studied by combination of electrochemical techniques. The growth and mechanism of the passive film formation on stainless steel 304 in fluoride solution were studied by cyclic voltammetry (CV). Under the assumption that the composition of the passive film is Cr_2O_3 the values of field strength, conductivity and half-jump distance were calculated. The electrochemical properties of the spontaneously passivated electrodes at the open circuit potential were studied by electrochemical impedance spectroscopy (EIS). The charge transfer resistance and the electrode capacitance were determined. The experimental data are interpreted in terms of the point defect model, assuming that predominant point defects on passive film of stainless steel 304 are cation vacancies. Diffusivity and concentration of point defects were estimated from the Warburg-type impedance. The CV and EIS results indicate that the increase of fluoride concentration leads to increase dissolution of stainless steel 304, while influence of pH value is negligible in studied experimental conditions.

EN60 **Session C**

CHANGES IN SAALE SEDIMENTS FROM 1994 TO 2010

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An extensive investigation of river sediments provides information about the medium- and long-term pollution of a river. It is also possible to determine geogenic and anthropogenic contaminations. To these belong historical as well as new industry inputs. The river Saale rising in Zell in the Fichtelgebirge, passes through the federal states of Bavaria, Thuringia and Saxony-Anhalt. After 427 kilometer it discharged between Breitenhagen and Barby left-handed into Germany's third-largest river, the Elbe.

The basis for our investigations provides two similar sampling campaigns. The first was accomplished from 1993 to 1994 from the Friedrich Schiller University of Jena [1]. The second from the Saxonian Academy of Sciences in Leipzig in 2000 [2]. In May/June 2010 a third sampling campaign was performed and the obtained results were compared to the first two campaigns. By means of this comparison 27 sampling points and 10 heavy metals (Cd, Co, Cr, Cu, Hg, Fe, Mn, Ni, Pb, and Zn) could be considered.

All metal contents were obtained for the particle size < 20 µm. Cobalt and nickel have their geogenic origin in the surrounding mountains in the upper reaches. The metal contents of cadmium, copper, and zinc did not vary very much for the three campaigns, but a significant increase can be detected for the last eight sampling sites and in probably caused by the industrial area around Halle or the tributary Weiße Elster.

The highest metal contents for manganese in the 1990s could not be detected in the two following campaigns. However, the mean manganese content after a barrage increases from the first to the third campaign. Chromium (Fig. 1) and mercury (Fig. 2) are the most interesting investigated metals. While chromium could be detected unchanged at the same sampling sites with the same metal contents from 1994 to 2010, the results for mercury pointed out a transfer and a reduction of the heavy metal contamination. This could be explained by some flood occurence in the 1990s and 2000s.

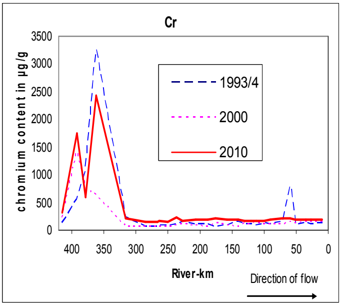

Fig. 1: Chromium content in the river Saale

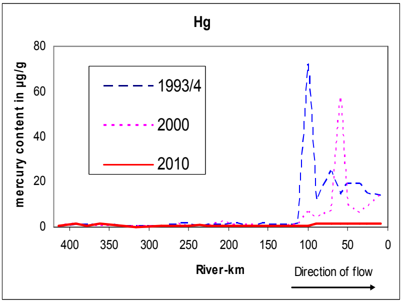

Fig. 2: Mercury content in the river Saale

Despite of the increase shutdown of industry factories next to the river Saale after the German reunification, the heavy metal contents did not decrease from the 1990s to 2010 as expected. Through these contaminations and since the Saale is one of the largest tributaries of the Elbe, there is an urgent need for further monitoring on these river sediments in periodical intervals.

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COMPARISON OF EXTRCATION EFFICIENCIES OF VARIOUS LEACHING SOLUTIONS FOR ASSESSMENT OF BIO-ACCESSIBLE TRACE METAL FRACTIONS IN AIRBORNE PARTICULATE MATTER

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In the last few decades, several studies have provided solid evidence for the association of particulate matter (PM) concentrations with both short-term and long-term health consequences including respiratory and cardiovascular diseases.[1] Many elements such as Ba, Co, Cu, Mn, Ni, Pb, V and Zn, some of them being known as potentially toxic, are largely present in atmospheric particles. [2] For risk assessment of metal toxicity, it is important to determine the bio-accessible trace metal fraction instead of total metal contents. A considerable number of studies have been reported in literature for assessment of bio-accessible trace metal fractions using different kind of leaching agents. Due to differences in their chemical behavior these leaching agents are assumed to extract different fractions of soluble trace metals in PM. Therefore, it is highly demanded to compare the extraction efficiencies of various leaching agents in order to find the best suitable conditions for the assessment of bio-accessible trace metal fractions in PM. For this task a highly sensitive method is required, since even total trace metal concentrations in PM are usually very small, distinctly reduced concentrations were reported in literature for leachable fractions. Furthermore it has to be considered that the composition of the leaching agents (organic and inorganic constituents with enhanced concentrations) may cause spectral and non-spectral interferences during multi-element analysis with ICP-AES or ICP-MS.

In present study, an in-vitro physiologically based extraction test is applied for extraction of trace metal fractions in PM using the leaching agents H_2O, NaCl, ammonium acetate, ammonium citrate, synthetic gastric juice and artificial lung fluid. The extracts were then measured using a newly developed ETV-ICP-AES procedure. Derived results indicated that the leaching agents containing organic constituents extracted higher amounts of “bio-accessible metals” as compared to extractants with inorganic nature e.g., NaCl.

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DIRECT ANALYSIS OF TRACE METALS IN AIRBORNE PARTICULATE MATTER USING ELECTROTHERMAL VAPORIZATION IN COMBINATION WITH INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY

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Airborne particulate matter (PM) contains a variety of metals. These metals are either contributed by natural sources such as crustal weathering, sea-salt aerosol generation, volcanism or anthropogenic processes such as fossil fuel combustion, industrial activity, incineration.[1] The high metal concentrations reported for contaminated sites such as urban areas can pose serious human health risks.[2] Thus routine monitoring of metals in PM is required for risk assessment, source identification and ambient level reduction. For the determination of trace metals in PM usually well established techniques like inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), or electrothermal atomic absorption spectrometry (ETAAS) are used. Application of these techniques requires the conversion of the solid samples into solutions, which is accomplished with the use of laborious and time consuming digestion procedures. Further drawbacks of this approach are loss of sensitivity and an increased risk of sample contamination.

Here we present an alternative procedure which allows the direct measurement of PM samples without a preliminary sample digestion step. For analysis aliquots of the investigated PM sample were measured using ETV-ICP-AES. The presented method is found to be more sensitive than conventional ICP-AES analysis of sample digests, additionally the sample throughput is enhanced and the use of hazardous chemicals could be avoided. Furthermore a comparison between the direct pyrolysis of sample including filter substrate inside the ETV furnace followed by ICP-AES measurement of the formed vapors and the preliminary treatment of the sample in a muffle oven followed by the ETV-ICP-AES measurement of the residue is presented. Applicability of the developed ETV-ICP-AES procedure has been demonstrated by the analysis of a set of rural PM10 samples.

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APPLICATION OF SEQUENTIAL EXTRACTION AND THE ICP-AES METHOD FOR STUDY OF THE DISTRIBUTION OF METALS IN FILTER ASHES

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The distibution of As, Al, Ba, Ca, Cr, Cu, Cd, Co, Fe, Ni, Mg, Pb and Zn in samples of filter ash obtained from various cassetes of Kolubara“ power plant (Lazarevac near Belgrade, Serbia) were studied by sequential extraction.

Filter ash was separated in electrofilters and transported through the pipelines, as water suspension, to the dump, where it could be exposed to different influences. Some relatively non-soluble elements could become soluble and potentially pollute surrounding surface, ground water and surrounding soil. The total of 60 samples of filter ash as well as the ash from active, currently filled and passive, previously filled cassetes of Kolubara“ power plant were studied.

Samples were subjected to a five-step sequential extraction, comparising of the following phases: distilled water, 1M ammonium acetate, 0.2 M ammonium oxalate/0.2 M oxalic acid, acidic solution of H_2O_2 and a 6 M solution of HCl. Concentrations of the elements in the extracts were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The distribution of metals in various fractions was investigated and discussed. Correlation analysis and two multivariate analysis methods (principal component analysis and cluster analysis) were carried out in order to provide better understanding of the nature of associations of trace elements with substrates.

The Certified Reference material CRM 684 (River Sediment) was used to provide accuracy of the instrument and analytical method.

The elements in filter ashes were grouped in inorganic associations except Ni and Ca, which are completely extracted in the last fraction and Mg was extracted in the first and fifth phase. Significant changes in diagenetic nature of ashes in the landfill were found on the basis of differences in the extracted concentrations and the results of correlation analysis of active and passive cassete.

MICROWAVE-ASSISTED PHOSPHORUS SEQUENTIONAL EXTRACTION USING BCR PROCEDURE

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Phosphorus, as essential nutrient, plays an important role in eutrophication of freshwater, one of the primary water pollution problems. Therefore, an important way to stop negative influence of phosphorus is the monitoring of phosphorus partitioning in sediments.

The Standards, Measurements and Testing procedure (formerly BCR) has been proved to be a useful tool for the study of phosphorus sequential extraction in freshwater sediments.

The SMT extraction protocol consists of determination five phosphorus fractions: non-apatite, apatite, inorganic, organic and total phosphorus, in three separated extraction steps applied to equal sample amounts. In order to accelerate the steps in determination of phosphorus forms, we have performed microwave extraction by using the microwave treatment. Phosphorus fractions were extracted from soil samples with different amounts of monoammonium-phosphate, Influence of different applied power and time of extraction were examined. Microwave extraction method for phosphorus determination provides similar results to the conventional procedure, but with significantly reduced treatment times. Also, using the reference material BCR-684 allowed validating the simplified microwave-assisted method.

DIAGNOSTIC OF CONTAMINATION BY HEAVY METALS IN MOLLUSCS FROM SÃO FRANCISCO DO CONDE, BAHIA, BRASIL

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Estuary environments are among the most ecologically productive in the coastal zones. However, with all of its environmental significance, it is still one of the most impacted ecosystems in the world. The main causes of degradation in the estuary environments are due to rapid and uncontrolled population growth in most coastal zones. Within the coastal environments, mangroves are the most vulnerable to metals contamination from urban and industrial sources. Mangrove supports a rich fauna, such as molluscs, fishes and shellfishes.

The objectives of this research is to determine metals (Cd, Cu, Hg, Ni, Pb, Zn) concentration in bivalves colected from 10 points in mangroves in the town of São Francisco do Conde, especifically in the town center and in the districts of São Bento and Muribeca.

An average of 120 specimens of each specie(mussels, clams and oysters) were collected. The sample were dried in the lyofilizador for 48 hour, then grounded with tungsten carbide container and spheres, and preserved in plastic container. For sample decomposition approximately, 0.5000g of sample and the certified reference (NIST 1566b) material were weighted.

A digester block were used in the sample preparation and 1.0 mL of HCl 37% and 1.0 mL of HNO_3 65% were added and coupled to “cold finger” and filled with water.

The system were allowed to react overnight at 50°C, then 3,0 mL of H_2O_2 30% `were added to the digestion tubes and the temperature gradually raised to approximately 140°C and reaction allowed to proceed for additional for 4 hours. All samples were analyzed for Cd, Cu, Ni, Pb and Zn in ICP OES.

The present study indicated that the concentration of Cd and Zn in the tissues were higher in the mussels when compared with clams. Oysters samples showed concentration of metals well above the level set by the Brazilian standards.

FAPESB

SOURCE APPORTIONMENT OF CARBONS AND HEAVY METALS IN SEDIMENTS

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The most of the metal industries and thermal power plants are running in the central India due to availability of the natural resourced materials i.e. minerals and coal. Sediments are sink for pollutants derived from various diffused sources. In the present work, contamination and sources of black carbon(BC), organic carbon(OC), carbonate carbon(CC), As, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb and Hg in the eight pond sediments of three industrial cities: Bhilai, Raipur and Korba of the central India are described. The sediments were collected in in the summer 2008. They were dried, crushed and sevied out of the particles of mesh size $\leq$ 0.1 mm. The sediments are alkaline in nature. The high content of BC, Fe and Mn was observed, ranged from 6.9 – 12.3, 2.9 – 8.1 and 0.12 – 0.32% with mean value of 8.7±1.2, 5.6±1.3 and 0.19±0.05%, respectively. The content of OC, CC, As, Cr, Co, Ni, Cu, Zn, Pb and Hg were ranged from 56 – 2015, 338 – 570, 8.6 – 28.2, 49 – 137, 27 – 65, 34 – 57, 36 – 99, 136 – 219, 55 – 148 and 0.02 – 0.26 mg kg^{-1} with mean value of 529±430, 458±62, 16.4±4.2, 97±21, 43±8, 45±6, 67±17, 173±20, 93±23 and 0.08±0.06 mg kg^{-1}, respectively. All species are correlated well with the BC content. The mobility, enrichment and fluxes of the carbons and metals are discussed. The sources of carbons and heavy metals in the sediments are apportioned by using cluster and factor analysis methods.

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DEVELOPMENT OF AN ELECTRO-THERMAL VAPORISATION INDUCITIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY - PROCEDURE FOR THE DETERMINATION OF PLATINUM GROUP ELEMENTS IN PLANTS

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In order to reduce noxious gas emissions of hydrocarbons, carbon monoxide and nitrogen oxides in exhaust fumes of modern vehicles, platinum group elements (PGEs) have been employed as catalysts in automobile catalytic converters in Europe since the late 1980s. Even though the air quality improved, concentration of PGEs in the environment increased significantly over time, caused by abrasion of the catalyst material during vehicle operation [1]. Despite PGEs being rather inert themselves, reactive and bio-available species arise by environmental transformations. This may lead to accumulation of PGEs in soil, sediments and plants, evoking concerns about their possible impact on the biosphere. Although elevated PGE levels were determined in road dusts and soils along heavily frequented roads [2], there is a lack of information on the availability of these metals to the biosphere [3]. The main reasons being difficulties in analysis, since relatively low PGE concentration levels in the presence of complex plant matrix have to be determined.

This contribution presents the development of an ETV-ICP-AES procedure for the direct analysis of PGE in *Brassica napus*. The plants were grown in a hydroponic setup containing incremental concentrations of Pt, Pd and Rh to study their respective uptake potential. Prior to ETV-ICP-AES analysis the dried and grinded plant samples were treated in a muffle furnace for two hours in an oxygen atmosphere to decompose organic material. For signal quantification three different strategies were investigated; namely external calibration with aqueous standard solutions, application of an internal standard and matrix-matched calibration. Compared to traditional approaches for analysis of PGEs in plants, the proposed ETV-procedure offers enhanced sensitivity, since the digestion step required for sample dissolution and the inherent dilution could be omitted. Furthermore the developed approach allows analysis of limited sample amounts which in some cases is due to restricted plant growth.

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POLAROGRAPHIC AND VOTLAMMETRIC DETERMINATION OF FOMESAFEN

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Fomesafen is a diphenyl ether light dependent peroxidising herbicide. It is used mainly in formulations for the protection of soybeans, beans and green peas against weeds[1]. It is a listed EPA group C carcinogen[1], compounds with similar structure exhibit toxicity toward aquatic organisms[2, 3], are endocrine disruptors and interfere with blood formation[4, 5]. Many methods have been developed for the determiantion of fomesafen, mainly HPLC with UV detection, but none used voltammetric determination on a silver solid amalgam electrode. Voltammetric techniques offer a cheap, reliable and sensitive way of determining important agrochemicals in various matrices[6, 7], this work is a continuation of many previous succesful efforts at monitoring electroactive polutants[8, 9].

The differential pulse voltammetric behaviour of fomesafen at a meniscus modified silver solid amalgam electrode has been studied in the pH range 2-12, the optimum conditions of the determination were in a mixture of BR buffer pH 8 – methanol 1:1. The behaviour has been investigated in the concentration range $1 \cdot 10^{-4}$ – $2 \cdot 10^{-6}$ $mol \cdot dm^{-3}$. The detection limit reached is $1 \cdot 10^{-6}$ $mol \cdot dm^{-3}$. The differential pulse voltammetric behaviour of fomesafen at a hanging mercury drop electrode has been studied in the pH range 2-12, the optimum conditions of the determination were in a mixture of BR buffer pH 2 – methanol 1:1. The behaviour has been investigated in the concentration range $1 \cdot 10^{-4}$ – $2 \cdot 10^{-6}$ $mol \cdot dm^{-3}$. The detection limit reached is $2 \cdot 10^{-6}$ $mol \cdot dm^{-3}$. The differential pulse polarographic behaviour of fomesafen has been studied in the pH range 2-12, the optimum conditions of the determination were in a mixture of BR buffer pH 2 – methanol 1:1. The behaviour has been investigated in the concentration range $1 \cdot 10^{-4}$ – $2 \cdot 10^{-6}$ $mol \cdot dm^{-3}$. The detection limit reached is $1 \cdot 10^{-6}$ $mol \cdot dm^{-3}$.

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LEAD IN NAIL AS ENVIRONMENTAL INDICATOR

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The search for biological indicators that designate Pb exposure has been one of the goals of various research groups. Nails, blood, urine and hair, for example, have been widely used for this purpose. Blood and urine tests are used to monitor contamination that occurred in a range that varies from days or weeks, while nails and hair show a history of contamination that occurred from months before the sampling. Nails and hair are materials collected so little invasive, and can be stocked for a long period than the other indicators. The aim of this work is to propose a method for Pb determination in nail by graphite durance atomic absorption spectrometry (GFAAS) allowing information about the environmental contamination. Hand and feet nails of forty adults (man and woman) were collected. Part of these nail samples (right hand and feet) were directly analysed without cleaning and other part (left hand and feet) were sequentially washed with acetone, water and 0.1 mol L^{-1} HCl and dried for 24 h (45^{o}C). Nails masses (0.5 to 34 mg) were digested in a Falcon® tube (15 mL) using 300 µL HNO_3 + 100 µL H_2O_2 and water-bath heating (90^{o}C for 60 min). With this sample preparation procedure it was possible to digest 60 samples simultaneously. Concentrations of lead varied from <40 ng g^{-1} (LOD) to 10 µ g^{-1} for washed samples and <40 ng g^{-1} (LOD) to 47 µ g^{-1} for non-washed samples. By using chemometrics tools (PCA and HCA) it was possible to eliminate the random results and conclude about contamination of the volunteers from environmental.

INTERPRETATION OF THE VARIABILITY OF PARTICULATE MATTER (PM) LEVELS IN THE EXTREMADURA REGION (SPAIN)

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The daily evolution of PM levels is regulated by the breeze circulation. PM levels are lower at the rural sites at night owing to the nocturnal drainage flows and to the lowering of the mixing layer height. These nocturnal low levels allowed us to estimate the continental background PM levels. At midday, the atmospheric pollutants accumulated are transported by the breeze, increasing PM levels (1). Furthermore, PM levels in the study area are also affected by the existence of a regional background, and local anthropogenic sources. So we aim here to demonstrate how PM levels are affected by these factors at different measurement stations in Extremadura region (South-Western Spain).
In the present study results on interpretation of the variability of PM (PM10 and PM2.5) levels in the Extremadura region are presented. Continental background, regional background and urban contribution are quantified in all the five stations present in this area. Through the analysis of the PM samples, the contribution of marine aerosol and African dust to the continental background is also quantified. Finally the temporal evolution of the continental and regional background is studied.

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COMPARISON OF TWO DERIVATIZING AGENTS FOR THE SIMULTANEOUS DETERMINATION OF ORGANOLEAD AND ORGANOMANGANESE COMPOUNDS BY GAS CHROMATOGRAPHY AND ATOMIC EMISSION DETECTION AFTER SOLID-PHASE MICROEXTRACTION

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The addition of lead additives to gasolines is banned in many countries, nevertheless in some parts of the world leaded gasolines are still used, the most popular additive being tetraethyllead (TeEtL). Tetraalkylead compounds can decompose to ionic forms persisting in the environment. Cyclopentadienylmanganese tricarbonyl (CMT) and (methylcyclopentadienyl)-manganese tricarbonyl (MMT) have been detected in gasolines as substitutes of organolead compounds, because of their lower toxicity. Two methods for the simultaneous determination of TeEtL, triethyllead (TEtL), trimethylead (TMeL), CMT and MMT in waters are proposed, both applying the combination SPME-GC-AED. The main difference between these methods is the derivatizing agent ($NaBPr_4$ and $NaBPh_4$) used to convert TEtL and TMeL into volatile compounds. The analytical procedures involved the addition of the derivatizing agent at a concentration of 0.05% (m/v) to 10 mL of water sample buffered to pH 4 and 7 for propylation and phenylation, respectively, and the reaction allowed to proceed for 5 min. The SPME enrichment was carried out by exposure of a 100 μm PDMS fiber to the sample headspace for 30 min at 80 ºC when using $NaBPr_4$ or during 15 min at 25 ºC for $NaBPh_4$. Sensitivities in the same order of magnitude were obtained for manganese compounds, detection limits in the range 0.05 – 0.15 μg L^{-1} being obtained with both methods. Nevertheless a markedly decrease of sensitivity was attained for lead compounds when using $NaBPh_4$. Water samples of different origin were analyzed and a marked matrix effect was attained in seawater matrices for lead compounds in all cases. To avoid the use of standard additions and since no significant differences were observed between different seawaters, one of these samples checked to be free of the target analytes was used for calibration purposes. Because of the propylation procedure showed best performance, it was extended to the analysis of soils, 0.25 g of sample being submitted to an extraction stage and then to the optimized procedure. Again calibration was carried out with a soil sample free of the organocompunds, because matrix effect appeared for lead compounds. Only CMT was detected in one of the seawaters analyzed at a mean concentration level of 0.18 ng L^{-1}.

IN SITU AQUEOUS DERIVATIZATION AND DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS BY SALTING-OUT-ASSISTED LIQUID-LIQUID EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Recent years have seen increased interest in the study of emerging pollutants in different environmental matrices. Within this group of compounds are pharmaceuticals, since their use is very broad in both animals and humans. Pharmaceutical residues have been detected in different environmental matrices.

Among pharmaceuticals, one group of particular interest are non-steroid anti-inflammatory compounds (NSAIDs). A new analytical method for the determination of trace levels of five NSAIDs: clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen in water samples is proposed. The analytical procedure involves *in situ* aqueous derivatization with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 2,2,2-trifluoroethylamine hydrochloride (TFEA) and salting-out liquid-liquid extraction (SALLE), followed by gas chromatography-programmed temperature vaporizer-mass spectrometry (GC-PTV-MS). The influence of several parameters on the efficiency of the derivatization (stirring time, reaction time, reagent concentration and pH), and the extraction (solvent, volume, salts and stirring time) and injection steps (liner, injection volume, liner temperature, injection time, venting time and venting flow) was investigated.

Quantification limits at low μg/L levels were achieved for all the compounds; enabling use of the method for the determination of NSAIDs in different water samples. Conversely to classic extraction strategies based on liquid-liquid extraction (LLE) or solid phase extraction (SPE), which require the concentration of large volumes of sample, only 2.5 mL of water is necessary to carry out the extraction. The volume of organic solvent (1.5 mL of ethyl acetate) is equally low.

The proposed method was successfully applied to the analysis of tap water, river and sea water and the influent and effluent water from a sewage treatment plant. The results obtained for real samples reveal the presence of ibuprofen and naproxen in the influent water of the sewage treatment plant. This sample, which was the most complex one, was also the only one in which a matrix effect was seen.

LIQUID CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-TANDEM MASS SPECTROMETRY OF SELECTED HUMAN STEROLS

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Human and animal waste is a significant contributor to deterioration of the water quality. As a consequence of incomplete removal of human sterols from urban effluents, they can be found in environmental samples. Human sterols can be used as indicators of sewage contamination and removal efficiency of sewage treatment plants. Gas chromatography–mass spectrometry (GC–MS) has been widely accepted as a reliable method for determination of sterols. However, derivatization step is necessary for thermally unstable sterols, because high GC temperatures can cause their degradation. Liquid chromatography–mass spectrometry (LC–MS) has become one of the most powerful techniques for analysis of compounds with wide polarity range, as well as for thermally unstable analytes in environmental samples. Moreover, tandem mass spectrometry (MS^2) is nowadays the key technique for determination and confirmation of analyte residues at trace levels, due to its selectivity and specificity. The standard ionization methods for LC-MS are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). APCI is more suitable for less polar and low molar mass analytes, as sterols. This study describes development of the analytical method for determination and reliable confirmation of five selected human sterols (cholestanone, cholesterol, α-cholestanol, β-cholestanol and epicoprostanol) at ng L^{-1} levels. The method was based on liquid chromatography–ion trap–tandem mass spectrometry with APCI. For each sterol, MS^n analysis was performed and precursor ions and fragmentation reactions, as the most sensitive transitions in selected reaction monitoring (SRM) mode, were selected for identification and quantification, as well as for confirmation purposes. APCI spectra of selected human sterols revealed that cholestanone and cholesterole can be distinguished by different SRM reactions, althought they have similar structures and the same molar mass. The stereoisomers (α-cholestanol, β-cholestanol and epicoprostanol) cannot be distinguished having the same MS^n fragmentations. However, it was determined that they can be chromatographically separated and successfully identified.

VOLATILE METHYL SILOXANES (VMS) AS CONTAMINANTS OF LANDFILL GAS AND BIOGAS FROM TREATMENT PLANTS. DETERMINATION AND QUANTIFICATION BY GC-MS.

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In this day and age, where great emphasis is placed on energy generation from renewable sources, combined with growing demand for and consumption of silicon (e.g. cosmetics, personal hygiene products and silicone oils), the problem of volatile methyl siloxanes (VMS) in biogas is becoming ever more important. The high energy potential of landfill gas (LFG) and biogas from waste water treatment plants (WWTPB) makes them an attractive renewable energy source, providing low-cost fuel for energy production.

Their main advantage is that they can be used to generate both electricity and heat in a cogeneration process, while at the same time reducing CO_2 emissions. Furthermore, the most popular and widely-used technology for LFG and WWTPB energy recovery is CHP plants.

There are currently a number of difficulties to be countered when using LFG and WWTPB; these are primarily related to the energy recovery equipment, biogas quality, technology used and the contaminant detection methods currently available. The presence of silicon compounds in raw biogas poses a serious problem for its economical use. Biogas containing VMS produces abrasive glass deposits during combustion in a motor. Being a thermo-electrical insulator, glass has a detrimental effect on the function of spark plugs and pistons. The interval between oil changes may be reduced to as little as 400 hours. Moreover, the presence of silica deposits significantly increases downtimes and shortens engine running times from 25,000 to 9,000 hours. VMS is one of the most important parameters for manufacturers and operators of biogas engines. In view of this problem, the measurement and control of VMS concentration is of vital importance for improving the efficiency of electricity generation and ensuring reliable complementary engine operation.

The best results are obtained by gas chromatography coupled with mass spectrometry (GC/MS). This combination offers a sensitive sampling technique. The sample investigated comes from Poland and Germany.

Methyl siloxanes from L_2 to D_6 were detected. VMS concentrations of up to 300 mg/m^3 were found.

Economic calculations, taking into account the costs of repairs, biogas analysis and frequent oil changes, indicate that a gas cleaning system is absolutely vital for raw biogas containing more than 10 mg $silicon/m^3$.

DETERMINATION OF TOTAL SULFUR CONCENTRATION IN COMPLEX ORGANIC MATRIX BY WD-XRF METHOD

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Results of an X-ray fluorescence determination of sulfur in organic materials are presented, obtained using a Spectroscan-V wavelength-dispersive spectrometer with the excitation of the fluorescence spectrum immediately in the solid samples under investigation.

It is shown that the amount of sulfur considerably varies not only in different types of waxes products, but also in different samples of the same product. An experimental evaluation of the metrological characteristics of the determination of sulfur in organic material products showed that they met the requirements of ASTM (American Society for Testing and Materials) D 2622 Standard test method for Sulfur in Petroleum Products by X-ray Spectrometry.

Spectroscan-V X-ray fluorescence spectrometer (Spectron-Optel Inc. Russia) equipped with a LiF (200), C (002), PET, KAP and ML analytical crystals were employed at voltage 40 kV and current of 1.5-4.0 mA. The method of Wavelength Dispersive X-ray fluorescence (WD-XRF) was used. Total sulfur was determined at analytical line S–K□ 5373 mÅ nm and the counting time 30 s. The calibration of the X-ray measurements was carried out with the external standard method using Di-n-Butyl Sulfide high purity grade standard, with a certified analysis, manufactured especially as a calibration material.

Optimum conditions will allow the direct determination of sulfur in essentially paraffinic samples at concentrations exceeding 0.0010 mass %.

WD-XRF analysis is universal and widely used with respect to different types of environmental and geochemical samples, such as soils, plants, atmospheric aerosol, waxes, water and alloys determined the traces of elements in organic samples using XRF and total reflection X-ray fluorescence.

The limit of detection (LOD) as presented in as: $m_{min} = 3\frac{I_B}{S} \cdot \frac{\sqrt{B}}{B}$

where: I_B is the background count rate, S is the sensitivity for elemental determination or the slope of the calibration curve.

It shows that the detection limit just depends on the ratio of the background and the signal count rates ($I_B/S = (I_B/I_0)m_0$), on the factor 3 which defines the confidence level or statistical criteria for the minimal intensity spectral line, and on the factor $\sqrt{B}/_B$, which is quite arbitrary and represents the relative standard deviation of the background under the spectral line.

ENVIRONMENTAL FRIENDLY ANALYSIS OF MICROPOLLUTANTS BY PRESSURIZED HOT WATER EXTRACTION – STIR BAR SORPTIVE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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This work presents the development, optimization and validation of a new method for the simultaneous determination of different pharmaceuticals (beta-blockers, lipid regulators, …) and personal care products (fragrances, UV filters, repellents, endocrine disruptors, phthalates, …) in both aqueous and solid environmental matrices. Target compounds were extracted from soils and sediments using pressurized hot water extraction (PHWE) followed by stir bar sorptive extraction (SBSE). The first stage was performed at 1500 psi during 3 static extraction cycles of 5 min each, testing the influence of extraction temperature (50-100ºC) and organic modifiers (methanol) in water, the extraction solvent. Later, aqueous extracts and water samples were processed using polydimethylsiloxane (PDMS) bars. Several parameters were optimized for this technique, such as extraction and desorption time, ionic strength, presence of organic modifiers and pH. Finally, target compounds were extracted from the bars by ultrasonic irradiation using a reduced amount of solvent (0.2 mL) prior to gas chromatography – mass spectrometry (GC-MS) detection. The optimized protocol showed acceptable recovery percentages (50-98%) for many of the analytes using a minimal amount of organic solvents compared to previous existing methodologies. It was then validated by measuring micropollutants in several groundwater samples from an aquifer located in Jerez de la Frontera (SW Spain).

Keywords: pharmaceuticals, personal care products, pressurized hot water extraction (PHWE), stir bar sorptive extraction (SBSE), gas chromatography – mass spectrometry

SORPTION ISOTHERMS OF SOIL IN COAL BASIN „KOLUBARA“

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The main goal of this thesis is to examine sorption characteristic of soil in landfills of slag, ash and other waste and to determine the necessity for improving protection of disposal sites and in which extent. We wanted to see if soil could adsorb metal ions and stop their further expand. Holes were drilled up to five meters depth, on sites determined for sampling, and samples were taken from every meter of depth. To determine soil sorption capability every sample was treated with standard solution of metal ion which concentration was a multiplied by maximum permissible concentration (MPC) of that element in water. We took a 20, 40 and 60 time higher concentrations and than amount of metal ion which was not adsorbed by the soil sample was measured i.e. concentration of metal in solution. Measurements were carried out by atomic absorption spectroscopy (AAS). Typical results for Ni, Cu and Zn are showing that adsorption is much better when concentration of these metals is low while for high concentrations maximum capacity for adsorption is reached. As and Cr have same ratio between free and adsorbed metal ion concentrations, hence soil is not good absorbent for As and Cr. In case of Cd and Pb, soil sample attaches several times higher concentration than solution does and therefore soil represents good adsorbent for these metal ions. Results that were obtained showing us that there is always certain amount of adsorption, but soil doesn't provide good protection from metal ions for underground water, especially on sites where underground water is on depth of one meter and additional actions for protection of underground water from pollution are essential.

ADSORPTION AND DESORPTION OF SELECTED ANTIBIOTICS ON RIVER SEDIMENTS

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Human and veterinary pharmaceutical compounds were recently raised great public attention as emerging contaminants in the aquatic environment. Because of their physical and chemical properties, many of them (or their bioactive metabolites) end up in sediments, where they can accumulate and induce adverse effects on aquatic organisms. That is the reason why the aim of this study was to investigated sorption-desorption behavior of some antibiotics on the Sava River sediments using laboratory batch systems. The selected compounds were ampicillin, amoxicillin (penicillin antibiotics), erythromycin (macrolide antibiotic) and doxycycline (tetracycline antibiotic). A preliminary sorption experiment was conducted to determine contact time required to attain sorption and desorption equilibrium. The contact time of 24 h was determined. The data obtained in the sorption-desorption experiments were well fitted with Freundlich model. The distribution coefficients varied with the tested pharmaceuticals. Ampicillin, amoxicillin and doxycycline were particularly strongly adsorbed while erythromycin was found to be weakly adsorbed. A sorption-desorption hysteresis effect was apparent for all compounds.

ANALYSIS OF SEVERAL HEAVY METALS IN WILD MUSHROOMS AND SOILS BY ENERGY DISPERSIVE X-RAY FLUORESCENCE AND ATOMIC ABSORPTION SPECTROSCOPY

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The aim of this study is to biomonitoring the polluted and non-polluted forest sites, from Dambovita County, Romania, on the large period, by using eight wild mushrooms, e.g. *Amanita sp*., *Hypholoma sp*. and *Boletus sp*. as bioaccumulator species for heavy metals. The principal objective of these researches is to achieve of complete data base concerning the pollution level of sixteen forest soils with heavy metals which affected directly the health. The Cr, Pb, Cd, Mn, Fe, Ni, Cu and Zn concentrations from fruiting body (cap and stipe) of mushrooms species and their substrate were determined using Energy Dispersive X-ray Fluorescence (EDXRF) and Flame Atomic Absorption Spectroscopy (FAAS). The validation methods of microwave digestion, FAAS and EDXRF, are important for the final results of this research. Periodic testing of standard solutions to verify of reliability of the measuring apparatus is performed. Accuracy has been checked using quality control test for fungi and their substrate to show the degree of agreement between the standard values and measured values, the difference was less than 5%. The coefficient of bioaccumulation of heavy metals has been calculated, as well. The iso-concentration curves of heavy metals in samples of mushrooms species and soil were realized with Surfer 9 Model.

PRECIPITATION OF CARBON AND IONS WITH RAIN

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Dissolved organic carbon (DOC) and nutrients (i.e. ions, trace metals, etc.) have a great role in the ecosystem dynamics and serve as a primary food sources for plant kingdom and aquatic webs. The DOC forms water-soluble complexes with trace metals, which can be transported and taken up by plants and organisms. In this work, the content, fluxes and variability of organic carbon(OC) and ions (i.e. Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, NH_4^+, Mg^{2+} and Ca^{2+}) precipitated with the rain during year 2008 in the Raipur city, India (21° 8′ 24″ N and 81° 22′ 48″ E) is described. The rain samples of Raipur city on event basis at resedential and industrial sites were collected during period: July – Oct., 2009. The organic carbon(OC) and ions were analysed by the thermal and ion chromatograhic method, respectively. The volume weighted mean(VWM) concentration of OC, Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, NH_4^+, Mg^{2+} and Ca^{2+} in the residential site was ranged (n = 15) from 49 – 293, 0.12 – 1.56, 0.31 – 4.89, 0.94 – 11.05, 0.06 – 0.70, 0.13 – 1.51, 0.05 – 0.50, 0.05 – 0.72 and 0.57 – 12.15 mg l^{-1} with average value of 260, 0.29, 0.56, 1.32, 0.15, 0.34, 0.10, 0.10 and 0.91 mg l^{-1}, respectively. The effect of meteorology i.e. temperature, humidity, acidity, wind speed and wind direction on precipitation of carbons is discussed. The correlation of the OC with the anions and cations are reported. The fluxes of OC and ions precipitated with the rain in Raipur city is discussed.

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SOURCE APPORTIONMENT OF CARBONS ANDTRACE ELEMENTS IN URBAN SOIL OF INDIA

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The urban soil quality is changing due to enormous anthropogenic activities in Asian countries. In the central India, several metal industries and coal based thermal power plants are running. In the present investigation, sources of black carbon (BC), organic carbon(OC), carbonate carbon (CC), Al, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Ba and Pb are apportioned using cluster and factor analysis in the urban soil of the most industrilized city, Raipur, India (21° 8′ 24″ N and 81° 22′ 48″ E). The surface soil (0-10 cm) samples were collected in March, 2010 from 17 sketches of Raipur city. The samples were crusded, dried and seived out of particles of mesh size ≤ 0.1 mm. The content of carbons and elements were analyzed by the thermal and TXRF methods, respectively. Significantly high contents of BC, Al, K, Ca, Ti and Fe were detected. The content of BC, Al, K, Ca, Ti and Fe was ranged from 0.98 – 19.32, 0.25 – 1.46, 0.32 – 0.78, 0.28 – 0.74, 0.24 – 0.48 and 2.83 – 5.73% with mean value of 5.52±1.90, 0.86±0.13, 0.49±0.07, 0.46±0.06, 0.32±0.03 and 3.78±0.36%, respectively. While, OC, CC, V, Cr, Mn, Ni, Cu, Zn, Sr, Ba and Pb content was ranged from 66 – 372, 51 – 310, 41 – 75, 75 – 141, 602 – 1620, 25 – 49, 23 – 38, 30 -100, 25 – 73, 285 – 527 and 31 – 90 mg kg^{-1} with mean value of 183±40, 128±33, 56±5, 99±9, 1034±151, 35±3, 31±2, 50±7, 41±6, 403±29 and 54±8 mg kg^{-1}, respectvely. Their contents are well correlated with the BC content. Their spatial variations, enrichment and sources are discussed.

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SEMI-SPECIFIC BIOSENSORS FOR BIOCHEMICAL OXYGEN DEMAND ANALYSIS IN PULP- AND PAPER INDUSTRY WASTEWATER

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Biochemical oxygen demand (BOD_7) is widely used universal parameter to describe organic pollution in water. Compared to BOD_7, a faster method is based on BOD biosensors. Unfortunately biosensors fail to estimate BOD resulting from specific refractory compounds found in industrial wastewaters. More precise measurements can be carried out using semi-specific microbial sensors. Semi-specific microorganisms can use certain compounds or compound groups as an energy source in addition to universal substrate spectrum. Thereby, semi-specific microorganisms are able to oxidize refractory compounds found in industrial wastewaters, that would be undetected by universal biosensors.

As the pulp and paper industry wastewater consists in a large proportion of cellulose fibers (40-60% of dry solids), the cellulose–degrading bacteria of *Bacillus subtilis* (isolated from decaying sawdust) and *Paenibacillus sp.* (isolated from rabbits manure) were used to construct semi-specific biosensors. The biosensors were calibrated with OECD synthetic wastewater and measurements with different wastewaters were conducted.

Response time of biosensors using steady state method was 20-25 minutes and service life was 96 days. Detection limit was 5 mg/l of BOD_7 while linear ranges extended up to 55 mg/l and 50 mg/l of BOD_7 for *B. subtilis* and *Paenibacillus sp.* based biosensors, respectively. Repeatability and reproducibility of both biosensors were within the limits set by APHA. BOD measurements carried out in cellulose-spiked OECD synthetic wastewater showed the biosensor's suitability for measurements in wastewater containing cellulose. Semi-specific biosensors were able to detect cellulose added to the OECD synthetic wastewater and good correlation was acieved between sensor-BOD and BOD_7. In comparison, both biosensors overestimated the BOD_7 values in paper mill wastewaters and underestimated BOD_7 in aspen pulp mill wastewater. The semi-specific biosensors are suitable for the estimation of organic pollution derived from cellulose, while the detection of pollution derived from tannins and lignins was minor.

SPECIATION OF METALS BY CONVENTIONAL, MICROWAVE AND ULTRASOUND ASSISTED SEQUENTIAL PROCEDURE IN SEDIMENTS FROM THE INDUSTRIAL AREA AND SURROUNDINGS OF PANČEVO, SERBIA

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Sequential extraction (the Community Bureau of Reference (BCR)), has been applied for the metals partitioning in surface and buried sediments in the vicinity of the petrochemical complex. The results obtained by conventional sequential extraction BCR method for Cd, Cu, Cr, Ni, Pb and Zn were compared with two alternatives: microwave and ultrasound assisted sequential extractions using identical operating conditions that were applied in each individual BCR step and analyzed by ICP/OES. Steps 1–3 of the sequential extraction (excluding the hydrogen peroxide digestion in step 3) were completed for 16 h in the conventional and for 120s within 90W of power in the microwave assisted sequential extraction and with 10 minutes in ultrasound bath. Digestion with the hydrogen peroxide for the digestion of organic matter was performed equally for all techniques, by heating the sediments two times for 1 hour with 10 ml of hydrogen peroxides on water bath, and the obtained residue were extracted by ammonium acetate at pH 2 for 16 h, with microwaves and ultrasound waves. The precision and accuracy of the proposed procedures were evaluated by using a certified reference material BCR 701. The acceptable accuracy for the most of metals was observed for all three steps of BCR protocol at 16 h total shaking period. After the first step with ultrasound waves Cd, Ni and Zn showed the acceptable accuracy, while lower accuracy were obtained for the metals extracted by microwaves. After the second steps lower accuracy were obtained after the microwave and ultrasound extraction of certificate material. This lower accuracy induced the significantly higher extracted values of the metals in the third step of the extraction by microwaves in the certificate material while for the extraction with ultrasound waves Cu, Ni, Pb and Zn showed the acceptable accuracy.

HPLC-DAD-MS/MS PHOTODEGRADATION STUDY OF SOLFONYLUREIC PESTICIDES IN WATER

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The environmental interest of sulfonylurea herbicides derives from the possibility of diffusion and penetration of these herbicides in the deepest layers of ground, in particular in sandy or clay-poor soils, up to reach ground waters; another interest regards the study of their natural degradation pathway that can bring to the formation of new species potentially more toxic and stable than the precursor herbicides. In this case a lower persistence in the environment unfortunately does not correspond to a lower toxicity: from hence the importance of the identification of the species that can potentially form.

Here, nicosulfuron and amidosulforon, typical sulfonylurea herbicides, are considered in order to outline the environmental fate of the molecules generating from the simulation of one of the natural processes that can occur, i.e. photoinduced degradation.

Aqueous pesticides solutions underwent a simulated sun irradiation: the new species formed during the degradation process were identified by HPLC-DAD-MS/MS and degradation pathways were proposed. The effect of temperature and the contribution of the hydrolysis were also evaluated.

Only by the use of chromatography hyphenated with ESI in both positive (PI) and negative ion mode (NI) and APCI in PI mode permits to obtain integrated information about the transformation products that can form: a study of the total ion chromatograms followed by the extraction of the SIM chromatograms of the most intense m/z signals have made possible the identification of five possible photodegradation transformation products.

EN88 **Session C**

FIELD STUDY OF ALKYL SULFATES IN AGRICULTURAL SOILS BY LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY

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Alkyl Sulfates (AS) are ionic surfactants1 that find widespread use in industrial, household cleaning products (laundry detergents, hand diswashing liquids and various hard surface cleaners), personal care products, etc. In spite of their great use, these compounds have received less attention and only a few data are available about the presence of residual AS in environmental samples2. We focused our attention in the linear-type alcohols sulfates. Such alcohols contain even carbon numbered alkyl chains only. The aim of this work is to study the role of the alkyl chain length in their adsorption of the sodium alkyl sulfates homologues in soils. This study was carried out for the seasons: Summer, Autumn, Winter and Spring, to evaluate their adsorption in agricultural soils. In this work the extraction of AS from the soil is carried out by Pressurized liquid extraction (PLE) with methanol followed by direct identification and quantification by means of reversedphase Liquid Chromatography - Mass Spectrometry (LC-MS/MS) with electrospray ionization (ESI) operating in negative mode. Validation parameters such as linear dynamic range detection and quantification limits, interday and intra-day repeatability and accuracy were established.

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DETERMINATION OF CHEMICAL CONSTITUENTS OF TROPICAL PEAT BY OFF-LINE PYROLYSIS-GAS CHROMATOGRAPHY/MASS SPECTROMETRY (PY-GC/MS)

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The analysis of chemical constituents provides essential information on materials derived from many origins, including a wide range of natural and synthetic substances. In the specific case of the humified organic matter present in peat, knowledge of its chemical structure can reveal the chemical and biochemical changes that occur as a result of both the decomposition environment and the nature of the original organic material. The objective of this work was to use off-line pyrolysis followed by gas chromatography coupled with mass spectrometry (PY-GC/MS) to determine the chemical constituents of peat soils. The use of high temperature and an oxygen-free atmosphere promotes the thermal degradation of macromolecules into smaller fragments, which can then be related to the composition of the original material. A peat sample from the Santo Amaro peatland (Sergipe State, Brazil) was pyrolyzed at 500 ^{o}C and found to contain three major chemical classes. Monoaromatic compounds such as benzene, toluene and xylenes were identified at retention times of 3.5-7.5 minutes, *n*-alkenes and *n*-alkanes at 7.5-28 minutes, and pentacyclic triterpene compounds at 28-38 minutes. The presence of the last class was indicative of the mainly plant origin of the peat, which was also supported by the greater proportion of triterpenes (67.9 %) relative to aliphatic and aromatic compounds (28.6 % and 3.55 %, respectively). The compounds identified in the pyrogram obtained using *in natura* peat were similar to those found for humin (the most refractory humic peat fraction, that is insoluble at any pH), indicating that the composition of the material was dominated by humin rather than by humic acids (the humic fraction that is insoluble at pH<2). This work demonstrates that off-line pyrolysis GC/MS is a valuable tool that can be used to investigate the composition and origin of peat.

APPLICATION OF LIGANDS BASED ON 3-ACETYL-6-METHYL-3,4-DIHYDRO-2*H*-2,4-PYRANDIONE IN ANALYTICAL CHEMISTRY: DETERMINATION OF BIOLOGICALLY ACTIVE ELEMENTS

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Transport of elements from place to place in soils, from water to soil using ligands on their way is of great importance. Ligands have an important role in mobility of elements. An element with ligand is able to create a complex and it can be mobile in environment. Many of microelements and toxic metals form bounds with some ligands in natural habitat. There is important use of some type of ligands for determination of elements from matrices as water or soils.

Recently, miniaturization of analytical techniques has been provided. There are new instrumentalmethods and also new extraction procedures are being developed. Conventional liquid-liquid extraction consuming a lot of organic solvent is being replaced by microextraction techniques as dispersive liquid-liquid microextraction [1]. These techniques use only microliter volumes of organic solvent which thus reduces the amounts of hazardous chemical waste .

Ligands based on 3-acetyl-6-methyl-3,4-dihydro-2*H*-2,4-pyrandione for determination of some biologically active elements have been studied. For the first time, 3-acetyl-6-methyl-3,4-dihydro-2*H*-2,4-pyrandione (L), was synthesized by Geuther in 1865 [2]. There are many articles written about synthesis of L as well as of some derivatives of L, written in [2,3,4]. However, there have been no articles found in literature about using 3-[*(E)*-3-(2-methoxyphenyl)-2-propenoyl]-6-methyl-3,4-dihydro-2*H*-2,4-pyrandione as ligand for determination of elements.

In this work, solubility of the 3-acetyl-6-methyl-3,4-dihydro-2*H*-2,4-pyrandione ligand and its derivatives in water and in organic solvents and application of ligands for determination of several biologically active elements were investigated as well as novel and simple methods for determination of some analytes using ligands and the microextraction technique mentioned will be presented.

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HEAVY METALS DETERMINATION IN BIOTIC AND ABIOTIC MATRICES FROM MINING AND INDUSTRIAL AREAS OF SOUTH-WESTERN SARDINIA

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The distribution of Cd, Co, Cr, Cu, Mn, Ni and Pb in selected biotic and abiotic hive compartments (i.e. bees, pollens, honeys, leaves and soils) has been evaluated by means a microwave-aided acid digestion and ETAAS measurements. Five apiaries have been placed in selected industrial and abandoned mining areas of South-Western Sardinia, whereas an additional apiary (the blank) has been placed in a neighbour site, not subject to any pollution phenomena. Sampling was accomplished on monthly basis (from March to November) for three consecutive years, in this period 659 samples were hence collected and analyzed. All analytical methods have been optimized and validated in terms of sensitivity, precision, and bias. The whole dataset has been evaluated by chemometrics (PCA, PLS2) and other statistical tools to obtain informations about correlations between variables as metals, matrices, sampling sites and periods.

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FOLLOW-UP OF Be-7, K-40, Cs-137 AND Ra-226 SPECIFIC ACTIVITY IN ENVIROMENTAL SAMPLES IN THE AREA OF BELGRADE IN THE 2009 TO 2011 PERIOD

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The gamma spectrometry analyses of air and soil samples from the area of the City of Belgrade, in the 2009 to 2011 period, are presented in this paper. Monthly average values of Be-7, K-40 and Cs-137 activity in the air are included. The values go from $0.03*10^{-3}$ Bq/m^3 in November 2010 to $11.2*10^{-3}$ Bq/m^3 in March 2009 for Be-7 activity, $0.2*10^{-4}$ Bq/m^3 in November 2010 to $4*10^{-4}$ Bq/m^3 in the spring of 2009 for K-40 activity, and $0.2*10^{-5}$ Bq/m^3 in January 2011 to $40*10^{-5}$ Bq/m^3 in June 2009 for Cs-137 activity. The values of annual effective dose which entered the human organism by breathing were calculated and included.

Additionally, analysis of clay-containing sandstone, clay and red clay samples as well as diatomaceous soil and calcified sandstone samples was performed. In 2009 values were from $1.33*10^{-2}$ to $4.74*10^{-1}$ Bq/kg, $9.25*10^{-2}$ to $1*10^{-1}$ Bq/kg, 3.85 to 5.74 $*10^{2}$ Bq/kg and from 1.61 to 99.4 Bq/kg, for Be-7, Cs-137, K-40 and Ra-226 activity, respectevly. In 2010 values were from $9.62*10^{-1}$ to 1.10 Bq/kg, $8.89*10^{-2}$ to $4.63*10^{-1}$ Bq/kg, 6.01 to $6.06*10^{2}$ Bq/kg, and from 7.51 to 14.8 Bq/kg, for Be-7, Cs-137, K-40 and Ra-226, activity respectevly. Obtained values are concordant with the published values characteristic for the City of Belgrade and the surrounding area. Measured radionuclide values are acceptable from standpoint of radiation load of population.

keywords: gamma spectrometry, air, soil, Be-7, K-40, Cs-137 and Ra-226

PRELIMINARY RESEARCH OF ORGANIC AND ELEMENTAL CARBON IN SUSPENDED PARTICLES IN THE AMBIENT AIR AT THE TERRITORY OF BELGRADE

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Determination of organic and elemental carbon is carried out in order to examine their impact on air quality. This paper presents the initial results of organic and elemental carbon in the location of Lazarevac, Grabovac and Slavija (teritory of Belgrade). The results of these measurements were aimed to determine directions of the future research. In order to determine organic and elemental carbon we used thermal/optical analysis of suspended particles up to 10 microns on filter paper. Samples were collected on quartz filter papers with diameter of 47mm using Sven Leckel SEQ47/50 sampler. It was found that the values of organic carbon in location of Lazarevac and Grabovac during summer months (August and September) were in the range of 7.2 to 76.0 μg/cm^2, and the values of elemental carbon were in the range of 1.2 to 16.0 μg/cm^2. In the winter months (November and December) measurements were performed in the same locations as well as at the Slavija location and it was found out that the values of organic carbon were in the range of 2.9 to 397.4 μg/cm^2, while the values of elemental carbon were in the range of 2.9 to 46.7 μg/cm^2. All these results imply that further investigation of the causes (pressure, temperature, humidity, wind speed, wind direction, etc.) that lead to changes in content both of organic and elemental carbon in ambient air at the investigated locations are necessary.

AEROSOL PHYSICAL AND CHEMICAL PROPERTIES IN A BIOMASS BURNING REGION: ANY EFFECT ON CLOUD DROPLET SIZE

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Human activities are changing the physical and chemical characteristics of atmospheric aerosols, which in turn is likely to affect cloud formation and consequently precipitation patterns. Any modification of the hydrological cycle would impact on the use of water resources for agricultural, industrial and domestic purposes, as well as for electricity generation. The goal of this study was therefore to investigate the influence of biomass burning on the regional atmosphere. We investigated the relationships between aerosol physical properties (number concentration and size distribution) and chemical composition in the municipality of Araraquara (São Paulo State, Brazil), which is a sugar cane burning region. Aerosols were collected during daytime and nighttime periods using a low-volume sampler. Particles (0.3-10 μm) were detected in six size fractions using an optical particle counter. From the database acquired it was possible to observe that in August 2010 the mean concentration of potassium (K^+, which is a marker for material derived from biomass burning) was nearly 65% lower than the mean concentration measured several years earlier (in August 2003). Interestingly, the numbers of fire foci in São Paulo State detected by satellite-borne sensors decreased by only 10% over the same period, despite the implementation of increasingly strict legislation designed to progressively eliminate sugar cane burning. However, exceptionally dry conditions in 2010 resulted in numerous outbreaks of accidental fires that were potentially detectable by satellite sensors. The data acquired will be analyzed together with cloud droplet size (as effective radius, r_e, obtained from NASA´s Terra and Aqua satellite overpasses of the region), in order to better understand the influence of lower tropospheric aerosols on the mechanisms of formation of cloud condensation nuclei.

PHOTODEGRADATION OF FLUOROQUINOLONES: BYPRODUCTS DETERMINATION IN SOIL AND STUDY OF THEIR ANTIBIOTIC ACTIVITY

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Pharmaceuticals and their potential toxic effects represent an emerging research area in environmental analytical chemistry. Fluoroquinolones (FQs) antibiotics are considered pseudo-persistant pollutants [1]. Their diffusion in natural ecosystems [2,3] poses the question of bacterial resistance. Little information is still available on the potential ecotoxicity and bacterial resistance of their photoproducts. Indeed, FQs are liable to photodegradation in aqueous systems [4-6].
In this research, the photodegradation in soil of six widely employed FQs, namely Ciprofloxacin (CIP), Danofloxacin (DAN), Enrofloxacin (ENR), Levofloxacin (LEV), Marbofloxacin (MAR) and Moxifloxacin (MOX), was studied. The degradation profiles have been obtained by analysis of different samples, enriched at environmental concentrations (0.5 $\mu g\ g^{-1}$), after exposure to natural sunlight for prefixed times. Soil samples were extracted according to the microwave-assisted extraction (MAE) procedure recently optimized in our laboratory [7], and then analyzed by HPLC-FD. Photodecomposition of the parent compounds gave rise to different products, identified by HPLC-ESI-MS. The results have been compared to those obtained by aqueous photolysis. The potential antibacterial activity of FQs byproducts was investigated on several well characterized bacterial strains, by testing FQs solutions before and after irradiation, and at the same time not-irradiated aliquotes containing the same amount of residue antibiotic as the irradiated solutions. The minimum inhibitory concentration (MIC) test proved that the photoproducts themeselves possess antimicrobial activity, especially in the case of ENR and DAN. For this reason, the previously cited MAE was evaluated for the determination in soil of their main photoproducts, separately produced by irradiation of antibiotic films and used for spikes. Satisfying recovery was gained (75-120%, n=4), with good reproducibility (RSD<15%).

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COMPOSITE POLYMER MEMBRANES FOR CARBON DIOXIDE SEPARATION

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The main task of the work is to construct the polymeric membrane that could be used for the waste gases treatment. For this purpose, membrane must have high permeability for the carbon dioxide and low permeability of the other gases commonly present in waste gases (hydrogen, oxygen, nitrogen and methane). The constructed membranes were of a dense type, based on a solubility/diffusivity mechanism. In this paper, feasibility of the application of poly(ethylene glycol)-copoly (butyl terephtalate) (random copolymer with number average molar mass 1500 g/mol and 77 mass % of polyethylene glycol), and poly(ethyleneoxid)-copoly(phtalamide). The commercial names of those polymers are polyactive and PEBAX 1657 respectively. In order to enchase the permeability of carbon dioxide, four different zeolytes were added, and in order to improve mechanical stability two different additives were tested. Three zeolytes were with the 3-dimensional pores (ZSM5 – MFI, pores 0,52x0,55 nm; Faujasite – FAU, pores 0,74x0,74 nm and Linde type A – LTA, pores 0,41x0,41 nm) and one was with the 1-dimensional pores (Linde type L – LTL, pores 0,41x0,41 nm). As an additive, n-tetradecyldimethylamonium bromide – n-C14TMABr was tested. The aim of an additive was to provide good wetting of a highly electrically charged zeolyte particle by the hydrophobic polymer chains. The other examined additive was dimethylaminopyridine (DMAP) which should improve the solubility of carbon dioxide due to its alkali properties. Beside those two additives, LTA zeolyte particles were surface-treated during the production procedure, and this type of zeolyte was tested as well. The best results in carbon dioxide/hydrogen selectivity and permeability were obtained with the membrane constructed with PEBAX 1657 and surface treated zeolyte. The obtained permeability of carbon dioxide was 148 Barrer, and the carbon dioxide/hydrogen selectivity was 11,8.

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ULTRASOUND-ASSISTED EXTRACTION FOR DETERMINATION OF MAJOR AND TRACE ELEMENTS IN SOIL SAMPLE

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The ultrasound-assisted extraction (UAE) was evaluated as more effective and accurate alternative to conventional methods for major and trace metal extractions. The extractions of major and trace metals from soil sample in deionized water was conducted by means of rotary mixer and ultrasonic bath. The UAEs were performed with sonication time of 10, 20, 30, 40 and 50 minutes. In order to assess the metal amounts associated with different soil phases, the sequential extraction according the BCR (Community Bureau of Reference of the European Commission) scheme was undertaken. Thus obtained soil extracts were subjected to the simultaneous multielement analysis by use of ICP-OES. For most of the examined elements considerable difference in the amounts extracted by use of rotary mixer and ultrasonic bath was noticed. The rotary assisted extraction was more efficient in the case of alkaline-earth elements (Ca, Mg). The UAE was more efficient in the case of matrix elements (Fe, Al and Mn), but for both, major and trace metals increase of the sonication time failed to improve extraction yields. The extracted amounts of element species varied with the extraction time increase, what was the most noticeable in the case of matrix elements (Fe, Al and Mn) and some trace elements associated with amorphous and crystaline Fe, Al oxides (Cr and Cu). For the UAE, the changes of the conductivity, pH value, redox potential, colloid particle size diameter and zeta potential of colloid particles, with the extraction time increase were continuously measured. The influence of ultrasonic energy on the soil sample preparation has been found to be significant, not only for possible decrease of the extraction time, but mainly, due to the expressed selectivity for metal species, observed through big differences in extracted amounts of Fe, Al and Mn ions by use of deionized water only.

COMPARISON OF CONVENTIONAL, ULTRASOUND AND MICROWAVE ASSISTED SINGLE EXTRACTION METHODS FOR ANION DETERMINATION IN SOIL SAMPLE

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The ultrasound assisted extractions (UAE) and microwave assisted extractions (MAE) of anions from a soil sample, type Ranker over serpentinite, were undertaken and results were compared with those obtained by conventional shaking. The extraction suspensions were prepared by adding 2g of soil sample into a 20 mL of deionized water. The extractions were conducted by use of: ultrasonic bath with extraction time of 10, 20, 30, 40 and 50 min; microwave digestion system with extraction temperatures of 50, 100 and 150 °C; and rotary mixer with extraction time of 22 hours. Extractable contents of NO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-} and F^- in thus obtained soil extracts were measured by Ion chromatography. During the ultrasonic treatment, redox potential, particle size diameter, zeta potential of colloid particles, conductivity and pH were continuously measured. The results have shown that MAEs are characterized by increased extraction yields and decreased time, while the average extracted amounts of anions achieved by use of ultrasound are comparable to those achieved by mechanical shaking. However, for UAEs no correlation between extracted amounts of anions and extraction time is observed, except in case of Cl^-. The particle size diameter analysis showed that increasing sonication time causes a decrease in mean particle size and zeta potential of colloid particles has ranged from -49.54 to -11.08 mV. Although it is less time consuming, the UAE is less reliable extraction method compared to conventional shaking, due to the interaction of ultrasonic energy with the aqueous soil suspension which is, alternately, influencing the processes of anion adsorption and desorption, and thus leading to change of extracted amount of anions as function of sonication time.

ENERGY DISPERSIVE X-RAY EMISSION FOR HEAVY METALS ANALYSIS OF SOIL SAMPLES

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In Energy Dispersive X-ray Fluorescence (EDXRF) practice, the measured X-ray characteristic for an element must be corrected for self-absorption and particle size of the samples. In this work, pellets with different aerial densities and different size of particles were prepared from CRM NIST 2710a and analyzed using an ElvaX spectrometer to asses the optimal aerial density of soil samples and the optimal size of soil particles. The experimental results indicate that the optimal aerial densities of the soil samples ranges between 0.2-0.3 g/cm2 and the size of soil particle must be less than 1 mm.

After determining the optimal parameters of soil samples the study was focused on distribution of some heavy metals (Mn, Fe, Ni, Cu, Zn and Pb) in the soils of Targoviste city (Romania), elements which are above natural levels in the soils of urban areas as a result of anthropic activities. The content in the investigated soils of Mn, Fe, Ni, Cu, Zn and Pb shows variations within quite large limits: 232.5-1474.2, 5370.1-42750.6, 18.2-127.3, 14.7-189.5, 92.7- 574.1 and 15.4-74.8 mg/kg. The heavy metals distribution maps were realized using Surfer software.

AMBIENT VOC$_S$ MEASUREMENT IN BELGRADE SEMI-URBAN AREA: WINTER CASE STUDY

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In order to assess the ambient levels and possible origin of volatile organic compounds (VOCs), concentrations of thirty one compounds were measured on-line, using Proton Transfer Reaction Mass Spectrometer (PTR-MS) in the semi-urban area of Belgrade. Measurements were conducted during the winter episode 2010. One-hour mean values were calculated and used for further analysis. UNMIX and Positive Matrix Factorization (PMF) receptor models were used to identify the sources of VOCs. Four common sources were reconstructed from both models and were identified as: biomass/biofuel burning, industrial solvents and painting, vehicle emissions and local solvent use. The transport pathways were investigated based on 48-hour backward trajectories of air masses computed by the HYSPLIT model. Hybrid receptor models, potential source contribution function (PSCF) and concentration weighted trajectory (CWT), were used for identification of source regions. Derived maps denoted that the sampling site was under the influence of several possible source regions, primarily from neighboring countries.

SPATIAL DISTRIBUTION AND REMOVAL MECHANISAMS OF BENZENE, TOLUENE, ETILBENYENE AND XYLENES CONCENTRATIONS IN AMBIENT AIR IN BELGRADE URBAN AREA

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Benzene, toluene, ethylbenzene, o-xylene and (m + p)-xylene (BTEX) are volatile mono-aromatic hydrocarbons which pose a serious risk for public health. BTEX are components of motor vehicle fuel, therefore the main source of BTEX in urban areas is combustion emissions from automobile exhaust. In the atmosphere BTEX participate in photochemical reactions and produce secondary air pollutants such as ground-level ozone, peroxyacethyl nitrate, free radicals and nitrogen oxides. Because of this BTEX compounds are regularly monitored in air monitoring network established by the Institute of Public Health of Belgrade. The network consists of 5 monitoring sites covering the urban area of Belgrade. Concentrations of BTEX are determined either continuously *in-situ* by automatic monitors or discontinuously with adsorption tubes. Discontinuously collected samples are prepared by solvent or thermal desorption in laboratory accompanied by GC-MS or GC-FID analysis, depending on type of desorption. Results obtained from monitoring network enable us to determine spatial distribution of BTEX compounds in city urban area and to identify particular removal mechanisms of BTEX compounds from atmosphere. The higher concentrations of BTEX are correlated with the higher traffic intensities, low wind speed and cloudy weather without precipitation, while lower concentrations coincide with clear skies or rainy days.

DISSIPATION OF ACETOCHLOR AND FLUROCHLORIDONE IN SOIL

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Acetochlor and flurochloridone are selective herbicides applied to soil. They are adsorbed mainly by the shoots, stems or roots of germinating plants and are used pre-, or early post-emergence to control annual weeds in several crops.

Field efficacy and dissipation studies with both herbicides applied as a mixture, were carried out over two growing seasons to make estimates of their persistence.

Eight experimental plots (25 m^2) were sprayed with commercial EC formulations of acetochlor (Relay, 900 g/l a.s.), and flurochloridone (Racer, 200 g/l a.s.). The application rates corresponded to 3.6 kg a.s./ha of acetochlor and 1.0 kg a.s./ha flurochloridone. Following application, soil samples in 5 cm increments (4 per plot/sampling time) were collected the same day after treatment (DAT-0) to determine the initial deposit and then five times over a period of 120 days.

Laboratory dissipation studies were carried out at room temperature simulating similar application levels and sampling times as the ones in the field.

The parent compounds were analysed by GC/NPD and GC/ECD folowing ASE extraction of the soil sample (cca 20 g), with methanol, or ethyl acetate. Good recoveries (>90 %, CV≤ 5%, n=9) were achieved even at the lowest spiking level (5 µg/kg).

The ratio of measured soil residues of acetochlor and flurochloridone at DAT-0 was > than 4 : 1, as expected according to the application rates of Relay and Racer EC formulations.

Assuming a first-order dissipation rate, the calculated field DT-50 values of acetochlor were 21-33 days, and the corresponding values for fluorochloridone were 50-54 days.

For acetochlor, the range of the DT-50 value was longer than usually reported (8-18 days), whilst that for fluorochloridone was within the reported range (9-70 days).

Estimates of dissipation rates of active substances (a.s.) in soil expressed as DT-50 are only rough indicators of field persistence.

DETERMINATION OF DIFFERENT STEROID HORMONES BY IN-TUBE SOLID PHASE MICROEXTRACTION AND LIQUID CHROMATOGRAPHY IN WASTEWATERS

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Endocrine disrupting chemicals (EDCs) in the environment have received a worldwide attention in recent years. Natural and synthetic steroid hormones have been regarded as the most important members of EDCs which could cause adverse effects on aquatic organism at the low ng/L level [1]. For example, the natural sex hormone estradiol, which has a high endocrine potential, its metabolites (estrone and estriol) and conjugates like glucuronides are mainly excreted by mammals. All of them are constantly discharged into environment due to their incomplete removal in wastewater treatment plants (WWTPs) or direct excretion and sewage runoff from agriculture and livestock.

In this study, a method combining in tube solid-phase microextraction and liquid chromatography with fluorescence and DAD detectors was developed for different EDCs including estrogens (estrone (E1), 17β-estradiol (E2), 17α-ethynyl estradiol (EE2), diethylstilbestrol (DES), estriol (E3), conjugates like estradiol-17-glucuronide (E2-17G) and progestagens like noretisterone (NOR)) and androgen steroids (tetosterone (TET)) in liquid environmental samples.

Good results for analytical parameters were obtained and the method was sucessfully applied in the determination of target analytes in samples of the different stages of a demonstration pilot facultative pond wetland system which are considered to be one of the most convenient methods for treating wastewater from small communities retaining large masses of organic matter and suspended solids [2].

Acknowledgments

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APPLICATION OF CHEMOMETRIC ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION (DLLME) FOR THE DETERMINATION OF SUSPECTED FRAGRANCE ALLERGENS IN WATER SAMPLES

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Fragrances are a group of chemicals incorporated in most cosmetic and other personal care products including baby care ones. Twenty six (26) ingredients of them have been identified as likely to cause contact allergies. Some of the suspected allergens do not only pose the risk of causing contact allergies, but also systemic effects.

A rapid and simple method for the determination of suspected fragrance allergens in water samples using dispersive liquid–liquid microextraction (DLLME) mated to chemometrics and coupled to GC–MS was developed. Volume of extraction and disperser solvent, pH, ionic strength, extraction time, centrifugation time as well as centrifugation speed were optimized in a 2^{7-4} Plackett–Burman design. The obtained significant factors were optimized by using a central composite design (CCD) and the quadratic model between the dependent and the independent variables was built. The optimum experimental conditions of the proposed method were: 120 μL carbon tetrachloride, 1.00 mL methanol, 7.5 min centrifugation time, natural pH containing 2,5% (w/v) NaCl, while keeping centrifugation speed fixed at 4000 rpm.

The calculated calibration curves gave high-level linearity for all target analytes with correlation coefficients ranging between 0.9910 and 0.9992. The repeatability and reproducibility of the proposed method, expressed as relative standard deviation, varied between 3% to 10% and 6% to 12%, respectively. The recommended method reached LODs to ppt levels and could be used for routine analysis of the environmental water samples.

EN107 **Session C**

CHEMOMETRICS-ASSISTED MATRIX SOLID-PHASE DISPERSION FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN INDOOR DUST BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Polycyclic aromatic hydrocarbons (PAHs) comprise a group of ubiquitous environmental contaminants that originate from different emission sources ,mainly associated with human activities, such as the incomplete combustion of fossil fuels, industrial processes or the use of motor vehicles. Since these pollutants exert toxic, mutagenic and carcinogenic effects, knowledge of their fate in indoor dust is critical. In the present study a simple and sensitive method for the determination of PAHs in indoor dust has been developed and validated. The samples were extracted by a simple and fast matrix-solid phase dispersion (MSPD) method. The influence of the main factors on the extraction process was evaluated with the aid of Response Surface Methodology and Central Composite Design. Under optimal conditions, indoor dust samples (0.5g) were mixed and dispersed using Florisil/C18 (2g) as the sorbent material. The blend was transferred on a polypropylene cartridge and analyte was eluted using 12,31 mL of acetonitrile. The extract was evaporated under a gentle stream of nitrogen till dryness. The residue was reconstituted in 100 μL acetonitrile: and analyzed by means of high performance liquid chromatography coupled to UV-diode array detector system (HPLC/UV-DAD).

Quality parameters including the limit of detection (LOD), repeatability, within-lab reproducibility, linear range as well as specificity were investigated. Good linearity was obtained with coefficient of determination (r^2) higher than 0.999. Repeatability and within-lab reproducibility less than 6% and 14%, respectively, was observed. The specificity of the method was tested by the analysis of blank samples. The absence of any chromatographic peak in the matrix, at the same retention time as target analyte, indicated that there were no matrix compounds that might give a false positive signal in these blank samples.

DETERMINATION OF MERCURY IN SEA WATER BY USING ISOTOPE DILUTION HIGH RESOLUTION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ID HR ICP- MS)

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Determination of mercury content in the low ng kg^{-1} range remains a fundamental analytical challenge and a great source of complexity for the realisation of reliable profiles of dissolved mercury.

Although the detection sensitivity of ICP-MS allows the direct measurement of mercury at sub-ng g^{-1} levels the physical deposition of salts on interface cones and polyatomic interference from the major ions in environmental matrices does not allow a simple analysis. Therefore, sample pre-treatment or matrix elimination prior to sample introduction into the plasma was often inevitable.

The present investigation represents comparative study of two approaches based on preliminary separation and preconcentration of mercury followed by High Resolution Isotope Dilution Inductively Coupled Mass Spectroscopy (HR ID-ICP-MS) determination. Could Vapour method featuring gaseous introduction of mercury via tin chloride reduction have been developed and applied as the first analytical method. Second approach is based on matrix separation and preconcentration of mercury on chelate resin with the direct APEX sample introduction.

To achieve SI-traceable values with small combined uncertainties measurement procedures, according to sound metrological principles, were developed. The measurement method in the second cases was based on direct isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) applied as a primary method of measurement. The correct assessment of the analytical procedural blank was of the crucial importance for obtaining reliable results for both methods. In order to achieve the lowest possible procedural blank, all sample-processing steps were accomplished in a class-100 clean chemical laboratory and class 10 ultra clean fume hood.

The estimation of the total uncertainty associated to each measurement result was fundamental tool for sorting the main sources of measurement biases. Preliminary forecast of the uncertainty budgets was used as a strategy to ensure that determination in mercury in seawater could be achieved with demonstrated traceability to a stated system of reference within less than 3 % expanded uncertainty (k=2).

The results for the determination of mercury by using two independent methods showed a degree of agreement 0.2%.

OPTIMIZATION OF THE ELECTROCHEMICAL TREATMENT OF CONCENTRATED COMMERCIAL AZODYES USED IN THE TEXTILE INDUSTRIES

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Textile industries produce large quantities of wastewaters, as a result of the dyeing and finishing processes, that cause severe pollution problems worldwide. Textile wastewater is strongly colored with high values of pH, temperature, COD and BOD while it shows low biodegradability. The release of colored effluents into the environment is undesirable, not only because of their color, but also because many dyes from wastewater and their breakdown products exhibit toxic, even mutagenic or carcinogenic properties, affecting the aquatic ecological system [1-3]. Azodyes are nowadays the main category of dyestuffs in the textile industry and they are extremely resistant to biodegradation; thus treatment of such wastewater is of crucial importance. Due to the large variability of the composition of the textile wastewater, most of the traditional methods are becoming inadequate. The electrochemical treatment is a relatively new trend in the wastewater management, giving very promising results because it is an effective method for color removal, with little or no consumption of chemicals; moreover, the degradation of recalcitrant pollutants can be achieved [4].

An electrochemical method for wastewater treatment based on the electrolytic degradation of azodyes is discussed in this paper. In a laboratory scale electrochemical cell made of Plexiglas, two cathodes made of stainless steel (SS) and one anode made of niobe/synthetic diamond doped with boron (Nd/D) were used. In order to study the effectiveness of the method, four commercial azodyes (Reactive Black XL, Reactive Black 5, Acid Black 172 and Disperse Red 167:1) at various initial concentrations were used. Moreover, we investigated the decoloration of the above azodyes and the optimized conditions affecting the procedure (concentration of supporting electrolyte, pH and applied current). UV-Vis spectra were recorded. The kinetics of the process was studied and Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) were measured.

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CHEMOMETRIC CHARACTERIZATION OF PAH POLLUTION PATTERNS IN SEDIMENTS FROM GUANABARA BAY, RIO DE JANEIRO, BRAZIL

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Sediment samples from Guanabara Bay (Brazil) were analyzed by GC-MS/SIM (i.e., gas chromatography – mass spectrometry with selected ion monitoring) as part of the Guanabara Bay Monitoring Program that has been carried out since 2005 by Petrobras SA. The bay is located in Greater Rio de Janeiro, an urban and highly industrialized ecosystem that receives intense chronic anthropogenic pollution. Furthermore, on January 18th, 2000, approximately 1300 m^3 of marine fuel oil (MF380) were released into the bay from a ruptured pipeline at the Duque de Caxias Refinery. Sections of selected ion chromatograms (SICs) relative to 3- to 6-ring PAHs were processed using a four-step procedure (viz., baseline removal, retention time alignment, normalization and concatenation) and analysed with Principal Component Analysis (PCA). The objectives were to characterize the pollution patterns for Guanabara Bay; to distinguish between chronic pollution from pyrogenic sources, biogenic input and petroleum related hydrocarbons (including the marine fuel oil from the Duque de Caxias Refinery) and to identify potential new indicator compounds for source apportionment and identification.

Five distinct sources of 3- to 6-ring PAHs was revealed. The harbour is the most contami-nated site in the bay, its plume stretches in a South-West to North East direction and the chemical profile indicates mainly pyrogenic sources mixed with a high-boiling point fraction of petrogenic PAHs. Rio São João de Meriti is the second largest source of PAHs, and introduces mainly a low-boiling-point fraction of petrogenic PAHs from the western region of Greater Rio de Janeiro. The sites close to the ruptured pipeline at the Duque de Caxias Refinery (e.g., Rio Iguaçu and Rio Sarapuí) show a distinctive pollution pattern indicating a heavy petroleum fraction with high ratios of pyrene and C_1-pyrenes to fluoranthene and C_1-fluoranthenes), benzo(e)pyrene to ∑5-ring PAHs and benzo[ghi]perylene to in-deno[1,2,3-cd]pyrene. They correlate well to the spilled marine fuel oil, used in this study as a petrogenic reference. Besides the apportionment of the pollution sources, the method also led to the identification of new potential indicator ratios also involving coeluting peaks (e.g., triphenylene and chrysene) and alkylated PAHs.

THE MATRIX SOLID-PHASE DISPERSION COMBINED WITH THIN-LAYER CHROMATOGRAPHY – DIRECT BIOAUTOGRAPHY FOR DETERMINATION OF FLUMEQUINE IN MILK

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Flumequine is a fluoroquinolone antibiotic, mainly used in veterinary and to some extent in human treatment. It can be found as a residue in milk and in other food products. Matrix solid-phase dispersion (MSPD) replaces solid-phase extraction as a pre-separation method for viscous, semi-solid or solid samples. The method involves blending of the sample with a solid support material, usually silica with bonded alkyl chains (e.g. C18) [1]. Our previous papers proved that bare siliceous sorbents with a low surface area could be used for MSPD of milk samples before TLC or HPLC analysis instead of expensive C18 sorbents [2]. Thin-layer chromatography-direct bioautography (TLC-DB) combines planar chromatography with microbiological detection. The developed TLC plates are dipped in a bacterial growth medium seeded with an appropriate bacterial strain. Visualization of antibacterial agents is usually carried out using tetrazolium salts, which are reduced into intensely colored formazan by dehydrogenase of living microorganisms. The location and size of growth inhibition zones give information about the type and quantity of antibiotics.

In the work presented, MSPD was combined with thin-layer chromatography-direct bioautography to obtain semi-quantitative results. Chromaton N-AW (silica with low surface area) was used as a sorbent for MSPD and *Escherichia coli* was used as a test bacteria in bioautography. The various modes of MSPD procedure were tested and the one giving the best recovery of the antibiotics from milk was chosen. In this variant, 20 mL of hexane was used for elution of milk lipids and additional layer of Chromaton was put at the bottom of the MSPD cartridge. The MSPD-TLC-DB method was tested for milk spiked with flumequine at MRL level, that is at 0.05 ppm. The mean recovery of flumequine obtained at this level equaled 84.47 ± 2.95 %.

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SIMULTANEOUS DETERMINATION IN COOKED FOOD OF THIRTEEN POLYCYCLIC AROMATIC HYDROCARBONS AND TWELVE ALDEHYDES BY AN AUTOMATED ON-LINE SOLID PHASE EXTRACTION ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD

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Alarm and awareness of possible health hazards associated with cooking processes is recently increasing. In particular, PAHs and aldehydes mainly originate from many kinds of cooking as smoking, roasting, baking, frying, and grilling. Up to now the interest in PAHs and aldehydes formed during food cooking has principally regarded the analysis of cooking fumes, but during cooking PAHs and aldehydes also form in the food itself. In this work we concentrated our attention in the analysis of cooked food, also comparing the results obtained for the same matrix after different cooking processes (grilled, cooked in butter, cooked in oil), that can likely affect the kind and the amounts of the species formed.
An on-line SPE UHPLC-MS/MS method has been developed for the simultaneous identification and determination of thirteen PAHs and twelve aldehydes (derivatized with 2,4-dinitrophenylhydrazine).
The chromatographic conditions have been optimized to obtain the maximum of sensitivity and resolution taking into account the different retention interactions and the different ionization conditions of PAHs and derivatized aldehydes. LOD values ranging from 0.028 to 0.768 μg L^{-1} for PAHs and from 0.002 to 0.125 μg L^{-1} for aldehydes were obtained. The resolution permitted the separation of four couples of PAH isomers. Sample pre-treatment and SPE were optimized in order to apply the whole methodology to the analysis of different food matrices as salmon, frankfurter, steak, and pork chop, subjected to different cooking modes (smoked, grilled, cooked in oil or in butter). Particular attention was devoted to the evaluation of matrix effect, that was significantly reduced through the on-line SPE treatment. For each food matrix the method detection limits and the recovery (*R)* were evaluated. *R* was shown not to depend on analyte concentration in the explored concentration range (LOQ-50.000 μg L^{-1}): the average *R* percent ranges from 70.6% to 120.0%.

OPTIMIZATION OF BIOAUTOGRAPHIC CONDITIONS FOR FLUMEQUINE SCREENING IN MILK

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Bioautography belongs to a group of the so-called effect directed analysis (EDA). These methods are based on biological activities, e.g. antibacterial, antifungal, antitumour and antiprotozoae of the tested substances. In many cases, EDA methods give higher sensitivity than any other methods. Moreover, they are simple, time-saving, cheap and do not require sophisticated equipment [1]. Bioautography hyphenated directly with thin layer chromatography (TLC-DB) enables separation and microbial detection on the same TLC plate. In this procedure, the TLC plate is dipped in a suspension of microorganisms growing in a proper broth and then incubated in a humid atmosphere. The visualization of the bioautograms is usually carried out using tetrazolium salts, e.g. MTT. The cream-white zones are observed on the purple background in the places where the antimicrobial substances were spotted.

Flumequine is a synthetic quinolone antibiotic with high activity against Gram-negative bacteria, such as: *Escherichia coli*, *Salmonella sp.*, *Pasteurella sp.*, *Haemophilus sp.*, *Proteus sp.*, *Klebsiella sp.* It is frequently used for treatment of enteric infections in domestic species, as well as used as water or feed additive to enhance weight gain of the animals. This presents a serious risk to public health; therefore monitoring of the residue levels of flumequine in foodstuff is necessary.

The aim of this work was to optimize the bioautographic conditions for screening of flumequine in milk samples. The TLC-DB was compared with commonly used for detection of antibiotic residues method, i.e. high performance chromatography with UV detection (HPLC-UV), and two microbiological methods, i.e. agar disc diffusion and agar cylinder diffusion.

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INVESTIGATION OF BIOLOGICAL ACTIVITY OF *VALERIANAE TINCTURA*, *MENTHAE PIPERITAE TINCTURA*, *HYPERICI TINCTURA* AND *AMARA TINCTURA* BY EFFECT DIRECTED ANALYSIS

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Valerianae tinctura, *Menthae piperitae tinctura*, *Hyperici tinctura* and *Amara tincture* are herbal tinctures a common remedy for digestive problems. The main reason for their application is that they can stimulate proper functioning of stomach and liver. These tinctures are for example used as the basic compounds of commercially available stomach bitter.

In the recent years, the popularity of the effect directed analysis (EDA) has increased; the method gives specific information about the type of biological activity characteristic for the tested compound. Depending on studied properties, e.g. antibacterial, antioxidant, anticancer, various EDA methods can be applied. TLC-EDA is a combination of the thin layer chromatography method with the effect directed analysis. Not only does this hyphenation allow to separate the tested compounds but it also helps to verify their biological activity.

This work presents the research results concerning the biological activity of *Valerianae tinctura*, *Menthae piperitae tinctura*, *Hyperici tinctura* and *Amara tincture*. The antimicrobial activity was tested using direct bioautography tests (TLC-DB) for Gram-negative bacteria, *Escherichia coli* and Gram-positive bacteria, *Bacillus subtilis*. Moreover, the antioxidant activity of the tinctures was studied using the DPPH test.

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPH QTRAP TANDEM MASS SPECTROMETER

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants derived mainly from anthropogenic sources. Contamination with benzo(a)pyrene and other PAHs in foodstuffs is topical problem for food market in Latvia. Processing procedures of food, such as smoking, drying and cooking are commonly thought to be the major source of contamination by PAHs. The assumption that benzo(a)pyrene is a good marker to all 16 PAHs is questionable. In the research project completed by EU the level for PAHs sum including benzo(a)pyrene was 20 times higher than benzo(a)pyrene itself. As it is known, until now the permitted level has been set for benzo(a)pyrene only. Recently the maximum level for 4 PAHs has been developed as sum of benzo(a)pyrene, chrysene, benzo(b)fluoranthene and benzo(a)anthracene.

PAHs can be measured using several analytical methods, including high performance liquid chromatography with fluorescence detection, gas chromatography mass spectrometry, gas chromatography tandem mass spectrometry methods and finally by advanced liquid chromatography tandem mass spectrometry. The ultra performance liquid chromatograph tandem mass spectrometer (UPLC/MS/MS) system is a highly sophisticated and novel analytical instrumentation, which allows the selective and sensitive analysis of chemical compounds in a wide range of sample matrices. Scientists are seeking ways to improve productivity of high performance liquid chromatograph for PAHs' methods using new technology such as shorter columns with smaller particle size. Column dimensions (length, inner diameter, particle size) are the key to achieve the best separation, depending on the sample matrix.

The aim of the current research is establish determination method for PAHs using UPLC/MS/MS. Compounds were detected with a Waters Acquit UPLC connected to the Applied Biosystems MDS Analytical Technologies AB Sciex Qtrap 5500 mass spectrometer operating in positive electrospray ionization using the compound-specific multiply reaction monitoring methods. Method is foreseen for the determination of 4 PAHs in foodstuffs. In the experiments the fallowing columns were used: Phenomenex, Kinetex C18 50 mm×3 mm, particle size 1.7 µm; Phenomenex, Kinetex C18 100 mm×4.6 mm, particle size 2.6 µm; Phenomenex, Luna Phenyl-Hexyl 150 mm×4.6 mm, particle size 5 µm.

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SUITABILITY OF ION CHROMATOGRAPHY TO DETERMINE ANIONS IN HIGH ACIDITY FLUORINATED SAMPLES

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The production of fluorinated compounds requires the control of the anionic impurities concentration levels for their commercialization. In this work, an ion chromatographic method has been developed to determine sulphate content in samples with high fluoride content and pH values lower than 2.5. These tough matrixes have never being studied with this technique before, being them analyzed by classical chemical method up to now.
The optimization of the method was carried out on industrial samples due to the lack of reference materials. Different parameters as dilution factor of the samples, injection time, eluent concentration, flow rate and time necessary for the recuperation of the base line were studied. The final chromatographic conditions were 9.0 mM Na_2CO_3 with a flow of 1.0 mL/min and an injection time of 2s.
In spite of the huge concentration of fluorinated species and the high values of acidity, the ion chromatographic method has showed the suitability for the determination of sulphate in different kinds of samples coming from fluorine industry in less than 30 minutes and without using any sample treatment apart from dilution. The sulphate concentrations obtained for the different fluorinated samples range from 0.14 to 2.73 mg/L with a precision in terms of % RSD between 1 and 15 %.

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DIFFERENT APPROACHES FOR EVALUATION OF THE MEASUREMENT UNCERTAINTY IN DETERMINATION OF THE ARSENIC CONTENT IN GROUND WATER

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As many important decisions are based on the results of analytical measurements, the uncertainty brings information on the reliability of the result, this being an indicator of the confidence for given result and in consequence for decision taken on the basis of the results. The evaluation of uncertainty requires the close look at all possible sources of uncertainty, hence improving the knowledge on the measurement procedure being in use, also very helpful in optimization of the measurement procedure.

In the present paper, methodology for evaluation of measurement uncertainty of result of arsenic determination in ground water is presented. Arsenic was determined using FAAS. The necessary relevant information was obtained from the method validation data, the quality control data and equipment calibration certificates. The method of measurement is described together with the measurement equation, selected traceable reference standards and the associated measurement uncertainty. The major sources of uncertainty of the result of measurement were identified and the combined and expanded uncertainty was calculated. Identification of the main uncertainty sources represent basis for target operation for reducing the measurement uncertainty of this determination.

DEGRADATION ANALYSIS OF SILICON ANTIFOAM BY THERMOGRAVIMETRIC ANALYZER

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Silicon antifoam is widely used to break down and prevent the formation of foam. Foam that arise in the production unit can cause various problems that would cause damage. The effectiveness of silicon antifoam can be determined by testing the degradation at a certain temperature according to the use of silicone antifoam.

The purpose of this study was to determine an effective silicon antifoam that is used at a given temperature by means of thermogravimetric analyzer (TGA).

Research was carried out by analyzing several kinds of silicon antifoam with different viscosity values and from different sources. Furthermore, the effectiveness of silicone antifoam tested with short residue sample.

Based on research that has been done can be concluded:

- Thermogravimetric analyzer can be used to determine the degradation of silicon antifoam.
- Silicon antifoam with higher viscosity are more resistant to degradation.
- Silicon antifoam which is more resistant to degradation is more effective in lowering the formation of foam.

METROLOGICAL ASPECTS OF THE DETERMINATION OF MERCURY IN ENVIRONMENTAL SAMPLES BY VARIOUS SPECTROMETRIC METHODS

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The determination of mercury is consider being an important issue in environmental science and the use of properly selected methods with evaluated uncertainty and well established traceability is of great importance for environmental monitoring studies.

The aim of this work is to provide critical comparison of the analytical performance of all those analytical procedure for trace levels of mercury determination in environmental matrices in respect of the uncertainty and traceability of the obtained results.

Graphite furnace atomic absorption spectrometry (GF AAS) and inductively coupled plasma mass spectrometry (ICP MS) were used for the determination of mercury in the solutions obtained after digestion of environmental samples. For the direct determination of mercury in solid samples slurry sampling ETAAS and direct solid sample analyzer AMA 254 were applied.

The experimental protocols are described with a set of model equations, which allow the computation of the analytical results and the corresponding combined uncertainties. Structured step by step uncertainty calculations according to the ISO Guide to the Expression of in Measurement Uncertainty are presented for several cases. The major performance characteristic of the experimental methods applied and practical approach for establishment of the traceability of the measurement results are also discussed.

INFLUENCE OF THE ANALYTE EFFECT ON RECOVERY INFORMATION IN ANALYTICAL MEASUREMENT

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According to the guidelines of the International Union of Pure and Applied Chemistry (IUPAC) accuracy of an analytical result may be assessed by evaluation of recovery (RV). RV may be estimated with the use of Certified Reference Material (CRM) or via addition of a portion of pure analyte to a given sample (Surrogate Recovery Method) [1]. The first way, called the Absolute Recovery Method [2], is based on subjecting a CRM to all procedures of preparation to measurement in a manner identical to the one for the analyzed samples. The second way, is based on subjecting to all procedures of preparation to measurement both the sample alone and the sample with analyte addition.

The analyte effect occurs when the chemical forms of an analyte (naturally present in a sample) and the one added to the sample, undergo diverse transformations leading to preparation of the sample to measurements. Neither the Absolute Recovery Method is free from this effect, since chemical forms of an analyte present in CRM, natural samples and standard solutions may be different. Regardless of the fact that the analyte effect has been known for years and that it is acknowledged and eliminated in some branches of atomic spectrometry, the possibility of its occurrence is most of the time disregarded and neglected.

The presented work covers the results of research on the analyte effect along with its influence on the estimated analyte recovery values (RV). Various organic and inorganic forms of selenium utilized in the study were determined with the use of hydride generation-atomic fluorescence spectrometry (HG-AFS).

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SYNTHESIS OF CdSe NPs IN HOMOGENEOUS PHASE IN AQUEOUS MEDIUM. CONTROL OF VARIABLES FOR OBTAINING OPTIMUM SIZES AND QUANTUM YIELDS.

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Unlike the organic synthesis of quantum dots, aqueous synthesis shows good reproducibility, low toxicity, low cost and, in particular, the products prepared in aqueous medium have excellent water solubility, stability and biological compatibility. However, perhaps owing to an excessive desire to obtain results with practical applications that will respond to the high expectations that have undoubtedly arisen concerning the use of QDs in biological media, there is still a long way to go in the study of their structure and chemical behaviour when they are synthesized in aqueous medium. The need to shed more light on these aspects is crucial if we are to optimize the bioconjugation processes involved.

The control of pH and temperature in the synthesis of CdSe NPs simultaneously in aqueous medium and the conjugation of both variables in its optimum values has allowed us to propose a new synthesis procedure in which particle formation is carried out in homogeneous phase, controlling in the solution the rate of release of the ions forming the particle with a view to obtaining highly fluorescent particles for as long as possible.

We studied the effect of the different experimental variables on the formation and growth rate of the initial NPs, which can be controlled kinetically. We obtained a very slow increase in size, with modifications of the spectral characteristics of great interest and the maintenance of NPs of the desired size over long periods of time and high fluorescence. We then determined the concentration of NPs in solution under the different conditions, observing that it allowed us to control possible aggregation processes. The NPs thus synthesized can be used in biological media.

SYNTHESIS TEMPERATURE AS A VARIABLE CONTROLLING THE SIZE AND LUMINESCENCE PROPERTIES OF CdSe QUANTUM DOTS OBTAINED IN AQUEOUS SOLUTION

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There is a broad field of enquiry with many perspectives for the future in the study of QDs for biological applications. Although important advances have been made in certain aspects, there are many lacunae as regards the procedures used for the synthesis of these nanoparticles, and their behaviour in different media must also receive more concerted attention.

In the methods for the synthesis of colloids in general, and nanoparticles in particular, one of the variables controlling or affecting their *nucleation* rate, through modification of the supersaturation conditions is *temperature*. From the point of view of *solubility*, the higher the temperature, the greater the solubility, the less the nucleation rate (a lower number of nuclei per unit volume), whereas at low temperatures solubility is lower (supersaturation is reached earlier), the nucleation rate predominating. The combination of both effects in *ageing processes* (a high initial temperature after which the solution is allowed to cool) elicits a predominance of the growth rate over the nucleation rate, larger particles being obtained.

These processes can be altered by kinetically controlling the concentration of one of the reagents in the solution (*colloid formation in the homogeneous phase)*.

Here we report an exhaustive study of CdSe NPs synthesized at different temperatures -namely, size, surface characteristics, fluorescence and stability over time- and show that the synthesis temperature becomes a decisive variable for obtained CdSe NPs of the desired size for each particular application.

SAMPLE PRECONCENTRATION IN MICRO CHANNELS

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One of the main obstacles analyzing biological samples is the low detectability induced by low concentrations and small sample volumes. Therefore, there is a vast demand for techniques for accomplishing preconcentration of minute, dilute samples.

In this work, a preconcentration platform using open micro channels (dimensions 50µmx50µmx3cm) manufactured on silicon chips is presented. For preconcentration studies the micro channels were filled with sample and a voltage was applied over the length of the channels. Negatively charged ions were attracted to the anode and positively charged ions were attracted to the cathode resulting in a concentrated analyte zone located in the channel according to charge.

There were other electrochemical effects that influenced the preconcentration, though. Electrolysis of water at the electrodes gave rise to a pH gradient in the channel. This could in turn change the charge of biomolecules like proteins, which affects the electromigration and position of the sample zone.

Preconcentration was performed in closed as well as open micro channels in order to investigate the effects of parameters such as initial pH of the solution, initial analyte concentration and the use of a zwitterionic buffer. Samples containing either a protein, Cytochrome C, or a fluorescent substance, tetramethylrhodamine isothiocyanate (TRITC), were studied using visible color, fluorescence or MALDI-MS for detection of the preconcentrated zones.

CERIUM OXIDE NANOPARTICLES EFFECTIVENESS AS RADICALS SCAVENGERS-AN ELECTROCHEMICAL APPROACH

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Cerium oxide nanoparticles are very interesting due to their electrocatalytic properties, high active surface ensured by nanoparticles use, non-toxicity (only in the case of nanoparticles having reduced dimensions), biocompatibility and oxygen storage capacity. During the last 5 years the use of cerium oxide became interesting in designing enzymatic biosensors, the scientific literature reporting biosensors for monitoring hydrogen peroxide [1], tributyrin [2], dopamine [3], etc.
The aim of the present work was to develop an electrochemical device based on cerium oxide nanoparticles able to be used in reactive oxygen species removal from physiological medium, based on proved cerium oxide non-toxicity.
The cerium oxide nanoparticles were synthesised in laboratory using two different protocols (wet synthesis and microemulsions synthesis).
Cerium oxides films were electrodeposed on conductive supports. CeO_2 efficiency as radicals scavengers was tested either in suspension or as deposed film, against superoxide and peroxide radicals, in aqueous solutions. Determinations were chronoamperometrical performed, at potentials corresponding to each investigated radical, the concentrations of investigated free radicals being compatible with phisiological conditions.
Using the calibration curve for superoxide radical Δi (nA)= 138.9xC (μmol·L^{-1}) – 147.33 (R^2 = 0.9988), it was proved that 100 ppm cerium oxide synthesised by microemulsions is able to scavange 0.8x 10^{-3} molL^{-1}, while that obtained by wet synthesis scavenged 0.68 x10^{-3} molL^{-1} both being more effective than commercially available one, possibly due to higher active surface and smaller nanoparticle size.
For peroxyl radical, using the calibration curve I(nA) = 0.924E6xC(μmolL^{-1})- 8.07 (R^2 = 0,995) it was found that 100 ppm CeO_2 is able to scavenge 5.7 μmolL^{-1}/min free radical for wet synthesis respectively 6.7 μmolL^{-1}/min free radical for microemulsions, even in this case being more efficient than commercial cerium oxide nano-powder.

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MICROSPECTROSCOPIC ANALYSIS OF METAL NANOPARTICLE ARRAYS PREPARED WITH HIGHLY ALIGNED DNA NANOFIBERS

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Metal nanoparticles (MNPs) such as Au or Ag can confine light (electromagnetic fields) to their dimensions on the order of or smaller than the wavelength. Such properties, called localized plasmon resonances (LPRs), are based on interactions between electromagnetic radiation and conduction electrons at metallic interfaces or in metallic nanostructures, leading to an enhanced optical near field of sub-wavelength dimension below the optical diffraction limit. Thus, it should be of great interest to construct various architectures of ordered MNPs, and to investigate the light localized on such architectures. Most recently, we developed a simple method to create highly aligned DNA nanofibers on a surface. This method is based on the processes of solvent vapor-induced buildup and controlled drying front movement and forms parallel aligned DNA nanofibers exceeding several hundred micrometers in length and 40 nm in diameter on a poly(dimethylsiloxane) (PDMS) sheet. Various MNPs could also be attached to such nanofibers, forming metallic nanoarrays with a longer scale. We then tried the formation of metallic nanoarrays with the above method. First, to attach gold nanoparticles (AuNPs) to DNA, 10 μL of positively charged AuNPs (143 μg mL^{-1}) and 100 μL of μ-DNA (4.5 ng μL^{-1}) were mixed. Then, 1 μL of the mixture was added to 8 μL of ethanol, this solution was deposited on a PDMS sheet. The PDMS sheet was tilted at 15° during solvent evaporation to move the drying front downwards. Solvent evaporation lead to a decrease in the volume of the solution, leaving behind line patterns. Line patterns of metallic nanoarrays were formed when AuNPs-attached DNA was continuously deposited at highly concentrated finger positions. A combination of dark-field microscopy and microspectroscopy were used to study the optical characteristics of the arrays. Higher optical responses were observed in the arrays when the light was polarized parallel to the arrays, indicating a uniaxial alignment of Au nanoparticles along the arrays.

DISTRIBUTION OF FULLERENOL NANOPARTICLES IN HUMAN SERUM

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Fullerenol $C_{60}(OH)_{24}$ can form hydrogen bonds with the surrounding water molecules and, depending on the conditions, result in formation of polydisperse polyanion nanoaggregates-fullerenol nanoparticles (FNPs). FNPs express many biological effects such as high antioxidative activity in cases when the oxidative stress was caused by xenobiotics and radiation injuries. The study concerning interactions between FNP and body fluids is of basic importance for revealing the mechanisms of action and further research on *in vivo* models.

In this study, there were investigated two concentrations of FNP in 0.9% NaCl and 10% solution of dimethyl sulfoxide (DMSO in 0.9% NaCl) in presence of human serum over a period of 2, 4 and 24 hours at 37°C. Size, charge and distribution of particles were measured by dynamic light scattering. Atomic force microscopic measurements of the same solutions were also done. There were no significant differences in size, charge and distribution of particles in examined solutions in comparison with human serum in 0.9% NaCl.

CdSe QUANTUM DOTS SYNTHESIZED *"À LA CARTE"* IN AQUEOUS SOLUTION. CONTROL OF SIZE AND FLUORESCENCE. pH AS A DECISIVE VARIABLE

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The QDs most widely used as biomarkers, such as CdS, CdSe and CdTe nanocrystals and their core/shell alloys are in most cases synthesized in organic phase, using high-boiling point solvents such as trioctylphosphine (TOP) or trioctylphosphine oxide (TOPO) or a mixture of TOP/TOPO. The synthesis procedures in aqueous medium are an attractive alternative to the synthesis of QDs in organic medium and is now an active area of interest.

Here we report the results obtained in a study of the effect of pH during the procedure of CdSe aqueous synthesis.The concentration of H^+ in the aqueous solution affects the solubility of CdSe by side reactions of Se^{2-} and Cd^{2+}. CdSe is a highly insoluble specie that is partially soluble at alkaline pH through side reactions of Cd^{2+} and at acid pH due to side reactions of the Se^{2-}. Synthesis is performed in the presence of mercaptoacetic acid, such that it is necessary to bear in mind that the concentration of the dispersant form, mercaptoacetate, will also depend on the pH of the solution. All these reasons lead to the conclusion that the pH of the synthesis conditions is a very interesting variable for the synthesis of NPs of the desired size and characteristics.

We present a study of the characteristics of CdSe NPs obtained at different pH values. The results obtained in an in-depth study of the variable allowed us to perform a control of size and the luminescence properties for the collection of the NP desired for each application and to know their evolution over time in each of the conditions, which are decisive factors from the point of view of future applications as a biological marker.

INTERACTION OF GOLD NANOPARTICLES WITH PLATINUM COMPLEXES

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Here we studied the interaction of a platinum complex derived from a bile acid with gold nanoparticles. As starting materials we used AuNp of 20 nm in suspension (c = 0.01 % in $HAuCl_4$) and the platinum complex $[Pt(GC)_2en]$, [1] where HGC is glycocholic acid, a bile acid conjugated with glycine.

500 mL of the nanoparticle suspension was incubated with 500 mL of a solution of the complex (c = 10^{-4} M). A shift towards a greater wavelength of the band in the visible spectrum was observed for the incubated product with respect to AuNp (≈38 nm). After one week, intensity decreased and at the same time a precipitate appeared. The evaluation of Np with increasing amounts of complex led to a constant maximum shift when the platinum concentration was $1.5\cdot10^{-4}$ M, such that it seems that the coverage of the nanoparticles at this concentration was optimum. The fluorescence of the complex and of the product of incubation with AuNp was measured by excitation at 350 nm. An increase in intensity was observed with respect to the free platinum complex, together with a variation in the structure of the band in the incubated product.

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ABOUT CONCEPTION OF NANOANALYTICS

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The interdisciplinary nature of nanoscience and nanotechnology has brought about wide-ranging collaborations between inorganic, organic, solid-state chemists, biochemists, physicists, specialists in material science etc. Analytical chemistry being a highly interdisciplinary discipline as well cannot set aside the attractive power and advances of nanoscience, nanoobjects and nanotechnology. In line with this tendency, the term “nanoanalytics” is very popular now but currently it has different meanings due to no agreement in analytical society about the matter of nanoanalytics [1-4]. It should be noted that in the case of nanoanalytics, creation of conceptual terminology, first of all, definitions and classifications, substantially delays from the speed of nanoanalytics development. For this reason, the analytical scientific community has with no postponement to agree on description and classification in the area of “nanoanalytics” that will exclude misinterpretation and misusing in the field.

It cannot be doubted that nanoanalytics deals with different nanotechnologies and different nanoobjects and consequently, this can outline two directions in the field. The nanotechnology methods in turn can be subdivided further into two groups. First one includes measuring technologies that are applied to characterization of the particle sizes as well as to surface and interface analysis even down to the atomic scale (scanning tunneling and atomic force microscopy) including local surface chemical composition. The design of new analytical instruments based on nanotechnological approaches can also be fit into this group. The second group includes technologies for development of nanoobjects (particles, layers, systems) or modification of their surface for analytical application.

The second direction is the use of different solid (surface and quantum effects) and liquid (surface, concentrating and supramolecular effects) nanoobjects as a tool in classical analytical methods: spectroscopy, electrochemistry, separation methods and sensors. This area is more familiar for the analysts and known for more than 30 years.

In this presentation, the definition and classifications in nanoanalytics will be considered. Also, a more detailed information concerning today’s situation in the application of solid and liquid nanoobjects in chemical analysis will be presented.

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OPTIMIZATION OF A SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY BASED METHODOLOGY IN ORDER TO ASSESS BREAST CANCER THROUGH URINARY METABOLOMIC PATTERN

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Solid-phase microextraction (SPME) has been introduced in the 1990s as a new sample preparation approach, which many offers advantages including simplicity, speed, and is a solvent free extraction, that presents good linearity and high sensitivity. Over the years, SPME has been widely applied in the characterization of biological matrices (blood, urine, plasma, saliva and hair) for the discovery of biomarkers or biomarker patterns of human diseases, which can allow a further understanding and characterization of such diseases. More recently, there has been an increased interest in the analysis of disease-related volatile organic compounds in biologic fluids with over 200 compounds being identified. The analysis and characterization of urinary volatile pattern is a recent approach with potential to provide valuable information about cancer and systemic diseases leading to a deeper knowledge of the human health status.

The purpose of this work was to optimize and validate a solid–phase microextraction extraction procedure for the detection and identification of several volatile organic metabolites described in literature as breast cancer volatile biomarkers. To fulfill this optimization several experimental conditions that might affect the SPME procedure such as stationary phases (CAR/PDMS, PEG, PDMS, PA and DVB/CAR/PDMS), extraction time and temperature, were tested and evaluated. The highest extraction efficiency, determined through total VOMs peak area, number of extracted compounds and reproducibility, was obtained using a CAR/PDMS coating fibre, at 50 ºC for 60 minutes.

OPTIMISATION OF HEADSPACE PROCEDURE FOR EXTRACTION VOLATILE CONSTITUENTS OF *THYMUS PANNONICUS* ALL. AND ANALYSIS BY GC-MS

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Clear designation of plant volatile constituents can be useful in reckognition of essential oil polymorphisms in plants belonging to taxa such as genus *Thymus*. Although some populations have a single chemotype in majority, many have two, three or more chemotypes. For obtaining plant volatiles, i.e. essential oils, collective sample of population is usually subjected to hydrodestilation (HD). On the other hand, the static headspace method (HS) for extracting plant volatiles enables anlyzing of individual plants and it is based on equilibrium between solid (plant) matrix and the gaseous phase above it, and therefore highly dependable on temperature and time of equilibration.

The headspace extraction of volatile constituents of *Thymus pannonicus* All. under four different equilibration temperatures (60 °C, 80 °C, 90 °C and 100 °C) and three different time periods (20, 30 and 60 min) was studied in this work. Additionally, the mode of the extraction of the volatiles using the headspace technique is compared to the classic hydrodistillation procedure.

Essential oil (EO) of *Thymus pannonicus* obtained by HD of aerial plant parts and HS extracts were analyzed by GC and GC-MS. Forty-six constituents were identified by comparing calculated Kovats retention indices and obtained mass spectra with those found in literature.

Correlation analysis revealed that headspace equilibration under 80 °C for 20 min, 80 °C for

30 min and 90 °C for 30 min gave HS extracts with a composition correlated the best with corresponding one of hydrodistilled EO (correlation coeficients: 0.64-0.67). Beside the fact that hydrodistillation requires large amount of plant material, this procedure is also time consuming and causes the loss of highly volatile components. Therefore, the composition of HS extracts was somewhat different then EO obtained by HD, but the HS method enabled fast extraction and analysis of volatile fraction of very small samples of *Thymus pannonicus*.

AN ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-BASED METHODOLOGY AS USEFULL TOOL TO DETERMINE STRESS OXIDATIVE BIOMARKERS

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There is increasingly intense scientific and clinical interest in oxidative stress and the many parameters used to quantify the degree of oxidative stress. Multiple oxidative stress markers have been suggested, but their measurement has posed substantial analytical difficulties in complex biological samples [1]. Thus, rapid, reliable and accurate analytical methods for determination of creatinine, uric acid and malondialdehyde (MDA) in human fluids are needed for both early diagnoses of these diseases and for studying the effects of these metabolites in the disease development and on human health.

Hence, the aim of this work is to develop and validate a fast and sensitive SPE-UHPLC–based methodology for the simultaneous separation and quantification of three different oxidative stress markers such as creatinine, uric acid and MDA in human urine. Abnormal concentrations of these metabolites in blood and urine are related to various medical conditions, such as gouty arthritis, hyperuricemia, hypertension, kidney damage, and are implicated in cardiovascular diseases.

Human urine samples were pretreated by dilution and filtration, followed by UPLC separation using an Acquity high strength silica HSS T3 analytical column (100 mm × 2.1 mm, 1.8 µm particle size) with an isocratic aqueous mobile phase of 20%methanol acidified with 0.1% formic acid (v/v).

This developed method provides a simple, rapid and sensitive detection for the species of interest within 3 min. The method was fully validated in terms of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), extraction yield, accuracy and inter/intra-day precision, using human urine spiked with bioactive metabolites at concentration levels ranging from 0.016 (MDA) to 25 µg. mL^{-1} (creatinine). A linear relationship between the ratio peak area of the standards to that of internal standard and the concentration of the standards was obtained for creatinine, uric acid and MDA with a square correlation coefficients > 0.998 for investigated analytes. The LOD varied from 0.07 to 0.87 µg.mL^{-1} and LOQ to 0.22 to 2.67 µg.mL^{-1}.

Acknoledgments:
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KINETIC AND EQUILIBRIUM STUDIES OF THE REACTIONS BETWEEN [Ru(terpy)(bipy)Cl]Cl COMPLEX AND BIOLOGICALLY IMPORTANT LIGANDS

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The studies in the field of anticancer characteristics of ruthenium(III/II) compounds have caused much attention for several years since some of them such as KP1019 (indazolium-tetrachloridobis(H-indazole)ruthenate(III)) i NAMI-A (imidazolium-*trans*-tetrachlorido (dimethyl sulfoxide)indazoleruthenate(III)) reached the level of clinical investigation.[1,2] Ruthenium complexes in the reaction with DNA molecule form preferentially the guanine- and adenine-adduct, as platinum complexes. Also, several studies indicate very strong interactions with proteins. However, Ru(III/II) complexes show generally less toxic effects than platinum(II) drugs3[2]

We studied the kinetics of the substitution reactions of [Ru(terpy)(bipy)Cl]Cl complex with biologically important ligands sach as: dimethylsulfoxide, guanosine-5'-monophosphate, thiourea and L-histidine. All reactions were studied by UV-VIS spectrophotometry in 0.1 M $NaClO_4$ with the addition of 10mM NaCl to prevent the hydrolisis of complex. The reactions are carry out at 298 K. The excess of ligand concentration was used to observed *pseudo*-first order conditions. The rate constants and activation parameters are calculated using computational program Origin 6.1. The order of reactivity of studied ligand is in good agreement with the structures of used ligans and depends of type of donor atom. Also we investigated the hydrolysis and complexation reactions for aqua analogue of Ru(II) complex with guanosine-5'-monophosphate and L-histidine. Potentiometric titrations were carried out at 298 K in inert atmosphere using a glass electrode. For the calculations is used computation program HIPERQUAD 2006. The acid dissociation constant of $[Ru(terpy)(bipy)(H_2O)]^{2+}$ complex is determined as well as the stability constants of the formed species in the different studied systems.

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DEVELOPMENT OF AN ELECTROCHEMICAL IMMUNOMAGNETIC SYSTEM FOR THE RAPID DETECTION OF CELIAC DISEASE IN SERUM AND SALIVA

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Celiac disease is one of the most common immune-mediated diseases in Western countries with an important problem of underestimation of the number of people actually suffering, due to a wrong diagnosis (Iceberg effect). For this reason, the scientific community focuses on the need for early diagnosis to prevent the development of more serious pathologies.

Currently, the main laboratory test is the assay of serum IgA anti-tissue transglutaminase (tTG) by ELISA-type immunoassay systems.

The purpose of this work is the development of an electrochemical immunosystem for the determination of IgA anti-tTG that can be applied both on the classic sample of serum and saliva samples, making the analysis non-invasive. In this way, the system can be used for preliminary screening diagnosis, even in the "doctor office and/or non-hospital facilities, realizing a "point of care testing” device (POCT).

The system (ELIME, Enzyme-Linked ImmunoMagnetic Electrochemical assay), is based on the use of magnetic particles with immobilized human tTG antigen, coupled with magnetized strips of 8 Screen Printed Electrodes (SPE).

The main features of the realized immunosystem are:
- high analytical sensitivity and rapidity, due to the use of magnetic beads and to the electrochemical detection;
- good accuracy and precision, evaluated by analyzing blank sera spiked with known concentrations of IgA anti-tTG;
- system validated by measuring serum samples of celiac patients and constructing a ROC curve for determining the optimal cut-off (2 AU/ml), the clinical sensitivity (94.74%) and the clinical specificity (100%).

Results obtained in adapting the developed system to saliva samples will be presented.

The research was conducted in collaboration with Radim SpA under the "Project for Industrial Research and Experimental Development Filas 2008: Biosensors for rapid determination (POCT) of celiac disease and allergies

ISOLATION AND IDENTIFICATION OF COMPOUNDS FROM HUMAN URINE TOXIC TO *VIBRIO FISCHERI* BACTERIA

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Kidneys generate 1-1,5 liters of urine a day. Urine contains variety of substances: noxious, toxic or unwanted products of metabolism. Highly developed analytical techniques enable isolation and identification of various substances in human urine. The literature provides a great deal of information about isolation and determination of specific (identified) compounds in urine by high performance liquid chromatography coupled with mass spectrometry HPLC-MS. There are few reports however about identification of compounds in human urine, present at low levels of concentration.

The aim of the study is isolation and identification of compounds toxic to *Vibrio fischeri* bacteria from human urine, confirming their identification and determination of toxicity parameters (EC50, EC20). To isolate urine compounds the high performance liquid chromatography (HPLC) technique was applied. The appropriate chromatographic system (column, phase conditions) was chosen. This system ensures washing and separation of urine compounds. The urine was fractionated in 28 fractions and toxicity of each fraction in the presence of *Vibrio fischeri* bacteria was determined. Both, toxic (> 75% toxicity) and non-toxic urine (< 25% toxicity) towards *Vibrio fischeri* bacteria were fractionated. The urine samples with widely varying toxicity come from the same person. The fractions of urine were liofilized, then they were dissolved in redistilled water. The liofilized samples were stored in a freezer until determination of toxicity using the Microtox® test. The preliminary studies showed that the second fraction of toxic urine is toxic toward *Vibrio fischeri* bacteria. The second fraction of non-toxic urine is also non-toxic. The next stage is the identification of toxic compounds in the second fraction. The toxic fractions will be separated again with usage HPLC-MS technique. The separation conditions must be chosen again (column, eluent gradient), to allow separation and pre-determination of toxic compounds structure. To confirm the initial identification of toxic compounds structure, the NMR and IR technique of these compounds is essential. The following step will be determination of identified substances toxicity in Microtox® test.

FLUOROMETRIC DETERMINATION OF CALCIUM IONS USING MINIATURIZED DETECTOR MADE OF LIGHT EMITTING DIODES

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Calcein is a standard reagent used for fluorimetric determinations of calcium. Both calcein and its complex with calcium show almost the same spectrofluorometric spectra. In alkaline solutions a quenching of the fluorescence in observed, however the effect is significantly stronger for free ligand than for the complex, and this difference is the fundamental for indirect fluorimetric determination of calcium ions.

In this contribution a simple two-channel FIA system for fluorimetric calcium determination in clinical samples is presented. The system is based on recently developed compact flow-through detector made of three ordinary light emitting diodes. Two of them are acting as the source of fluorescence excitation lights, and have emission spectra compatible with absorption spectrum of calcein-calcium complex, whereas the third LED plays a role of detector of LED-induced fluorescence.

Doubling of LEDs emitters (fluorescence inductors) significantly improved stability and sensitivity of the developed optoelectronic device (when compared with earlier prototypes of paired emitter detector diodes for fluorimetric measurements [1, 2]). The presented FIA system has been optimized and applied for determination of calcium ions in some real sample important in food and primarily in blood serum samples.

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FLOW ANALYSIS SYSTEM FOR PROTEIN DETERMINATION BASED ON COMPACT OPTOELECTRONIC DETECTORS

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Determinations of total protein content in various physiological fluids, such as urine or blood serum, belong to the mostly required bioanalytical assays in clinical diagnostics. In this contribution two simple flow injection systems dedicated for protein determination are reported.

In the fluorimetric FIA system the reaction of fluorescamine with free amine groups in proteins is applied. Product of this reaction -fluorescent pyrrolinones is excited with UV radiation of a wavelength of 390 nm and emits blue light (480 nm).

The photometric FIA system is based on conventional Bradford method. This protein assay is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 in which under acidic conditions the red form of the dye is converted into its blue form to bind to the protein being assayed (the absorbance maximum for acidic solution of dye shifts from 465 nm to 595 nm when binding to protein occurs).

For both systems have been developed dedicated, compact, optoelectronic detectors constructed of light emitting diodes specially selected for each analyte. The operation of both optoelectronic detectors is based on the concept of paired emitter detector diode- so called PEDD [1]. The developed detectors and FIA systems were optimized and characterized with model proteins -albumins and globulins, as well as with serum standards. Finally, the systems have been applied for the analysis of real clinical samples. Results of analysis of human serum and urine samples are comparable with those obtained using routine analytical methods recommended for clinical diagnostics.

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PM12 **Session C**

APPLICATION OF SPECTROSCOPY IN ANTIOXIDATIVE ACTIVITY ANALYSIS OF TWO INEDIBLE GANODERMA SPECIES FROM SERBIA

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In the last decades wood decaying fungi of the genus *Ganoderma* (fam. Ganodermataceae, cl. Basidiomycetes) have been recognized as therapeutic fungal biofactories. Although the most analyzed species is *Ganoderma lucidum* the others such are *Ganoderma resinaceum* Boud.inPat. and *Ganoderma pfeifferi* Bres. became of great importance recently. The fruit bodies of *G. pfeifferi* are woody and perennial and have some resemblance to the annual ones of *G. resinaceum* according to varnish-like resinous surface layer. As traditionally used medicinal macrofungi they could be used as new natural sources of biomolecules expressing various pharmacological effects: antioxidative, antitumor, cardiovascular, hepatoprotective and immunomodulating.

Since antioxidative activity of bioactive substances that act as delaying or preventing agents (antioxidants) is associated with prevention of many chronic diseases including cancer and diabetes, the main goal of this study was to detect an antioxidative potential of two inedible species of genus *Ganoderma* using spectroscopy analytics on Multiskan Ex (ThermoLabsystems). Spectroscopy analytics is the most frequent analytics used in such studies due to its simplicity, cheapness and rapidity, especially when using micro-plates for large number of samples.

Regarding this, antioxidant effects of ethanolic extracts of two fungal species obtained by maceration with 95% ethanol were examined. The antioxidative activity was evaluated by the total redox potential of crude extracts (FRAP assay), together with the free radical scavenging activity using $DPPH^{\cdot}$ and $OH^{\cdot}$ assay. The total phenol content in the extracts was determined colorimetrically with the FC reagent (2.03-4.78 mg/g). The highest $DPPH^{\cdot}$ and $OH^{\cdot}$ scavenging activity was found in the extract of *G. pfeifferi* (83.22% at 60 µg/ml), and 60.56% at 0.166 µg/ml for $OH^{\cdot}$ assay, respectively. The extract of *G. resinaceum* showed 88.18% at 80 µg/ml. The antioxidative activity was in correlation to the total phenol content.

BBIOREFCODE - A NMR DATABASE FOR URINARY METABOLITES

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NMR spectroscopy based metabonomics offers an accurate and rapid method to bodyfluid analysis and screening for applications like

(1) identification and quantification of single metabolites or specific biomarkers in urine, plasma, and cerebrospinal fluids
(2) diagnosis of metabolic disorders in newborns and children by urine screening for clinical research[1].

The composition of urine not only reflects metabolism per se, but also the effects arising out of the intake of specific food, drugs or (toxic) chemicals, and various metabolic diseases. The characterization of the "abnormal" deviations within the natural variation of healthy peoples' urine is a task best solved by the conjunction of dedicated NMR methods, statistical models, and databases.

BBIOREFCODE (Bruker Biofluid Reference Compound Database) is a database of presently 600 reference compounds of biofluid metabolites. It contains 1D and 2D NMR spectra of each compound, and also covers a pH value range from 3 to 8 in 0.5 pH steps. This adds up to a database volume of more than 17'000 single NMR spectra so far.

The BBIOREFCODE database significantly facilitates the analysis of urine as a mixture of >1000 NMR-detectable compounds, and allows to safely identify and quantify single compounds, like specific biomarkers.

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PAIRED EMITTER DETECTOR DIODE BASED FLOW SYSTEM FOR PHOTOMETRICvDETERMINATION OF CREATININE

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Creatinine is a waste product formed in muscle because of the break-down of a high-energy storage compound, creatine phosphate. The concentration of creatine phosphate in muscle is approximately four times larger than the concentration of adenosine triphosphate. In muscles it spontaneously undergoes degradation to form a cyclic anhyride-creatinine. The concentration of creatinine in blood and its excretion in urine are remarkably constant in normal individuals. In modern clinical analysis, creatinine has become one of the most requested analytes. Determination of this metabolite in various biological fluids is useful for evaluation of dysfunctions of many organs such as: kidneys, muscles and thyroid. Such analyses are helpful for biomedical diagnosis of acute myocardial infarction as well as for quantitative description of hemodialysis therapy.

Usually, creatinine is determined by Jaffe's reaction where the target analyte creates an orange compound with picric acid in alkaline medium. The changes in color are the source of analytical signal. This method of creatinine determination has been applied in the presented MCFIA system coupled with paired emitter detector diode (PEDD) [1,2] as a dedicated flow-through detector. Flow system was constructed with the microsolenoid devices that enable simple sample handling in the system in fully mechanical mode. Operational conditions of the developed MCFIA system and PEDD detector have been optimized

The developed analytical system has been used for creatinine determination in real samples such as urine and serum. The obtained results have been compared with those obtained using routine method applied in clinical laboratory.

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MICROPROCESSOR-CONTROLLED FLOW ANALYSIS SYSTEM FOR SIMULTANEOUS DETERMINATION OF ACID AND ALKALINE PHOSPHATASE IN HUMAN SERUM SAMPLES

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Many enzymes which are located in human serum are the targets of routine clinical analysis. Especially phosphomonoesterases like alkaline phosphatase (ALP, EC 3.1.3.1) and acid phosphatase (ACP, EC 3.1.3.2) belong to the group of enzymes that are the most commonly assayed in clinical laboratory. A rise in ALP activity occurs with diseases of many systems: skeletal (Paget's disease, osteomalacia), renal (Cushing's syndrome), digestive (cholestasis, particularly obstructive jaundice). The measurements of ACP activity shows significantly elevated serum values in case of prostate cancer. Thus, determinations of ALP and ACP serum activities are particularly important in oncological diagnostics.

A mechanical, multicommutated flow analysis system based on solenoid microdevices: valves and pumps have been developed for simultaneous photometric determination of alkaline and acid phosphatase activity in human serum. The system is based on newly developed compact flow-through detector dedicated for selective photometric detection of the product of enzymatic reaction p-nitrophenol formed in the course of assays. The detector has been constructed using ordinary light emitting diodes, implementing the PEDD concept [1,2].

The proposed analytical procedure based on kinetic methodology of enzyme activity detection (Bessey and Lowry method) eliminates interferences caused by intense color of serum samples. After optimization the system allows analysis of human serum with optimum parameters such as: high sensitivity, selectivity and relatively short time of determination. The system validated with real clinical samples is useful for determination of both enzyme activity in human serum at physiological and pathological levels. The results of analysis obtained using the developed bioanalytical system are well-correlated with those offered by reference methods recommended for clinical analysis.

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MICROEMULSION ELECTROKINETIC CHROMATOGRAPHIC ANALYSIS OF SOME ANTI-DIABETIC DRUGS

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Microemulsion electrokinetic chromatography (MEEKC) is an electrophoretic technique particularly suitable for the separation and analysis of pharmaceuticals. We have studied the possibilities to use this technique for the separation, identification and quantification of cationic and anionic anti-diabetics (metformin, glibenclamide, gliclazide) in complex mixtures.

Several U/A microemulsions were tested, using different proportions of n-heptane and acetyl acetate as oil, sodium dodecylsulfate (SDS) as surfactant, 1-butanol as co-surfactant, and aqueous buffer solutions (borate and phosphate) with pH values in the range 7 to 10. The optimum microemulsion composition was established: 0.8% w/w n-heptane, 3.31% w/w SDS, 6.61% w/w 1-butanol and 89.27% w/w pH 9 buffer solution (a mixture 95/5 v/v of 25 mM borate buffer and 25 mM phosphate buffer solutions).

The influence of the experimental parameters (cassette temperature, separating voltage, type and the pH of the running buffer) on the electrophoretic mobility of the analytes and on the separation efficiency was studied. Efficient separation of anti-diabetic drugs like glibenclamide, gliclazide and metformin (with different lipohylicity and distinct acid-base function) in mutual or complex mixtures was achieved using a 20 kV applied voltage at 25°C (cassette temperature). The UV detection wavelengths used were 230 and 300 nm (with full spectra recorded between 200 and 600 nm).

The newly established method was thoroughly validated and it proved to be linear in the concentration range between 10 and 100 microg/mL for glibenclamide and gliclazide (r = 0.9995 and 0.9991, respectively) and between 20 and 200 microg/mL for metformin (r = 0.9992). The detection limits are 7 microg/mL, 6.3 microg/mL and 4 microg/mL, respectively, when using a hydrodynamic injection 5 s at 50 mbar.

The proposed method is simple, fast and reliable, with very good results in the assay of the three antidiabetic drugs in various mixtures.

NEW HPLC METHOD FOR THE ASSAY OF SIMVASTATIN AND ITS IMPURITIES

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Cholesterol lowering drugs (simvastatin is one of the first and still most used in therapy) are frequently prescribed to reduce morbidity and mortality related to coronary heart disease. The methods used for the assay of simvastatin as literature presents are mostly liquid-chromatographic, RP-HPLC using C8-C18 stationary phases and mixtures of aqueous buffer solutions and polar solvents as mobile phases.

We have developed a new alternative chromatographic method for the assay of simvastatin and the detection of its possible impurities, with respect to their neutral character (due to the lactone form) and the high structural similarities between them, using a less explored porous graphitic carbon stationary phase (the specific retention mechanism consists of dispersive analyte-graphite interactions, with higher retention for the more hydrophobic molecules).

We have studied the behavior of simvastatin and its impurities in a chromatographic system consisting of a Hypercarb column (100x4.6 mm) and a water/acetonitrile mobile phase with a specific gradient programm and a 2.5 mL/min flow rate. The aquisition channels were 238 and 254 nm, with full spectra registered between 200 and 600 nm. The critical parameters for the newly developed method (mobile phase composition, flow rate, temperature) were studied, and the optimum experimental conditions in terms of efficiency and resolution were established with the final goal of testing method's suitability for the analysis of simvastatin in bulk and pharmaceutical dosage forms.

The method was validated with respect to the linearity, precision, accuracy, selectivity, detection and quantitation limits and was compared to the official assay method indicated in the Ph. Eur. Simvastatin monograph.

STRESS STABILITY STUDY OF OLANZAPINE IN ITS DOSAGE FORMS: PROJECTION OF DRUG PRODUCT SHELF-LIFE

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The objective of the present study was to investigate the stability profile of Olanzapine, second generation antipsychotic agent. Olanzapine film-coated tablets were exposed to different stress conditions, which were used as a main tool for identification of degradation products and pathways, as well as prediction of shelf life. According to preliminary stability results level of impurities and degradants were shown to be the most restrictive parameter and were therefore used in this stability evaluation. Three identified impurities, unidentified and total impurities were monitored. Analysis of samples was performed using RP-HPLC. Separation was achieved on a C_{18} column (150 mm x 4.6 mm, 5µm) with a mobile phase composed of acetonitrile, methanol and citrate buffer pH 5.9 (20:8:72, v/v/v). Detection was performed at 260 nm using DAD detector. The results demonstrated that Olanzapine film-coated tablets were sensitive to temperature and moisture. The influence of temperature on drug product shelf-life was studied through kinetics of formation of the main degradation product (impurity at RRT 1.35). The increase of the main degradation product was shown to be highly temperature dependant and for product protected from moisture followed zero order kinetics. Observed rate constants at each stress temperature were calculated. Using Arrhenius equation predicted rate constant at temperatures below 25°C was determined. Based on the results of only 3 months a shelf-life of two years could be assigned. Formal stability studies conducted in the latter phase of development confirmed the results of preliminary tests. Furthermore, identification of this impurity enabled extension of shelf-life for Olanzapine film-coated tablets to three years.

DETERMINATION OF BIPHOSPHONATES AND RELATED SUBSTANCES IN PHARMACEUTICAL FORMS BY RP-HPLC WITH UV DETECTION

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A new RP-HPLC method has been developed for simultaneous determination of zolendronic, alendronic and pamidronic acids and their related substances (phosphites and phosphates) in pharmaceutical forms. Separation of analytes was achieved on IC-Pak Anion HR, 75 x 4,6 mm, 6 µm column, using 3.0 mmol/dm^3 HNO_3 as a mobile phase at flow rate 1.0 mL/min and 30 µL injected volume. The column was thermostated at 30^{0}C. The detection method was UV at 240 nm. The method was validated with respect to linearity, trueness, precission, limit of quantification and specificity. The linearity of determination was in the concentration range 0.08 – 0.12 mg/mL, with average recovery values 101.9% for zoledronic acid, 102.5% for pamidronic acid and 104.3% for alendronic acid. Relative standard deviation in peak areas (n = 10) was less than 0.5%. Linearity of determination of related substances was between 0.002 and 0.02 mg/mL. The content of phosphates in all forms was less than 0.2% while content of phosphites was less than 0.05%. The developed method allows the determination of any phosphonic acid (ie no change of method is required for different acids) and its related substances in pharmaceutical forms with minimal sample preparation, short analysis time and very simple mobile phase and detection method. The obtained results are precise and accurate and represent significant advancement in comparison with parmacopoeial methods.

VOLATILE CONSTITUENTS OF THE ESSENTIAL OIL FROM THE AERIAL PARTS OF *FERULAGO ANGULATA* (SCHLECHT.) BOISS.

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The genus *Ferulago* consists of 35 species, seven of which are found in Iran, two of these being endemic: *F. contracta* Boiss. et Hausskn. and *F. phialocarpa* Rech. f. et H. Riedl [1,2]. The chemical composition and biological activities of some species of the genus *Ferulago* have been the subjects of some earlier studies [3-5]. In this study, the essential oil obtained by hydrodistillation of the aerial parts of *Ferulago angulata* (Schlecht.) Boiss. (Umbelliferae) , growing wild in Khorasan province (Iran), were analyzed by GC and GC/MS. Forty five compounds representing 93.2% of the aerial parts oil of *Ferulago angulata* were identified, of which α-pinene (10.5%), limonene (9.6%), β-myrcene (5.5%) and fenchyl acetate (4.2%) were the major ones. Comparison this results with previous report on the aerial parts of *Ferulago angulata* shows significant differences. The major components of previous report were α-phellandrene (24.2%), β-phellandrene (14.9%), α-pinene (14.7%), p-cymene (10.3%) , δ-3-carene (6.7%) and (Z)-β-ocimene (5.8%). [3]. The present investigation revealed that the aerial parts oil of the plant consist of monoterpenes as major constituents (75.1%)

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VALIDATION AND APPLICATION OF A MODIFIED RP-HPLC METHOD FOR THE QUANTIFICATION OF RELEASED DESLORATADINE DURING INTRINSIC DISSOLUTION TEST

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The main aim of the present study was to develop a simple, sensitive and rapid RP-HPLC method for the determination of desloratadine for the purposes of the intrinsic dissolution test. The HPLC system consisted of HPLC apparatus Agilent Technologies 1200 series equipped with a DAD detector. The analyses were carried out in a reverse phase column Zorbax SB C18, 250 x 4.6 mm, 5 µm particle diameter. Mobile phase was mixture of methanol and trifluor acetic acid buffer (pH 3.5 ± 0.05 adjusted with triethyl amine) in volume ratio 76:24. The HPLC analyses were performed at 35°C, DAD detector was operated at a wave length of 238 nm and the flow rate was 0.8 ml / min. Sample aliquots were filtered through 0.45 µm pore-sized regenerated cellulose syringe filter and were injected in volume of 100 µl. The retention time of desloratadine was about 5 minute. The method was validated in term of linearity, precision and accuracy. The linear calibration curve was obtained over the range of 0.1 to 0.7 µg /ml (r^2 =0.9998). The accuracy was within 99.56% and 101.04%. The intra-day and inter-day RSD were all less than 1.02%. The limit of quantification was 0.1 µg /ml and the limit of detection was 0.05 µg /ml.

This method was simple,rapid and suitable for determining the low content of released desloratadine during intrinsic dissolution test.

FIRST DERIVATIVE UV –SPECTROPHOTOMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF METOPROLOL AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORMS

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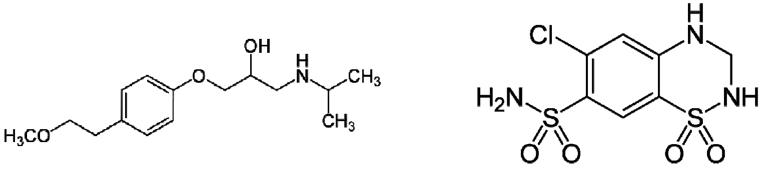
β-blockers are clinically important drugs and are used in the treatment of disorders such as hypertension, angina pectoris and arrhythmia (1). Metoprolol (Figure 1) is a relatively selective β-1 adrenoceptor antogonist that has been used extensively for more than 25 years to treat such cardiovascular disorders as hypertension, arrhythmia and heart failure (2).



I II

Figure 1. Chemical structures of metoprolol(I) and Hydrochlorothiazide(II).

Metoprolol as well as hydrochlorothiazide in complex drugs were determined with chromatographic methods (3). In this study a new spectrophotometric method for simultaneous determination of hydrochlorothiazide and metoprolol is presented. The first derivative absorption spectra at 270,4 nm were used for metoprolol and at 279.7 nm were used for hydrochlorothiazide. No interferences were found between both determined constituents and those of matrix. The recovery of individual constituents under established conditions is very high and ranges from 98.79% to 99.39%. Linearity is maintained within a wide concentration range from 100.0 $\mu g.mL^{-1}$ to 300.0 $\mu g.mL^{-1}$ and from 12.5 $\mu g.mL^{-1}$ to 37.5 $\mu g.mL^{-1}$ for metoprolol and hydrochlorothiazide, respectively. The method gave satisfactory results with regard to accuracy and precision and at the same time, selectivity was established which was evident from the excellent values of mean percentage recoveries and low values of standard deviation.

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EFFICIENCY OF ENDOCRINE DISRUPTORS REMOVAL IN THE POMERENIAN VOIVODSHIP WWTPs (POLAND)

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Results of numerous studies conducted during last years may constitute the basis for the statement that pollutants emitted from anthropogenic sources may pose new kind of threat, from which people didn't realise before. These pollutants are substances belonging to EDC group defined as any agent that is able to affect synthesis, secretion, transport, binding, action or excretion of natural hormones present in the organisms and that are responsible for homeostasis, reproduction or behaviour. Chemicals belonging to EDC group occur naturally in the environment, unfortunately many synthetic compounds or products released during production of the consumption goods also possess hormone-like properties. Also numerous factor influences the toxicity level of the endocrine chemicals. One should mention rate of intake and assimilation, decomposition in the organism, bioavailability, mobility, timeframes of exposure, diet, individual diversity.

Application of the procedure of collecting samples, their treatment and methodological solutions presented are the effect of both literature studies and own elaborate. Elaborated analytical methodology constitutes scientific novelty due to:

- possibility of using in order to determine EDC at trace levels in aqueous samples of different level of contamination,
- small volumes of reagents used,
- application of non-toxic compounds,
- high recovery of analytes.

It was proved that analysis with the aid of LC/MS/MS is the most rational solution aiming at detection of endocrine disrupting compounds belonging to different categories and chemical classes. One of restrictions of the chemical monitoring is difficulty in assessing the results of co-existence of numerous chemicals at different concentration levels and their inter-relationship. Application of biotests as the indicators of the environmental pollution eliminates these difficulty and enables obtaining information about the final threat posed and resulting from the presence and the sample's level of contamination, pollutants' bioavailability and interactions between them.

SPECTROFLUORIMETRIC DETERMINATION OF ERYTHROMYCIN IN PURE AND DOSAGE FORM

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Erythromycin (EM) and erythromycin estolate (EME) are a broad spectrum macrolide antibiotics that exhibit high activity against nearly all Gram-positive and Gram-negative bacteria, hence, it is widely used in the human and animals therapy.

In our studies we propose simple, fast and accurate spectrofluorimetric method of erythromycin determination in pure and dosage forms.

Fluorometric measurements were performed on a Hitachi F-7000 Fluorescence Spectrophotometer. Excitation and emission slit widths were 5 nm. Individual and three-dimensional spectra were recorded in the range 200–900 nm. The spectra were evaluated with the PC software package supplied with spectrophotometer (FL-Solution 2.1 for F-7000). Pharmacopoeial and HPLC methods were applied as reference one.

The influence of pH solution and presence selected cations on fluorescence intensity were studied too. It was ascertained that the highest fluorescence intensity take place at pH=7. Moreover, the increasing of fluorescence intensity was observed at presence Na^+, K^+, Ca^{2+} and Mg^+ in contrast to Fe^{3+} which caused quenching of intensity. Moreover, the fluorescence intensity of erythromycin estolate was higher than erythromycin. Under optimized experimentation conditions, EM and EME demonstrated good linearities between the concentrations and fluorescence intensities. The examined concentrations were from 1 to 50 mg/dm^3. The correlation coefficients of calibration curves were between 0.9995 and 0.9999. These curves were used to calculate the unknown concentrations in the studied pharmaceuticals. The obtained recovery were from 95,6% to 98,9%. Moreover, the relative standard deviation was below 0.54% confirmed high precise of the method. The statistical test (t-Student and F-Snedecor) used for comparison spectrofluorometric, pharmacopeial and chromatographic methods pointed that they are comparable in respect of precision but not of accuracy.

LIPOPHILICITY OBTAINED BY TLC IN PREDICTION OF ACE INHIBITORS ABSORPTION

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Since, oral dosing is the most common route of drug administration the final drugs plasma concentration depend on the first place of its absorption. Lipophilicity is one of the essential properties of biologically active substances and has significant influence on drugs absorption. The more lipophilic molecules exert better absorption. For drugs absorption the important influence has the formulation.

Angiotensin converting enzyme (ACE) inhibitors are complex organic molecules in the form of prodrugs. Following administration, these esters undergo hydrolysis into diacidic active metabolites, with exception of lisinopril (already diacidic form). The substances investigated were: *Enalapril, Quinapril, Fosinopril, Lisinopril, Cilazapril and Ramipril.* These ACE inhibitors can be found in the form of tablets (enalapril, fosinopril, lisinopril, ramipril) or film-coated tablets (quinapril, cilazapril).

Continuing our previous TLC studies of ACE inhibitors lipophilicity, this work presents applicability of chromatographically obtained lipophilicity data in absorption prediction.

The examination of ACE inhibitors lipophilicity, were performed by NP and RP TLC, as previously studied, with adition of ramipril for absorption prediction. The relationships between the chromatographically obtained hydrophobicity parameters R_M^o and C_0 and recalculated (KOW WIN) log*P* values (in the range from -0.94 to 6.61) as well as the relationships between R_M^o and C_0 and literature absorption values (25% to 60%) were investigated. The good correlations indicate that both NP and RP TLC methods, can be successfully used not only in ACE inhibitors lipophilicity evaluation but also in prediction their absorption. In conclusion chromatographically obtained hydrophobicity parameters of ACE inhibitors, R_M^o and C_0 values, can indicate prodrug absorption.

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DETERMINATION OF THE ACTIVE COMPONENTS OF HORSE LINIMENTS BY MICROEMULSION LIQUID CHROMATOGRAPHY

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Liniments are commonly used by trainers and owners of horses both in everyday training and in complex medical treatment. The largest group of these preparations are the Analgesic Liniments. Analgesic horse liniments are the strongest variety, typically possessing ingredients such as natural aroma oil, capsaicin or menthol. Unfortunately, due to the high content of the active component, allergy reactions take place very often. That's why the control of the concentration of the active compound in the liniments is very important.

The technique of microemulsion liquid chromatograhy (MELC) was described as a perspective one for a simultaneous determination of both the hydrophilic and hydrophobic compounds in an isocratic mode. Due to high content of aqueous phase oil-in-water microemulsions are compatible with usual reversed-phase columns.

The main disadvantage of the microemulsion mobile phases is their high viscosity. Different ways to reduce the pressure in the chromatographic system were investigated and compared. The technique of the microemulsion preparation was optimized. Microemulsion of 3.3% SDS, 1% heptane, 8% n-butanol and 0.05% TFA was shown to be the most suitable for the determination of capsaicin and menthol in liniments.

In current work a number of horse liniments from ZooVip® company were succsessfully analysed by MELC. As it was shown, the suggested technique may be easily transferred to the analysis of the oil-based ointments without any changes in the procedure of the sample pretreatment and the separation conditions.

RAPID DETERMINATION OF VALSARTAN IN PHARMACEUTICAL DOSAGE FORMS BY HPLC

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HPLC method for the determination of valsartan in tablets is developed and validated. The liquid chromatography was performed on an column LiChrospher 100 RP Selekt B (250 mm × 4.0 mm, 5μm particle size), the mobile phase consisted of acetonitrile − 10 mM ammonium hydrogen phosphate, pH 3.0 (70:30, v/v). The run time was 2.7 min. The flow rate was 1.0 ml/min, with UV detection at 230 nm. The proposed method was validated as international conference on harmonization (ICH) guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy and precision. This method was also suitable for the assay determination of valsartan in pharmaceutical dosage forms.

CHAOTROPIC USE IN FAST SIMPLE 4 COMPONENTS QUANTIFICATION OF SALBUTAMOL SYRUP

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Understanding of the mechanism of action of chaotropic anions in basic molecules containing N-atoms in their structure, as huge majority of active pharmaceutical ingredients are, can be very useful in some HPLC method developments, especially when components are very differ in their polarities or solubility's in different solvents. For instance, the liquid pharmaceutical dosage form, Salbutamol syrup which contains active ingredient Salbutamol and three preservatives like K-sorbate, Nipagin and Nipasol , which differ between themselves in their hydrosolubility, polarity, can not be simultaneously separated in fast and reliable manner without use of long isocratic acidic chromatographic run, or use of gradient separation. Other idea could be an ion-pairing of Salbutamol with alkyl-sylphonates for increasing its retention, but this will change the column`s characteristics and extend the equilibration time and cost of analysis.

We considered and performed the use of most powerful chaotropic PF6- anion for increasing Salbutamol as most polar component in the syrup formulations, and created very simple, short, accurate, reproducible and rugged method for simultaneous quantitative determination of Salbutamol, Potassium Sorbate, Nipagin and Nipasol in less than 6 minutes with RP Select B (L7) column with 125 x 4 mm with 5um particles, with minimal Resolution 3.95 for the nearest peaks. Increasing the percentage of organic for 5% shortens the run time in les than 3.8 minutes without significant scarifying of minimal peak resolution to Rs min= 2.97. The method can be modified further for large number of analysis per day if we use the same chromatographic matrix with column dimensions of 75 x 4 mm with 5um particles, and almost

halving the run time, keeping in mind that halving the column length reduces the resolution with the square root of it, generating final reducing the Resolution values for about 1.42.

ASSESSMENT OF INDOXYLACETATE AS A SUBSTRATE FOR ACETYLCHOLINESTERASE

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Biochemical assessment of acetylcholinesterase (AChE) activity is a standard protocol in biochemistry, pharmacology, and toxicology. In 1960s, Ellman´s method was developed for the purpose of AChE activity assay and it became routine after some optimizations. The method has however some disadvantages and limitations in its performance. Firstly, the used reagents are labile and fresh reagents have to be prepared at least every day. Secondly, some compounds interfere in the assay. Glutathione, thiol containing compuonds and oxime containing drugs are the most relevant. We infer to introduce indoxylacetate based assay for assessment of AChE activity. The assay was tested and basic parameters were established for the indoxylacetate. Michaelis constant and maximum reaction velocity were calculated for electric eel AChE. The assay was also performed for estimation of oxime rectivators obidoxime, pralidoxime, and HI-6 efficacy after paraoxon ethyl inhibition. The whole experiment was repeated using Ellman´s method and the both methods were compared. Significant advantages of indoxylacetate as a substrate are reported.

NOVEL APPROACHES TO KINETIC DETERMINATION OF ANTIMALARIAL COMPOUNDS

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Malaria remains a relevant problem, especially in Third World countries. Today the pharmaceuticals for the treatment of malaria are based on the endoperoxide compounds, which contain peroxide bridge in their structure. The most widespread antimalarial compound (AC) is artemisinin (AM), the natural extract from leaves of *Artemisia Annua* herb growing in China; in addition, new synthetic endoperoxide compounds are developed for the improvement of antimalarial drug efficiency.

Unfortunately, there are no accredited or recommended procedures for the determination of natural or synthetic AC; the existing procedures employ liquid and gas chromatography. The difficulties of the determination of these compounds are caused by their insolubility in water, absence of absorbance in visible region of spectrum, chemical stability, and poor redox properties. These problems especially refer to synthetic compounds. Thus, the development of novel procedures for the determination of AC in pharmaceuticals and blood is a topical problem.

In this work novel approaches are proposed for the determination of natural (AM) and synthetic (1,2,3,4-tetraoxyne, TO) compounds using kinetic non-catalytic method.

The kinetic procedure for the determination of AM is based on two coupled reactions: the first one is the reaction between AM and HCl with a formation of H_2O_2; the other is the reaction between H_2O_2 and iodide ions. The rate of the indicator reaction is controlled by spectrophotometric method by the absorbance of I_3^- ions at 350 nm as an indicator substance. Under optimal conditions the analytical range of AM is 150 – 1000 µM with RSD 0.8%. The developed procedure was applied for the determination of AM in pharmaceutical formulation.

The oxidation of *o*-dianisidine by H_2O_2 in the presence of horseradish peroxidase has been proposed as an indicator reaction for the determination of TO where the latter plays a role of co-oxidizer along with H_2O_2. Under the optimal conditions the limit of TO quantification is 200 µM with RSD 1.5%.

Therefore, the novel indicator systems were proposed for the rapid and inexpensive kinetic and enzymatic determination of AM and TO.

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A SIMPLE METHOD FOR THE AMPICILLIN DETERMINATION IN PHARMACEUTICALS AND HUMAN URINE

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The kinetic–spectrophotometric method for the determination of micro quantities of ampicillin (Amp) in solution has been developed. It was based on its reaction with Ni(II) ions in alkaline medium in the presence of KCl, as the electrolyte regulating ionic strength of the solution. The reaction rate was monitored at 265 nm. A differential variation of the tangent method was used to process the kinetic data. Examining the impact of each reactant on the reaction rate, optimal working conditions for kinetic determination of Amp at 25 ºC in solution were determined: $c(Ni^{2+}) = 3.0 \cdot 10^{-4}\ mol \cdot dm^{-3}$; $c(NaOH) = 4.0 \cdot 10^{-2}\ mol \cdot dm^{-3}$; $c\ (KCl) = 1.5 \cdot 10^{-2}\ mol \cdot dm^{-3}$. Relative standard deviation for the Amp concentration interval from 3.49 to 55.84 $\mu g \cdot cm^{-3}$ ranges from 7.79 to 3.20 %. Detection limit, calculated according to the 3S criterium, is 2,58 $\mu g \cdot cm^{-3}$. The kinetics equation for reaction was given on the basis of tgα dependance on reaction components concentration. The relative reaction rate constant was calculated for temperatures 19, 22, 25 and 28 ºC. According to the relative reaction rate constant values for four temperatures, the thermodynamic parameters were obtained for the proposed method: activation energy ($Ea^* = 21.05$ $kJ \cdot mol^{-1}$), activation enthalpy ($\Delta H^* = 18.57\ kJ \cdot mol^{-1}$), activation entropy ($\Delta S^* = -197.94\ J \cdot K^{-1} \cdot mol^{-1}$) and Gibb's energy ($\Delta G^* = 77.56\ kJ \cdot mol^{-1}$). The effects of some metal ions, aions, amino acids and other molecules which can be found as excipients in pharmaceuticals were investigated in order to assess the method selectivity. The method was applied to determining micro qunatities of Amp in capsules, powder for oral suspension and human urine. The obtained results are in accordance with the referent HPLC method (*British Pharmacopoeia, 2005, p.85; Jugoslovenska farmakopeja 2000, 2001, p. 42*) results.

SPECTROPHOTOMETRIC THERMODYNAMIC STUDY OF HISTIDINE CATALYTIC IMPACT ON AMPICILLIN DETERMINATION IN THE PRESENCE OF NI(II) IONS

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This paper presents results of spectrophotometric study of histidine (His) effect on the reaction between ampicillin and Ni(II) ions in alkaline medium in the presence of KCl, as the electrolyte regulating ionic strength of the solution. The reaction rate was monitored at 265 nm with respect to the blank reaction rate. A differential variation of the tangent method was used to process the kinetic data. Examining the impact of each reactant on the reaction rate, optimal working conditions for kinetic determination of His in solution at 25 ºC were determined: $c(Ni^{2+}) = 1.0\cdot10^{-4}$ $mol\cdot dm^{-3}$; $c(NaOH) = 3.5\cdot10^{-2}$ $mol\cdot dm^{-3}$; $c\ (KCl) = 7.5\cdot10^{-2}$ $mol\cdot dm^{-3}$; $c(Amp) = 8\cdot10^{-5}$ $mol\cdot dm^{-3}$. The system obeyed Beer`s law in the range from 0,31 to 1,55 and from 1,55 to 10,85 μg·cm^{-3}. Limit of detection is 0,05 μg·cm^{-3}. Relative standard deviation for the His concentration interval from 1.55 to 10.85 μg·cm^{-3} ranges from 8.05 to 2.02 %. The kinetics equations for both reactions were given on the basis of tgα dependance on reaction components concentration. The relative reaction rate constant was calculated for temperatures 20, 25 and 30 ºC. According to the relative reaction rate constant values for these temperatures, the thermodynamic parameters were obtained for the proposed method: activation energy (Ea* = 27.75 kJ·mol^{-1}), activation enthalpy (ΔH* = 25.27 kJ·mol^{-1}), activation entropy (ΔS* = 43.57 J·K^{-1}·mol^{-1}) and Gibb's energy (ΔG* = 12.29 kJ·mol^{-1}). Comparing thermodynamic characteristics of reaction system with and without (ΔS* = – 197.94 J·K^{-1}·mol^{-1}; ΔG* = 77.56 kJ·mol^{-1}) His it can be assumed that this aminoacid show catalytic role in reaction between ampicillin and Ni(II) in alkaline medium.

LED-BASED DETECTOR AND SENSOR FOR VITAMIN B2

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Riboflavin is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It is main component of flavin mononucleotide and flavin adenine dinucleotide cofactors and is therefore required by all flavoproteins. Riboflavin is necessary for a wide variety of cellular processes and plays a key role in energy metabolism. Deficiencies of the riboflavin cause damage to the skin, eyes and mouth. Riboflavin is vitamin exogenous, deliver to the organism mainly in the form of milk products or vitamin preparations and nutrients.

Riboflavin, also known as B_2 vitamin or E101 food additive, imparts the orange color to solid B vitamin preparations, and the yellow color to vitamin supplement solutions. Moreover it is used as to food coloring, as well as to fortify some foods like fruit and energy drinks.

In this contribution an optoelectronic riboflavin detector is presented. Its operation is based on the concept of fluorometric paired emitter detector diode [1], where the fluorescence of analyte is induced and detected by selected light emitting diodes (LEDs). The developed miniaturized fibreless detector made of three LEDs has been adapted for measurements performed under FIA conditions. The range of determination and detection limit offered by the device is sufficient for determination of riboflavin in pharmaceutical products and vitamin-enriched drinks. Further improvement of analytical characteristics of the developed FIA system can be done by coupling of the detector with separation/preconcentration flow-through units like C18-microcolumns.

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HPLC DETERMINATION OF SYNTHETIC ANTIOXIDANTS IN MICROEMULSIONES FOR ORAL USE

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Synthetic antioxidants are a considerable group of food additives which are used for preservation of foods. They protect oxidizable nutrients and thus extend the shelf-life of foodstuffs. Many biologically active compounds beneficial to human health i.e. vitamins, carotenoids, flavonoids are prone to oxidation and degradation, and some of them have poor biopharmaceutical properties i.e. water solubility, bioavailability. To improve water solubility of some poorly soluble bioactive compounds we prepared microemulsions, but their stability in these systems was insufficient. In order to increase stability of the bioactive compounds we used synthetic phenolic antioxidants *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), *tert*-butylhydroxyanisole (BHA). To follow and quantitate these antioxidants in nutraceutical delivery systems a reversed-phase HPLC method was developed. Different reversed-phase columns (BDS Hypersil C18, Phenomenex Luna C18 and Gemini C6-Phenyl) and various mobile phases containing different ratios of organic solvents (acetonitrile, methanol, THF) and water were tested. Optimisation of the selectivity and run time was performed. Intermediate repeatability of injection of standards, range, limit of detection and quantitation were determined for the best HPLC separation. Determination of antioxidants in microemulsions is easier than in foodstuffs, because no extraction of antioxidants is needed. Samples were prepared by simple dilution of microemulsions.

LIQUID CHROMATOGRAPHY COUPLED TO INDUCTIVELY COUPLED MATRIX - MASS SPECTROMETRY (LC-ICP-MS) FOR THE STUDY OF IN-VIVO METABOLITES OF ETHACRYNIC ACID

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Recently, LC-ICP-MS has been introduced to deal with some applications in the field of pharmaceutical, biomedical and clinical analysis. In the case of drug research, the number of drugs and its metabolites containing detectable elements such as metals, Cl, S, P and Br is quite limited (1). The conjugation of drug related compounds with endogenous molecules such as glutathione opens up great possibilities to study phase II biotransformations.

In this communication, the GSH adduct formation of ethacrynic acid has been characterized in vivo. Biological samples consisting of bile of rats have been analyzed by LC-ICP-MS for GSH derivatives. In this case, ethacrynic acid is directly conjugated to GSH. All drug related species have been separated by reversed-phase HPLC using an elution gradient consisting of a linear increase of methanol percentage in the mobile phase. For ICP-MS detection, glutathione derivatives and related compounds have been detected from SO^+ at m/z 48 amu. Complementarily, Cl-containing compounds can also be monitored via Cl although the sensitivity for Cl ions is comparatively lower.

Apart from qualitative information aimed at detecting metabolites, quantitative data are also available. For quantification purposes, it has been encountered that the percentage of organic solvent in the mobile phase strongly affects the sensitivity. Here, corrective strategies based on calibration curves established at different solvent concentrations and post-column solvent gradient corrections have been proposed to circumvent sensitivity variations. Results obtained have shown that suitable calibration models have been built for any compound regardless the solvent percentage at which is eluted.

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PM40 **Session C**

ANALYTICAL STUDIES OF SOME CEPHALOSPORIN ANTIBIOTICS FROM AQUEOUS SAMPLES

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Cephalosporins can be considered to be one of the most important and most frequently used groups of the antibiotics applied in medicine, which are grouped into "generation" by their antimicrobial properties. For pharmacokinetic studies, development of rapid, sensitive and selective analytical methods for the determination of drugs in biological fluids is required.

The aim of the present work was to develop reliable methods, such as HPLC (a rapid and specific method widely installed in clinical settings), UV spectrometry, voltammetry applicable to various cephalosporin's determination from aqueous samples. For HPLC determinations optimized method proved a sensitivity of $4.5\cdot10^{-4}$ µg mL^{-1} for cefotaxime sodium, $8.5\cdot10^{-4}$ µg mL^{-1} for cefuroxime sodium, and, respectively $6\cdot10^{-4}$ µg mL^{-1} for cefalotin sodium, the recovery of the active compounds ranging between 96 to 106 %, depending on the analysed artificial samples.

The electrochemical behavior of the antibiotic drugs in Britton–Robinson buffers (pH 2–11) at different working electrodes (platinum, glassy carbon, gold) was studied by means of cyclic voltammetry, diffential pulse voltammetry and square wave voltammetry.

Also, the behaviour of the studied cephalosporins (cefotaxime sodium, cefuroxime sodium, cefalotin sodium, cefepime and cefpirome) in fluid body was studied.

The specific parameters of the used methods in the view to establish the range of applicability for pharmaceutical formulation were optimized.The results obtained for these 5 cephalosporins by different analytical methods were compared.

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DETERMINATION OF ENALAPRIL *CIS/TRANS* ISOMERS BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY- ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Enalapril as Angiotensin I-converting enzyme (ACE) inhibitors, are generally used as effective drugs in the treatment of hypertension and heart failure [1]. In the recently time [2] it is proposed that the rotational isomers containing a proline tertiary amide bond should be described as either *Z*-(*cis*) or *E*-(*trans*) according to the Cahn–Ingold–Prelog (CIP) rules as encoded in IUPAC.
The structural studies using X-ray analyses proved that enalapril in the solid state, such as a crystal or powder, usually exists as the *Z*-(*cis*) form [4]. On the other hand, enalapril in solution forms *Z*-(*cis*)/*E*-(*trans*) mixtures by undergoing reversible interconversion. The *Z*-(*cis*)/*E*-(*trans*) isomeric composition of enalapril in polar solution has been investigated by NMR [5], which indicated that the *Z*-(*cis*) isomer is predominant in aqueous solution.
As a result persisting two isomers of enalapril, determination of enalapril maleate using RP HPLC technique with photodiode ray detector (PDA) on C18 and C8 columns, gives broad peak of enalapril, usually with tailing larger than 2. Since Ultra High Performance Liquid Chromatography (UHPLC_PDA) offers better quality of obtained data: resolution, peak shape, and efficiency, the goal of this paper was to transfer HPLC method for determination of enalapril to UHPLC. However, during the transfer, using BEHC18, 1.7 μm, 150 x 2.1 mm column and ACN:0.1% HCOOH (25:75), instead of one broad peak of enalapril, two peaks appeared, separated with elevated baseline.
In the further investigation it was necessary to confirm the two peaks of enalapril. For such a purpose, UHPLC system Accela, with Triplequadrupole mass detector was used. Identification and conformation of MS / MS analysis was done based on two specific SRM transition of 337.1-> 303.1 and 337.1-> 234.1. The chromatograms are identical to the chromatograms obtained UHPLC with PDA detector. Chromatograms for selected transition gives two separate peaks, which suggests that this is the same compound different izomers. The results will be subject to further analysis.

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DETERMINATION OF FLAVONOIDS IN VARUMIN® ORAL SOLUTIONS

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Varumin® is a herbal medicinal product, dedicated primary for treatment of different cancer types. Herbs are adjusted in aspect of composition and content, and thus producing superior synergistic action of the products. Origin of raw materials and also technological procedure are without presence of chemical or biological contaminants. Flavonoids or bioflavonoids are a class of plant secondary metabolites or yellow pigments. They are most commonly known for their antioxidant activity. Consumers and food manufacturers have become intersted in flavonoids for their possible medicinal properties, especially their putative role in prevention of cancers and cardiovascular diseases.
Because use of herbal medicines on the market is tendy, it is necessary to control product quality. The aim of this work is to test chemical properties of product. Specifically, to determine flavonoids in Varumin® oral solutions. Rutin and quercetin were analysed flavonoids, because both have strong antioxidant properties. High performace liquid chromatography with diode array detector was used to determine compounds separatelly. Gradient elution was done using mobile phase: acetonitrle and 0.01 M H_3PO_4 (pH 2.1). Quantification of analytes was made at the wavelength of 367 nm.To determine total flavonoids spectrophotometry were used as a technique. The products were filtered through Sep-pack C_{18} cartridges to exclude chlorophyll and carotenoid pigments. Spectrophotometric measurements of the absorbance were made at 360 nm. Molar extinction coefficient of quercetin (ε_{360}=13.6 $mM^{-1} \cdot cm^{-1}$) was used to determine total flavonoid contents. Obtained results from tested oral solutions shown satisfactory values of total flavonoids presence.

POTENTIOMETRIC DETERMINATION OF GALANTAMINE BASED ON THE INHIBITION OF THE ENZYMATIC HYDROLYSIS OF ACETYLCHOLINE

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Acetylcholinesterase (AChE) is an important enzyme that is target of many neurotoxins and drugs that specifically bind to its active site resulting in a total inactivation of the enzyme. The controlled reversible inhibition caused by certain drugs, as is the case of galantamine, has interesting therapeutic uses including the treatment of Alzheimer's disease. Numerous methods for the determination of both AChE activity and inhibition have been reported, including several pH potentiometric sensors. To our knowledge, there are not reported in the literature any potentiometric method for determining AChE inhibitors by monitoring the concentration change of the substrate, acetylcholine (ACh), in the inhibited enzymatic reaction.

In the present work we applied an ACh-selective electrode, developed in our research group, to the study of the inhibition caused by galantamine in the AChE-catalyzed hydrolysis of ACh. The aim of the work was to characterize the inhibition process (i.e., competitive, noncompeitive or uncompetitive), to determine the inhibition constant (K_i) and to use the linear Dixon plot to the galantamine analytical determination.

The Dixon plot (reciprocal of initial rate *vs.* inhibitor concentration at different substrate concentrations) is frequently used for both identification of the likely mechanism of enzyme inhibition and for estimation of the K_i value. Galantamine obeyed the Dixon plot shape corresponding to a competitive inhibitor and the K_i was estimated to be 2.7×10^{-7} M.

Our next goal is to apply these results to the kinetic-potentiometric determination of galantamine in pharmaceuticals and biological fluids.

SIMPLE AND RAPID APPLICATION OF FIRST DERIVATIVE RATIO SPECTROPHOTOMETRIC FOR SIMULTANEOUS ESTIMATION OF EPROSARTAN AND HYDROCHLOROTHIAZIDE

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In this work; simple, and rapid application of first derivative ratio **s**pectra and first derivative spectrophotometric methods were been presented for the determination of eprosartan and hydrochlorothiazide in binary mixtures and its pharmaceutical dosage forms. On the first derivative spectrophotometric method ; solutions of standard and samples in methanol derivative absorbance (dA/dλ) signals were evaluated at 224.9 and 241.6 nm's for eprosartan and 233.1 and 255.1 nm's for hydrochlorothiazide, respectively. This method showed that there are linear in a range for eprosartan and hydrochlorothiazide 9.6 -60.0 μg/mL and 0.8 – 4.8 μg/mL ,respectively. The coefficient of correlation were calculated as 0.9898 and 0.9991 for eprosartan and 0.9985 and 0.9984 for hydrochlorothiazide, respectively.
On the first derivative spectra ratio spectrophotometry is used solutions of standard and samples in methanol first derivative spectra ratio spectra absorbance signals were measured at 237.7 and 243.5 nm's for eprosartan and 259.5 and 277.6 nm's for hydrochlorothiazide, respectively. In this method linear range were found as 9.6 -60.0 μg/mL for eprosartan and 0.8 – 4.8 μg/mL for hydrochlorothiazide ; the correlation coefficients, r, were calculated 0.9999 ve 0.9999 at 237.7 and 243.5 nm for eprosartan and 0.9994 and 0.9996 at 259.5 ve 277.6 nm's for hydrochlorothiazide,respectively . In this presented method; The limit of detection were calculated as 0.058 μg/mL and 0.047 μg/mL at 237.7 and 243.5 nm's for eprosartan and 0.082 μg/mL and 0.095 μg/mL of 259.5 nm , 277.6 nm's for hydrochlorothiazide. Besides , the limit of quantitation were established as 0.191 μg/mL and 0.151 μg/mL for eprosartan and 0.271 μg/mL and 0.313 μg/mL for hydrochlorothiazide..
These presented spectrophotometric methods as being simple,and rapid , without pre-analysis procedures and giving reproducable results, are shown to be suitable for the routine analysis.

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ADSORPTION OF ARSENATE BY IRON(III) OXIDE COATED ETHYLENEDIAMINE FUNCTIONALIZED MULTI-WALLED CARBON NANOTUBES

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In accordance with the World Health Organization recommendation, maximum allowed concentration of arsenic in drinking water has been reduced from 50 to 10 µg L^{-1}. The low restrictive limit for arsenic implies development of new sorbents of higher selectivity and capacity. In this work, iron(III)-oxide coated functionalized multiwall carbon nanotubes (MWCNT) were tested as potential sorbent. MWCNT were modified by oxidation (o-MWCNT) and by ethylenediamine functionalization (e-MWCNT), followed by coating of hydrous iron(III)-oxide on e-MWCNT surface by two protocols. First method involves adsorption of iron(II) on e-MWCNT, oxidation by $KMnO_4$ and precipitation by sodim-hydroxide, producing iron(III)-oxide coverage on e-MWCNT surface (e-MWCNT/Fe^{2+}). Equilibration of iron(III)-chloride and e-MWCNT system and subsequent precipitation of iron(III)-oxide gave e-MWCNT/Fe^{3+}, by the second method. Adsorbents were characterized by TGA, FTIR, XRD, BET, SEM and TEM techniques.

Sorption characteristics of e-MWCNT/Fe^{2+} and e-MWCNTs/Fe^{3+} were investigated in a batch system with respect to pH, equilibration time, mass of sorbent and initial arsenate concentration. The influence of solution pH was studied in a range 3 – 10 and As(V) initial concentration from 50 to 4000 µg L^{-1}. The concentration of As(V) has been measured using an ICP-MS system (Agilent Technologies 7500ce). The obtained adsorption equilibrium data were correlated by the use of Langmuir and Freundlich isotherms, and best fitting was obtained by the use of Freundlich model. Adsorption capacities obtained were 23.47 mg g^{-1} for e-MWCNT/Fe^{2+} and 13.70 mg g^{-1} for e-MWCNT/Fe^{3+}.

APPLICATION OF POTASSIUM FLUOROBROMATE(III) FOR SEPARATION OF PALLADIUM-TUNGSTEN ALLOYS

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The aim of this research is to show the principal possibility of potassium fluorobromate (III) $KBrF_4$ (a very prospective fluorinating agent) application for separation of rare and noble metal ores on the example of palladium-tungsten alloy. Such alloys are widely applied in electronics mainly as a material for contacts of relays, slide-wires, etc. The main problem of separation of this alloy is its high resistance to oxidizing agents. For example, aqua regia is not suitable for this purpose due to shielding palldium by tungsten. The background to possible $KBrF_4$ application is a relative readiness of tungsten to turn into volatile hexafluoride, while palladium turns into soluble hexafluoropalladate.

In this study we used samples of the alloy (80 % of Pd and 20 % of W) in the form of small plates. After prior preparation and sintering of the samples with $KBrF_4$ the composition of the solid residue was determined by X-ray fluorescence analysis. The content of tungsten was found out as (1,5±0,5) % from the initial one, i.e. 0,3 %. Therefore, separation coefficient on this stage is C_{sep} = ~66. Gaseous WF_6 can be chemically adsorbed for further treatment.

After hydrolysis of the residue, Pd can be precipitated by ammonia solution as a complex $(NH_4)_2PdCl_6$ [1]. This precipitate is the end product which can be processed further to metallic Pd. Small amounts of tungsten remain in the solution in the form of $(NH_4)_{10}[H_2W_{12}O_{42}]$.

Quantitative analysis of obtained $(NH_4)_2PdCl_6$ showed that the content of W in the end product was not more than (0,02±0,01) %. The total separation coefficient after the two stages is C_{sep} = ~1000.

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REMOVAL OF Cr(VI) FROM AQUEOUS SOLUTIONS USING NOVEL GRAFTED POLYMERIC ADSORBENT

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Rapid industrialization and increase in population cause toxic heavy metals to release into the environment. As a consequence, these metals are found well above the tolerance limits in aquatic environment. Chromium is widely used in electroplating, leather tanning, paint, cement and photography industries producing large quantities of effluents containing toxic metals. In environmental waters, chromium exists predominantly in two chemical forms: Cr(III) and Cr(VI). It is well known that the toxicological and biological properties of most elements depend upon their chemical forms. Cr(III) is considered as an essential nutrient for human metabolism, whereas Cr(VI) has definite adverse effect on human health, a suspect carcinogen. Therefore, removal of chromium from environment is important. Several methods are used for the removal of Cr(VI) from aqueous solutions, such as, electrolysis, ion exchange, reverse osmosis, solvent extraction, adsorption, complexation and precipitation. Among all these, adsorption is considered as an effective and economical method to remove Cr(VI) from aquatic systems.

In our study, the metal adsorbents are synthesized by using polyethylene-coated polypropylene nonwoven fabrics as the trunk material. PE / PP nonwoven fiber (NWF) was irradiated by accelerated electrons in inert atmospheres for grafting of dimethylaminoethyl methacrylate. Grafting conditions were optimized by a systematic investigation of the effects of absorbed dose, monomer concentration, reaction temperature and duration(1-2). 150 % grafted copolymer was later modifed by quaternization of poly(dimethylaminoethyl methacrylate) chains by dimethylsulphate. The PE/PP based adsorbent thus prepared was used for its suitability of removing chromate ions from aqueous systems. Adsorption/removal studies were carried out in both batch and continuous flow type systems. The selectivity of adsorption of chromate ions in the presence of other competing anions were also studied showing the enhanced selectivity for chromate ions.

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FLUOROQUINOLONE ANTIBIOTIC DETERMINATION IN BOVINE MILK USING CAPILLARY LIQUID CHROMATOGRAPHY WITH DIODE ARRAY AND MASS SPECTROMETRY DETECTION

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Fluoroquinolones (FQs) are a group of synthetic antimicrobial compounds widely used in human and veterinary medicine, and used at sub-therapeutic levels to promote growth for food-producing animals. Residual antibacterials in food constitute a risk to human health because they can contribute to the transmission of antibiotic-resistant pathogenic bacteria through the food chain. To ensure food safety, regulatory agencies have established tolerance levels for authorized veterinary drugs and have set requirements about the performance of analytical methods.

Several Liquid Chromatography (LC) methods with diode array detection (DAD), fluorescence or MS detection have been reported for FQs determination in milk, but most of them require complex sample treatments. Capillary LC (cLC) has been established as an alternative to conventional LC. It is environmentally friendly and, in general, better suited for coupling separation techniques to each other and with detection techniques such as MS.

The aim of this work was to develop and optimize a cLC-DAD-MS method and a sample preparation procedure for determination of ofloxacin, ciprofloxacin, enrofloxacin, sarafloxacin, difloxacin and flumequine in commercial bovine milk. Separation was carried out in a Zorbax SB-C18 column (150 x 0.5 mm, 5 μm). A mixture of acetonitrile-5 mM ammonium formiate buffer at pH 3.7 was used as mobile phase in gradient elution mode. Flow rate and temperature were set at 20 μL min^{-1} and 25 ºC, respectively. For focusing purposes on the head of the capillary column, 20 μL of buffer solutions were injected.

A fast and simple extraction method based on milk deproteinization was developed. Samples (5 g) were shaken with 0.5 mL of 15% trichloroacetic acid aqueous solution and centrifuged at 4200 rpm for 25 min. Supernatant was collected, and the precipitate rinsed and centrifuged again with 1 mL of ammonium formiate buffer (5 mM, pH 3.7). The resulting solution from combined extracts was shaken, passed through a 0.45 μm nylon filter and directly injected in the LC system.

Validation of the proposed methodology was performed according to the European Commission Decision 657/2002/EC. Sample detection limits were between 2.8 and 25 μg·g^{-1}, and good linearity was observed until 250 μg·g^{-1} for all analytes. Acceptable and constant recoveries between 58% for sarafloxacin and 90% for ofloxacin with RSDs (n=3) lower than 9.7% were obtained at the 100 μg·g^{-1} spiked level.

In conclusion, the optimized sample extraction procedure combined with cLC-DAD-MS has provided a fast and simple method for the FQ determination at concentrations of the maximum residue levels established by the EU Council Regulation in bovine milk.

SEPARATION AND DETERMINATION OF ARSENIC SPECIES IN WATER BY SELECTIVE HYBRID RESINS

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A simple and efficient method for separation and determination of inorganic arsenic (iAs) and organic arsenic (oAs) species in drinking, natural and wastewater was developed. If arsenic is present in water prevailing forms are inorganic acids of As(III) and As(V). oAs can be found in traces as monomethylarsonic acid, MMA(V), and dimethylarsinic acid, DMAs(V). Three types of resins: a strong base anion exchange (SBAE) and two hybrid (HY) resins: HY-Fe and HY-AgCl, based on the activity of hydrated iron oxides and a silver chloride were investigated. It was found that the sorption processes (ion exchange, adsorption and chemisorptions) of arsenic species on SBAE (ion exchange) and HY resins depend on pH values of water. The selective bonding of arsenic species on three types of resins makes possible the development of the procedure for measuring and calculation of all arsenic species in water. Proposed method was established performing standard procedures: with external standard, certified reference material and standard addition method. ICP-MS was applied for the determination of arsenic in all arsenic species in water, detection limit was 0.002 µg L^{-1} and relative standard deviation (RSD) of all arsenic species investigated was between 3.5-5.1 %.

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ARSENATE REMOVAL FROM WATER BY COPPER(II)-LOADED DI-(2-PICOLYL)AMINE CHELATING FIBER ADSORBENT

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Recently, the use of polymers based on crosslinked polystyrene having polymeric ligand exchanger (PLE) properties to remove arsenate in water has attracted much attention. A PLE consists of a polymer with chelating functional groups that can bind tightly to a transition metal, which can remove variety of ions from aqueous systems by complex formation. Although a number of polyvalent metal ions, including Cu(II), Fe(III), Zr(IV), Mo(VI) and Ti(IV), have been successfully immobilized on chelating resins for arsenate removal, the use of PLE nonwoven fibers prepared by means of radiation induced graft polymerization has been very limited [1-3].

In this study, a novel PLE nonwoven fiber selective for arsenate was prepared by means of radiation induced graft polymerization which is different from conventional anion exchange resins based on crosslinked polystyrene matrices used for the removal of arsenate from aqueous solutions. For this purpose, glycidyl methacrylate (GMA) was grafted onto polyethylene coated polypropylene nonwoven fibers in emulsion medium by using radiation induced graft polymerization. Epoxy groups of grafted GMA were then modified with di-(2-picolyl)amine molecules. Modification was conducted in 15 % (w/w) di-(2-picolyl)amine solution. Modified nonwoven fibers were treated with 1000 mg/L Cu(II) solution in order to bound Cu(II) ions which convert nonwoven fibers into Cu(II) bound forms thus conferring the properties of PLE. PLE nonwoven fibers consist of Cu(II) bound to di-(2-picolyl)amine functional groups. This is the first attempt to use PLE nonwoven fibers carrying di-(2-picolyl)amine bound to Cu(II) ion for the removal of arsenate from aqueous solutions. Arsenate adsorption property of the prepared Cu(II) bound PLE adsorbent was conducted in aqueous solutions by studying different adsorption conditions such as effect of pH, effect of flow rate on As(V) adsorption and elution of adsorbed As(V) in column mode.

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SP01 **Session C**

MULTI-WALLED CARBON NANOTUBES-DISPERSIVE SOLID-PHASE EXTRACTION COMBINED WITH NANO-LIQUID CHROMATOGRAPHY FOR THE ANALYSIS OF PESTICIDES IN WATER SAMPLES

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Carbon nanotubes (CNTs) have found different applications in Analytical Chemistry since their discovery in 1991. Their strong binding affinity for hydrophobic molecules, their internal tube cavity and surface area as well as their ability to establish π-π electrostatic interactions are the main characteristics that have attracted their use, especially as solid-phase extraction (SPE) stationary phases.

In general, the bottleneck of conventional SPE is the sample loading step. One of the variations of the technique which considerably reduces the time and simplifies the extraction is the so called dispersive SPE (DSPE), in which extraction is not carried out in the column, but in the bulk solution, becoming faster and easier than conventional SPE. Although DSPE is highly used for matrix clean-up purposes, sorbent materials have been less used in the dispersive mode with the aim of trapping the target analytes, which are later eluted or desorbed with an appropriate solvent.

In this work, the simultaneous separation of a group of twelve pesticides (carbaryl, fensulfothion, mecoprop, fenamiphos, haloxyfop, diclofop, fipronil, profenofos, fonofos, disulfoton, nitrofen and terbufos) by nano-LC with UV detection is described. For the analyses, a 100 µm I.D. capillary column packed with silica modified with phenyl groups was used. Experimental parameters, including the use of a trapping column for increasing the sensitivity, were optimized. A preliminary study of the applicability of a rapid and practical DSPE procedure, was developed for the extraction of some of these pesticides from Milli-Q water samples using multi-walled CNTs. The method was validated through a recovery study at three levels of concentration, obtaining LODs in the range 0.016-0.067 µg/L (below European Union maximum residue limits) for the majority of the analytes. DSPE permitted a clear diminution of the total sample treatment time with respect to conventional SPE. MWCNTs were reused up to 5 times, representing an important reduction of the waste of stationary phase [1].

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DETERMINATION OF METALS IN GASOLINE AND NAPHTHA AS MICROEMULSIONS USING A PISTON PROPULSION FLOW-BATCH AND GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

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The determination of metals in fuel samples are generally carried on using manual procedures. These procedures, however, are subject to some problems such as large reagent and sample consumption as well as high contamination risks. These problems may be overcome by using automatic analyzers. Flow-Batch Analyzers (FBA) [1] has been successfully applied in automation of several analytical procedures. A new FBA approach was recently proposed: a low cost and compact analyzer based on pneumatic propulsion of the fluids [2]. In this work the association of Piston Propulsion Flow-Batch (PFBA) with microemulsions as an automatic method for the determination of metals in gasoline and naphtha under microemulsions form by GF AAS was proposed. The composition of the quaternary microemulsions for gasoline samples was: water, gasoline, Triton X-100 and tert-buthanol. For the naphtha analysis ternary microemulsions were prepared, whose composition was: water, naphtha and n-propanol. Analytical curve technique using inorganic standard solutions was adopted for the determination of Copper, chromium and lead. All microemulsions were prepared manually and automatically in order to compare the behavior of the proposed system. The automatic method was validated by using ANOVA. PFBA was applied successfully for the preparation of gasoline and naphtha microemulsions for determination of metals by GFAAS. The microemulsion systems studied in this work furnished low sample dilutions, providing low LOQ and LOD values.

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Capes, CNPq, INCTAA, Petrobrás

RAPID DETERMINATION OF ANTIHELMINTIC BENZIMIDAZOLES AND METABOLITES IN MILK BY ON-LINE RESTRICTED ACCESS MATERIAL – LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY: DEVELOPMENT AND VALIDATION OF THE METHOD

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The present work describes the development and validation of an analytical method based on liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) that allows the determination of anthelmintic drugs in milk. The veterinary residues studied included twelve benzimidazoles and their metabolites: albendazole, albendazole-sulfoxide, albendazole-sulfone and albendazole-2-aminosulfone; febantel, fenbendazole, oxfendazole (fenbendazole-sulfoxide) and fenbendazole-sulfone; flubendazole and 2-aminoflubendazole; oxibendazole; and carbendazim.

The method incorporates a restricted access material (RAM), coupled on-line with the LC–MS/MS system, that allows direct injection of the complex matrix into the system, efficient cleaning being achieved together with a reduction of prior manual preparation of the sample to a simple, rapid process of precipitation, centrifugation and filtration.

The on-line RAM–LC–MS/MS methodology developed was validated according to European Union Decision 2002/657/EC. The validation parameters included verification of linearity, repeatability, reproducibility, specificity, recovery, stability, ruggedness, decision limits and detection capabilities. Repeatability and within-laboratory reproducibility, measured as intraday and interday precisions, were evaluated at three concentration levels, being 20.0 % or below for the lowest concentration (5 ng mL^{-1}). The decision limits (CC_{α}) and detection capabilities (CC_{β}) were in the range of 0.3–2.4 and 1.1–9.3 ng mL^{-1} respectively. Recoveries varied around 100% for the three concentration levels studied. Performance was assessed, at 10 ng mL^{-1}, in seven different matrices: whole and nonfat commercial cow's milk, unfortified and fortified with calcium, soy, several vitamins and/or fiber; and raw cow's and goat's milk.

The results of the validation process show that the on-line methodology developed is a useful, rapid and safe tool for the quantification and confirmation of the presence of benzimidazoles and their metabolites in milk at low ng mL^{-1} levels.

SILICA-BASED SOLID PHASE ANALYTICAL REAGENTS FOR DETERMINATION OF MONO- AND POLYNITRO AROMATIC COMPOUNDS

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Nitroaromatic compounds (NC) are used to manufacture drugs, fungicides, insecticides, as dyes and explosives. Acute (short-term) inhalation or ingestion of 4-nitrophenol in humans causes headaches, drowsiness, nausea, and cyanosis. Contact with eyes causes irritation in humans. Therefore preliminary control and reliable methods for determination of NC in the environment is important. For selective analytical determination of nitroaromatic compounds the reaction between them and π–donor molecules are used because of formation of intensively colored charge-transfer complexes. We used this approach to develop new solid-phase analytical reagent (SAR) that can be used for reagent-less monitoring of water contamination by nitro and polynitro compounds. The proposed SAR is based on silica gel with covalently immobilized carbazole. The SAR analytical properties were examined on determination of 2,4,6-trinitrophenol traces in contaminated water. It was demonstrated that the SAR adsorbs 2,4,6-trinitrophenol (PicrOH) from water because π–complex formation on the adsorbent surface.

In the range of solution pH=1-2, effective (>92%) and selective adsorption of PicrOH is observed. For the solutions with PicrOH concentration $3{,}5\cdot10^{4}$ $\mathrm{mol\cdot L^{-1}}$, the equilibrium is set after 60 minutes. In the pollutant content range of 0.1 - 20 µmol per gram of adsorbent, its distribution is constant. Total capacity of the SAR to PicrOH is 23 μmol/g with distribution coefficients up to 10^{3} $\mathrm{mL\cdot g^{-1}}$. The PicrOH adsorption isotherm can be attributed to Langmuir L-type isotherm. 1-263 µg of analyte can be easily eluted by 1-8 mL of DMSO with overall recovery 92%. Intensity of the SAR color is increased with PicrOH concentration. The SAR with adsorbed analytes has absorption bands with maxima at 496 and 466 nm for PicrOH and 4-nitrophenol correspondently. Contrary fluorescence of the SAR decreases with the pollutant content. These were used to develop visual test-method for monitoring of water contamination with PicrOH in the concentration range 1-10 MAC. Also adsorbed contaminant can be eluted to solution by DMSO for further quantitatively determination by HPLC with LOD 0.1 MAC. Application of proposed SAR for SPE of other nitroaromatic compounds is also reported.

SP08 **Session C**

SAMPLE PREPARATION FOR THE HPLC DETERMINATION OF ANTIBIOTIC RESIDUES IN MILK

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Milk is one of the globally most consumed foods of great importance for human growth, as it contains essential nutritional components like saturated fat, protein, and calcium. Its quality can be impaired due to the prevalent use of antimicrobials as feed additives in veterinary medicine. Analysis of antibiotics residues plays a key role in ensuring food safety.

Matrix solid phase dispersion (MSPD) is a sample preparation technique especially suitable to the extraction of solid, semi-solid and/or highly viscous food and biological matrices. Isolation of target analytes is performed by dispersing tissues onto a solid support, thus avoiding many of the difficulties encountered by employing the classical SPE approach. Although milk is liquid and sample homogenisation is actually not a problem, it requires sophisticated sample preparation to isolate target analytes due to its matrix complexity.

We applied ultrasound assisted MSPD for the extraction of cephalosporin antibiotics (cefaclor, cefadroxil, ceftiofur, cefuroxime, cefoperazone, cefazolin, cephalexin and cefotaxime) from milk samples prior to HPLC-DAD analysis. Nexus sorbent was used and its preconditioning found to improve its performance. Moreover, sonication provided an efficient contact between the solid and the extractant, thus resulting in higher recovery rates of the target analytes. The optimum method using a monolithic column achieved separation in less than 16 min. Recovery rates of examined antimicrobials from milk ranged from 93.4 to108.6%. Decision limits (CCα) were 53.9 to 113.3 μg kg^{-1} and the corresponding results for detection capability (CCβ) were 58.3 to 120.3 μg kg^{-1}.

A modified MSPD procedure was applied to the extraction and clean-up procedure of antibiotics using STRATA-X by Phenomenex and the half the sorbent of a 2mL QuEChERS tube for the isolation of five penicillins (cloxacillin, dicloxacillin, oxacillin, amoxicillin and ampicillin) and three amphenicols (chloramphenicol, thiamphenicol and florfenicol) in milk. Analysis was performed on a Kinetex^{TM}-C$_{18}$ core-shell analytical column within 18 min. Recovery rates of examined compounds ranged from 86.8 to 110.6%. Decision limits (CCα) ranged from 35.2 to 56.3 μg kg^{-1} and the corresponding results for detection capability (CCβ) were 39.9 and 61.9 μg kg^{-1}.

Both methods were validated according to the European Union decision 2002/657/EC, determining linearity, selectivity, stability, decision limit, detection capability, accuracy and precision.

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ANALYTICAL REAGENTS FOR PRE-CONCENTRATION OF ACTIVATED AND DEACTIVATED PHENOLIC COMPOUNDS

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New silica-based analytical reagents (SARs) for selective pre-concentration and visual monitoring of activated and deactivated phenols were proposed. To selectively detect traces of activated phenols in contaminated water their ability to form azo-dyes was used. For this, silica with covalently bonded diazonium salt of м-aminophenilarsonic acid was obtained. Such modified silica reacts with traces of phenolic compounds in water forming immobilized azo-dyes. Capacity of the adsorbent to phenols is up to 400 μmol/g of adsorbent with distribution coefficient about 10^4 ml/g. For selective pre-concentration of deactivated phenols two other SARs were proposed. One was based on silica-immobilized 2,3,5-triphenyltetrazole and other on silica-immobilized carbazole derivative. In both cases immobilized molecules are capable to form stabile π-complexes with mononitro- and polynitrocompounds. It was demonstrated that model trinitrophenol is fully adsorbed from solution by the proposed SARs at pH range 4 – 6. It was found that beige color of carbazole-containing SAR turns to brownish-red if immersed to solution containing thrinitrophenol or 2-nitrophenol in the pH range 2-8.0 with the adsorption bands at λ = 496 and 466 nm, respectively. The capacity of the SAR toward triphenyltetrazole is up to 250 umol/g of adsorbent. All proposed SPEs are suitable for their application in HPLC determination of phenols. Analytes can be quantitatively desorption to solution by acetonitrile or DMSO. Test-system for visual monitoring of phenols under MAC was developed using proposed adsorbents.

EXTRACTION AND SEPARATION OF ANTICANCER DRUG LEADS FROM MICROALGAE BY USING SUPERCRITICAL FLUIED CARBON DIOXIDE

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The supercritical fluid is any compound at a temperature and pressure above the critical values. The supercritical CO_2 extraction has focused on advocating on reduction in the use of organic solvents. Besides ecological benefits, one of the most interesting properties of supercritical CO_2 is the high diffusion coefficients lipid in supercritical fluid, far greater than in organic solvents.

Marine dinoflagellates *Amphidinium* species are known to produce a lot of bioactive secondary metabolites such as cytotoxic macrolides and polyketides.[1,2] Our continuing research for bioactive metabolites from these dinoflagellates, we discovered several novel polyketide-like metabolites, named iriomoteolides and amphirionins.[4,5]

Usually, our separation scheme of these polyketides is follows. The harvested algal cells obtained by mass-cultivation were extracted with organic solvents (MeOH/toluene). The extract was partitioned between toluene and water. The toluene-soluble materials were subjected to three-step separation with column chromatographies (SiO_2 gel, C_{18}, and then NH_2-SiO_2 columns) and C_{18} HPLC. Contents of these metabolites in the dried cells were less than 1%, while major components in the cells are lipids such as glycerolipids, fatty acids, and steroids. In our investigation on bioactive substances from natural products, we developed supercritical CO_2 extraction for separation of these macrolides in the lipid-rich cells of cultured dinoflagellates. So, we set up the sequential system of supercritical CO_2 extraction and trap HPLC.

In this symposium, we describe the comparison between usual extraction with organic solvents and of supercritical CO_2 extraction and application of the sequential extraction/separation system.

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LIQUID CAPILLARY MICROEXTRACTION OF DIPHENYLKETONES

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Benzophenone and its derivatives are used as photostabilizers so that they can protect other substances and materials from harmful UV radiation. Thus these substances can be used in the majority of sun protection makeup and also they are additives in some dyes, enamels and pigments for protection against UV light. Benzophenone derivatives provide effective protection of PVC and other polymers. Also, these chemicals act as preservatives, they are used in perfume industry as smell fixing agents they are applied as additives in soaps, shower gels, some perfume etc.

As a result of wide industrial application, diphenylketones can penetrate the environment and the human body. Diphenylketones have some toxic properties. It is known that these substances can accumulate in the body, causing destruction of the endocrine system, leading to allergic effects: swelling of mucous covers and sore throat, skin irritation.

We have developed the liquid capillary microextraction (LCME) of benzophenones from aqueous solutions and their subsequent GC/PID detection. For capillary microextraction we have applied polypropylene capillary from Membrana GmbH "(Wuppertal, Germany) with an inner diameter of 1175 microns, wall thickness 300 microns, pore size - 0.2 microns and a length of 2 cm. Conditions of LCME have been optimized. Some organic solvents were investigated: toluene, hexane, chloroform, benzene, n-octane, n-octanol, xylene, cresol. It was shown that the best efficiency of LCME is reached using toluene. The influence of time was investigated on microextraction of diphenylketones. The results showed that extraction equilibrium is reached after 20-25 min. at stirrng rate of 500-800 rev/min for 0.3 mg /l aqueous solution of analyte.

Extraction recovery of benzophenones in equilibrium conditions is 18-22%. Also the effect of some salts as NaCl, K_2SO_4, KNO_3 and surfactants of different nature has been investigated on the efficiency of LCME of diphenylketones Working range for benzophenones determination by GC / PID after LKME is 0.1-1 mg / l, detection limit by the 3s-criterion - 0.05 mg/l. The pre-concentration (partition) coefficients for microextraction of benzophenones are 60-100, RSD is 5-10%.

IN SITU CHEMICAL FIBER MODIFICATION AND HEADSPACE-SOLID PHASE MICROEXTRACTION/DERIVATIZATION/GC/MS FOR AUTOMATIC DETERMINATION OF ALDEHYDES IN BIO-OIL. COMPARISON WITH HEADSPACE–GC/MS DETERMINATION

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Bio-oil is a dark brown liquid produced by fast pyrolysis. It can be used as a fuel, as a source of chemicals or as food additive. Bio-oil contains 20 - 30 wt % of water, pyrolytic lignin (15-20 %), organic acids (10-15 %), anhydrosugars (5-10 %) and aldehydes (10-20 %). The aldehyde fraction is toxic, causes instability of bio-oil during storage, but can be extracted and used in phenol/formaldehyde resin formulations.

Aldehyde derivatization is usually carried out using *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA), providing thermal stable and volatile oximes to be analyzed by GC. These oximes derivates can be extracted using different approaches, such as Headspace (HS), Solid-Phase-Micro-Extraction (SPME), and HS-SPME.

The principal aim of this work is to integrate 3 analytical steps (chemical fiber modification, derivatization and extraction) in only one automatic HS-SPME step for aldehyde determination in bio-oil samples (OFD-HS-SPME-GC/MS).

The automated procedure was achieved using a CTC CombiPal autosampler with CycleComposer software. The procedure starts with the chemical fiber modification by PFBHA impregnation (3 mg/mL, 250 rpm, 40°C and 10 min), followed by the exposure to HS-sample (30 min). This was possible by creating two methods, the first “Desorbed into virtual injector” and the second “Desorbed into real injector”, enabling the sequential fiber impregnation and derivatization/extraction of aldehydes in the same autosampler.

The OFD-HS-SPME-GC/MS conditions were optimized applying an experimental design methodology. The optimal analytical properties were: LDs 0.2 ug L^{-1}, 4.4 ug L^{-1} and 15.2 ug L^{-1} for formaldehyde, acetaldehyde and propionaldehyde, recovery 85.6% (from bio-oil sample) and reproducibility 8.3%. These properties were compared with those obtained for another optimized method (in solution derivatization and oxime extraction by HS-GC/MS), being the first more appropriate for aldehyde determination in bio-oil due to its better sensitivity and selectivity.

A NOVEL APPLICATION OF MICROWAVE-ASSISTED EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM BREWER'S SPENT GRAIN

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Brewer's spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated. It is available in large quantities all over the year, although its main application has been limited to animal feeding. Nevertheless, it is a lignocellulosic material and could be better used since it is rich in oligo- and polysaccharides, as well as in phenolic compounds [1]. Recently, attempts have been made to use BSG as a source of these last compounds [1, 2]. Polyphenols are of great interest to the food industry because they improve the quality, the nutritional value and the antioxidant properties of foods [3]. Such compounds are included in different major groups, among them phenolic acids, such as ferulic and *p*-coumaric acids, which are associated with cell wall constituents, especially with arabinoxylans and lignin [1].

The present work aimed at develop a microwave-assisted extraction (MAE) method for the extraction of antioxidant compounds from BSG. According to our knowledge, this is the first application of MAE for the extraction of these type of compounds from BSG. For this purpose, BSG was subjected to various experimental conditions (extraction time, temperature, type of solvent and solvent-to-solid ratio) in order to investigate its effect on the phenolic composition of the extract. This study demonstrated that applying MAE higher amounts of ferulic acid were extracted comparing to the obtained with alkaline hydrolysis proposed by Mussatto *et al.* [4]. Moreover, the MAE approach proved itself to be a good alternative, as it requires less energy and solvent than the alkaline hydrolysis, while generating fewer wastes. Work is in progress in order to identify and characterize by HPLC-ESI-MS other antioxidant compounds, offering new possibilities for the re-use of this brewery by-product in the food, cosmetic and/or pharmaceutical fields.

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SAMPLE PREPARATION FOR ANALYSIS BY RCC

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Rotating coil columns (RCC) applications for sample preparation of oil/oil products are proposed in this work. The hybrid method of RCC pre-concentration of the trace elements and consequent ICP-MS or ICP-AES detection was developed in this research. RCC method of fractionating of oil products and their mixtures is also proposed.

It is known that more than 60 elements were detected in crude oils. The element concentrations are in wide range (from 0.1 wt.% to ng/kg). Despite small amounts these elements play important role in oil producing and recovering, ecological monitoring and reconnaissance investigations. In the meantime determination of trace elements in oil is very difficult because of ultramicro content and absence of methods with direct concentration.

Thus RCC applications look very promising for pre-concentration of trace elements from oil into an aqueous stationary phase. An oil sample is continuously pumped through CCC column as the mobile phase whereas an aqueous acidic solution (defined volume till 90% of total column volume) is retained as the liquid stationary phase. Design features enable retention of small volumes of the stationary aqueous phase and variable volumes of the mobile phase (from 10 mL to 1 L and more) to obtain higher concentration factors. Two phases are kept well mixed and agitated but there is no emulsion on the water–oil interface. The acidic aqueous solutions used as stationary phase could be analyzed with ICP-MS, AAS, AES without any additional preparation [1]. It should be stated that pre-concentration step disables bleeds of the volatile compounds and offers to be very fast (20-50 min).

In fact, sample preparation step is a limitation one for oil elemental analysis. In this case CCC preconcentration step has an exceptional place as sample preparation technique for oil elemental analysis. Elemental analysis of different types of oil (Tengizskaya, West-Siberian, Novoachinskaya), oil products (diesel, gasoil, straight distillates) and oil residues are presented. This method is characterized by very low DL (0.001–1 µg/kg), suitable for most oil types. Results obtained could be very useful for unique research in theoretical modeling of Earth’s origin and oil genesis.

Fractionating of oil products using RCC is suggested for the first time. It should be stated that there is no any alternatives to fractionating towers/columns at high temperatures and pressures. First results of separations of oil product mixtures and oils are presented.

1. A.V. Soin, N.S. Musina, T.A. Maryutina // J Chromatograph Separat Techniq 2011, 2:106

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MICROWAVE ASSISTED EXTRACTION COMBINED WITH DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AS A SENSITIVE SAMPLE PREPARATION METHOD FOR THE DETERMINATION OF HALOANISOLES AND HALOPHENOLS IN CORK STOPPERS AND OAK BARREL SAWDUST

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A method based on microwave assisted extraction (MAE) in combination with dispersive liquid-liquid microextraction (DLLME) has been proposed as a new approach for the sensitive determination of cork taint responsible compounds in cork stoppers and oak barrel sawdust. For this purpose, haloanisoles (2,4,6-trichloranisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromoanisole (TBA)) and halophenols (2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), pentachlorophenol (PCP) and 2,4,6-tribromophenol (TBP)) were extracted from the solid samples using a MAE method. Subsequently, a DLLME-derivatisation procedure was applied on the MAE extracts and the analytes were determined by gas chromatography-electron capture detection (GC-ECD). Parameters affecting the DLLME-derivatisation method were exhaustively investigated. Firstly, the type of extraction solvent, the ionic strength and temperature conditions were selected. Then, experimental design methodology was applied to study the concentration of K_2CO_3 and the volumes of aqueous solution, extraction solvent and derivatisation agent. Once optimised, the proposed method showed satisfactory linearity, repeatability and inter-day precision. Detection limits obtained were similar or even lower than previously reported. In addition, the proposed method was successfully applied to the analysis of real samples. The results obtained proved the suitability of the combination of MAE with DLLME as a sensitive sample preparation methodology for the analysis of haloanisoles and halophenols in solid enological matrices.

DEVELOPMENT OF AN ULTRASOUND ASSISTED EMULSIFICATION MICROEXTRACTION METHOD FOR THE DETERMINATION OF THE MAIN COMPOUNDS CAUSING BRETT CHARACTER IN WINES

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Volatile phenols are usually present in wine aroma and contribute positively to it, but if some of them are present at high concentrations they cause wine off-flavours that negatively affect wine quality. The yeast Brettanomyces/Dekkera can decarboxylate trans p-coumaric acids to obtain vinylphenols. These vinylphenols are later transformed by reduction reactions into ethylphenols. These compounds, 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol and 4-vinylguaiacol, are associated with the known Brett character, with a large spectrum of flavours and aromas including cloves, bready, barnyard, horsy and medicinal. Hence, their determination is of great interest in the enological industry to guarantee wine quality and avoid economic losses.

The aim of this study was the optimisation of an ultrasound-assisted emulsification-microextraction (USAEME) method, coupled with tandem mass spectrometry (GC-MS/MS), for the determination of the Brett character responsible compounds in wine. The influence of the type of solvent, the solvent volume, temperature, time and salt addition on the USAEME extraction efficiency for volatile phenols was evaluated. The multivariate optimisation strategy used including experimental design and response surface methodology, allowed the appropriate determination of the optimal USAEME conditions. The analytical characteristics of the optimised USAEME method were evaluated and, subsequently, the optimised and validated method was applied to the analysis of the compounds responsible for the studied off-flavour in real wines. To our best knowledge, no approaches applying USAEME procedure to the determination of Brett character responsible compounds in wine have previously been published.

CARBON ISOTOPE MASS SPECTROMETRY IN DOPING CONTROL

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The abuse of doping substances that are close analogs of hormones produced in the human body or their metabolic precursors (testosterone, dehydroepiandrosterone, androstenedione, androstenediols, etc.) should be detected in the current practice of doping control. This cannot be made based on the results of classical techniques – gas chromatography coupled with mass spectrometry, and only so-called atypical changes in the hormonal profile of an athlete can be detected.

The use of synthetic testosterone in sport was prohibited more than 25 years ago. Previously, the determination of the concentration ratio of testosterone (T) and its inactive isomer epitestosterone (E) in urine was the only way to detecting the use of this substance. Nevertheless, this approach has a number of important limitations because the T/E ratio in population varies over a wide range (from 0.1 to 4.0 or higher). Moreover, prohormones (substances that are testosterone precursors or metabolites, which cannot be determined by traditional doping control methods) became widespread recently in the sports nutrition market. Because the vast majority of synthetic steroids are prepared from plant raw materials with a carbon isotope composition of about –30‰ or lower (with reference to the belemnite international standart), the use of this substances can be detected by means of isotope mass spectrometry. The method is based on the measurement of the $^{13}C/^{12}C$ isotope ratio of endogenous steroids in the human body; this ratio lies in a range from –17‰ to –26‰, depending on the residence and diet of the individual, and it is never lower than –27‰.

In 1994, Becchi et al. was the first to propose a procedure for selective extraction of testosterone metabolites from the urine followed by analysis using gas chromatography–combustion–isotope mass spectrometry (GC–C–IRMS); at the same time, this procedure required 50 mL of a sample.

In 2004, the World Anti-Doping Agency (WADA) published a technical document to regulate the use of GC–C–IRMS; however, currently, there is no universal approach for the analysis procedure in anti-doping laboratories, because this analysis is complicated and labour-intensive. Multistep solid-phase extraction (SPE) and SPE in combination with semi-preparative liquid chromatography are most frequenly used.

The aim of this work was to develop a procedure for the determination of the $^{13}C/^{12}C$ isotope ratios in steroids separated from human urine using gas chromatography in combination with isotope mass spectrometry, and to determine intralaboratory reference population values for the evaluation of the results of analysis.

SELECTIVE DETERMINATION OF ESTROGENIC COMPOUNDS IN WATER BY MICROEXTRACTION BY PACKED SORBENTS AND A MOLECULARLY IMPRINTED POLYMER COUPLED WITH LARGE VOLUME INJECTION-*IN-PORT*-DERIVATIZATION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The European Water Framework Directive (WFD) recognized some of the emerging compounds such as synthetic hormones used as drugs, or biologically active technical products as priority contaminants due to their presence in environment, persistence, bioaccumulation and their potential action as estrogenic compounds. However, the determination of these substances is often difficult and requires some analytical effort to detect the traces within a complex matrix. The present work studied the fully automated procedure of “Microextraction by packed sorbents” (MEPS) coupled with large volume injection (LVI)-in-port-derivatization-GC-MS for the determination of several estrogen-active substances, such as alkylphenols, bisphenol A, estradiol related substances and sterols, in river and wastewater samples. Extraction parameters such as the addition of an inert salt to the water sample, the extraction regime, the volume of organic solvent used for the elution/injection step, the elution solvents and the selectivity of the sorbents were studied during the MEPS optimisation. For optimum in-port-derivatization, 10 µL of the derivatization reagent N,O-bis(trimethylsilyl)triufloroacetamide with 1% of trimethylchlorosilane (BSTFA + 1% TMCS) was used. In parallel experiments, a 17β-estradiol-molecularly imprinting polymer (MIP) and silica gel (modified with C-18) sorbents were examined for the enrichment of the target analytes from water samples and the obtained results revealed the high selectivity of the MIP material for estrogen-like structures. Recovery values for most of the analytes ranged from 75 to 109 % for the C18 sorbent and from 81 to 103 % for the MIP material except for equilin (on C18 with only 57-66 % recovery). Precision (n=4) of the entire analysis protocol ranged between 4 % and 22 % with both sorbents. Limits of detection (LODs) of the target analytes ranged between 0.02-87 ng L^{-1} for C18-sorbent and 1.3-22 ng L^{-1} for the MIP material using small sample volumes. The full automated protocol was applied to analyse river water and wastewater for the occurrence of the target analytes. Concentrations measured in treated wastewater samples exceeded those in river water whereas some of the analytes could not be detected in any of the samples above the LOD.

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EVALUATION OF LOW-COST DISPOSABLE POLYMERIC MATERIALS FOR SORPTIVE EXTRACTION OF ORGANIC POLLUTANTS IN WATER SAMPLES

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Sample preparation is often considered to be a fundamental step in analytical procedures, because it helps not only to achieve detection limits that are as low as legislation requires but also to clean up the sample matrix. Usually sample preparation procedures consisting of several extraction and clean-up steps consume noticeable amounts of organic solvents, labour-effort and time due to a low degree of automation. Polymeric materials can be used as extraction medium instead of organic solvents as demonstrated by stir bar-, silicon rod- or solid phase micro extraction methods which reduce or even avoid the use of organic solvents. Miniaturizations of extraction devices facilitate its integration in fully automated analytical protocols thus, in general, polymer materials with their ability to ad/absorb substances seem to be promising tools for solvent-reduced substance extraction from aqueous samples. In our investigations diverse polymeric materials were evaluated for their suitability to extract semi-polar and polar analytes from complex aqueous matrices. The feasibility of low cost and disposable polymeric materials were examined for the extraction of polar and non-polar contaminants present at trace concentration in environment and are endowed with a wide range of properties (log $K_{w,o}$ = -0.07 – 6.88, from caffeine to octocrylene, respectively). Thus, materials commercially available for industrial or analytical purposes such as silicon, polyethersulfone, PTFE (Teflon®), PDMS, low density polyethylene and polypropylene in different formats (tube, rod or film) were investigated for their potential application as sorptive materials. The extraction ability of these materials was evaluated, optimised and compared with each other to select finally the material best suited for the selected analytes. Parameters that affect the extraction process such as pH, ionic strength of the solvent, sample volume, extraction time and desorption conditions were also evaluated. A set of experimental partition coefficients (K_{ppw}) were estimated for all of studied materials (2 mg of each one) and compared with the theoretical log $K_{o,w}$ of the analytes. Finally, a novel and simple approach using PES tube material was validated and applied for the simultaneous determination of the target compounds in real water samples. Moreover, the influence of the matrix particularly in the wastewater analysis was specified.

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APPLICATION OF CARBONIZED HEMP FIBERS AS A NEW SOLID-PHASE EXTRACTION SORBENT FOR ANALYSIS OF PESTICIDES IN WATER SAMPLES

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There is a growing interest in utilizing the use of the low-cost and abundantly available materials as precursors for the preparation of carbon materials from bio-mass or industrial byproducts. Different carbon materials have been widely used in the sample preparation procedure as a solid-phase sorbents. In this work, short and entangled hemp fibers, acquired as waste from textile production, were used as low-cost precursor for production of activated carbon. The first step in carbon material preparation was carbonization of the waste hemp fibers in inert atmosphere. In the next step, during the activation of carbonized material with potassium hydroxide, the porosity of the material was increased and semi-polar oxygen surface groups, as sorption sights, were introduced. Activated carbon material, with high surface area, obtained in this way was used in the sample preparation, as a sorbent in the solid-phase extraction, for pesticide analysis in water samples. The following parameters that may affect the solid-phase extraction procedure efficiency were optimized: different elution solvents, the sample pH and the sample volume. Extracts were analyzed by liquid chromatography–tandem mass spectrometry technique. For this study pesticides belonging to the different chemical classes as triazine, neonicotinoid, carbamate, organophosphate and sulfonylurea were chosen. Obtained results indicate that carbonized hemp fibers could be successfully applied as a solid-phase sorbent for the pesticide analysis in water samples.

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN FISH: MICROWAVE ASSISTED EXTRACTION vs QuEChERS METHODOLOGY

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Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds which consist of two or more condensed aromatic rings. Currently, about 10,000 compounds have been found; only a few occur in considerable amounts in environment and food. Sixteen are considered by the U. S. Environmental Protection Agency (EPA) as priority pollutants due to their carcinogenic and mutagenic properties.

The goal of this study was to develop an accurate and sensitive methodology for the determination of 18 PAHs (16 PAHs considered by EPA as priority pollutants, dibenzo[a,l]pyrene and benzo[j]fluoranthene) in fish species based on liquid chromatography with photodiode array and fluorescence detection. Two extraction methods, microwave-assisted extraction (MAE) and QuEChERS [1], were optimized and compared regarding their efficiencies and reproducilities. Validation of the overall methodologies was performed by spiking assays and using standard reference material SRM 2977 (Mussel tissue). The optimized methodologies were applied to assess the safety concerning PAH contents of sardine (*Sardine pilchardus*), chub mackerel (*Scomber japonicus*) and horse mackerel (*Trachurus trachurus*) which are regularly eaten in Europe. PAHs can bioaccumulate in fatty tissues and fish is not free from exposure to these contaminants from the environment. Benzo(a)pyrene, the marker used for evaluating the carcinogenic risk of PAHs, was not detected in the analysed samples. The predominant compounds in the three different fish species were naphthalene, acenaphthene, fluorene and phenanthrene.

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ROTATING-DISK SORBENT EXTRACTION OF ORGANOCHLORINE POLLUTANTS FROM LIQUID SAMPLES

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One of the first extraction techniques employing polydimethylsiloxane (PDMS) is solid-phase microextraction (SPME). The evolution of this extraction technique has been noticeable in the recent years. One of the drawbacks of SPME, however, is its efficiency limitation due to the small volume of PDMS coating. More recently, with the purpose of increasing the extraction capability by increasing PDMS volume in relation to that used in SPME, new sorption techniques have been described, including stir-bar sorptive extraction (SBSE), PDMS rod extraction, and thin-film microextraction. One problem associated to SBSE is that increasing the sorptive bar stirring rate may cause physical damage in the extraction phase due to direct contact with the sample vial bottom.

The novel technique proposed in this study consists in extracting low-polarity pollutants onto a rotating Teflon disk coated with a PDMS film on one of its surfaces. We refer to this procedure as rotating-disk sorptive extraction (RDSE). The disk configuration allows immobilizing a larger exposed surface area of PDMS than with the stir bar used in SBSE, and because there is no contact between the extraction phase and the container while the disk rotates, this can be stirred at high velocity without damaging the phase, thus facilitating analyte mass transfer to the PDMS surface. In this work, RDSE technique has been applied to the determination of organochlorine pollutants such as: pesticides (OCPs) and polychlorinated biphenyls (PCBs).

Optimization of the extraction was carried out in water samples. Variables such as: rotational velocity of the disk, extraction time, salting out effect, amount of methanol, and sample volume were studied. After the extraction, the analytes were desorbed from the PDMS by using 2 ml of methanol under stirring or by using thermal desorption. Final determination was performed by GC-MS.

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MICROPOROUS MEMBRANE LIQUID-LIQUID EXTRACTION TECHNIQUE COMBINED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE DETERMINATION OF PIRETHROID PESTICIDES IN WATER SAMPLES

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A simple, easy-to-use, efficient and environmentally friendly method has been established for the simultaneous analysis of nine pirethroid pesticides in water samples by the combination of microporous liquid-liquid extraction (MMLLE) and gas chromatography-mass spectrometry (GC-MS). Nine pirethroid compounds including esbiothrin, prallethrin, tetramethrin, phenothrin, permethrin, cyfluthrin, cypermethrin and deltamethrin were preconcentrated and well separated under optimal conditions. A MMLLE extraction process using 1-octanol as extraction solvent was optimized by means of experimental design. The analytes of interest were concentrated from 4 times for cyfluthrin up to 76 times for prallethrin. The proposed method has good linear relation in the range of 10-1000 ng/ml with related correlation coefficients between 0.985 and 0.999. Overall enrichment factors for the optimized method ranged from 81 to 230 times except for cyfluthrin. Detection limits of the chromatographic method (S/N = 3) were in the range of 0.01 - 0.78 ng/ml with RSD values between 4.2 and 18.4%. The recoveries changed in the range of 4.2 – 76.2% with relative standard deviations between 11.2 - 27.0% for intra and inter-day experiments at different concentrations (0.5 ng/ml, 1 ng/ml and 10 ng/ml). Once analytical method was optimized, it was applied to solutions of synthetic encapsulated samples of some of pyrethroid insecticides in order to quantify the dissolved free fraction. Surface water samples collected in agricultural zones (Salburua, Gazteiz) were also analyzed.

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COMPARATIVE STUDY OF SOLID PHASE EXTRACTION PROCEDURES FOR PARACETAMOL, PARACETAMOL-GLUCURONIDE AND P-AMINOPHENOL IN WASTEWATERS

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In the last years, the release of pharmaceuticals and their metabolites into the aquatic environment has received an increasing attention of scientific community. SPE has been the technique of choice to be used for preconcentration and clean-up of environmental samples. Nowadays, there is in the market a great variety of sorbents that can be used for this purpose. In this work, a comparative study of different SPE procedures is presented for determination of paracetamol, paracetamol-glucuronide and p-aminophenol in wastewaters. Due to its high consumption in the treatment of pain and fever and the possibility of being used by several patients, including children, pregnant women and the elderly, paracetamol and their metabolites may be found in environmental samples. For instance, in 2009, more than 3 millions of packages were sold in Portugal.

The aim of the work was to compare the efficiency of different types of polymeric cartridges, namely Oasis® HLB, MCX, MAX and WAX, for simultaneous determination of the above mentioned compounds in wastewaters. Parameters such sample's pH, sorbent quantity, elution solvents and elution volume were compared in order to obtain the best recoveries for all compounds. Two different extraction/purification strategies, using single SPE columns or two of them in tandem were tested.

The results obtained showed that all polymeric cartridges tested had good results for paracetamol, contrarily to p-aminophenol, which had low recoveries for all sorbents analyzed. On the other hand, for paracetamol-glucuronide only the anionic sorbents (MAX and WAX) were able to present good recoveries. For their simultaneous determination and in order to improve the recovery of p-aminophenol, tandem MCX-WAX was evaluated, achieving recoveries of 28% for p-aminophenol, 98% for paracetamol-glucuronide and 104% for paracetamol.

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DETERMINATION OF TRACE AMOUNTS OF LEAD BY SINGLE-DROP MICROEXTRACTION COMBINED WITH ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Sample preparation plays an important role in the field of trace element analysis. In recent years, miniaturized sample preparation methods have been extensively used for the separation and preconcentration of metal ions in various samples. Single-drop microextraction (SDME) is an alternative method for preconcentration and matrix separation prior to detection. The present work describes a new method for the preconcentration and determination of lead by SDME combined with electrothermal atomic absorption spectrometry (ETAAS). The method was based on the formation of an ionic pair between lead iodide-Rhodamine B in phosphoric acid medium. In the presence of a large excess of KI, anionic lead iodide was complexed with Rhodamine B as an ion-association complex [1]. In the preconcentration step, lead iodide-Rhodamine B complex was extracted into a 10 µL drop of diisobutylketone-carbontetrachloride mixture. The extracted complex was directly injected into the graphite furnace. Some effective parameters on extraction and complex formation, such as type and volume of organic solvents, concentration of phosphoric acid, KI and Rhodamine-B, extraction time and stirring rate were optimized. The calibration graph was linear in the range of 0.025–1.00 μg L^{-1} with correlation coefficient 0.9997 under the optimum conditions of procedure. The enhancement factor defined as the slope ratio of two calibration curves with microextraction and without microextraction was 426. The effects of interferences were also investigated. The proposed method was successfully applied for the determination of lead in water samples.

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APPLICATION OF NOVEL DOUBLE LAYER SORBENT SYSTEM IN MEMBRANE-SOLID-PHASE MICROEXTRACTION TECHNIQUE FOR THE ISOLATION AND PRECONCENTRATION OF ORGANIC POLLUTANTS IN WATER SAMPLES

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According to the legal regulations, the allowed content of compounds, having an adverse effect on human health, in drinking water is very low. Therefore accurate monitoring of an environment requires application of additional isolation and/or preconcentration steps prior to the final determination of the critical compounds. In addition, analytical procedure and analytical tools applied, should meet requirements of the concept of sustainable development and green chemistry.

One of the most popular techniques of sample preparation, which fulfils the requirements of green analytical chemistry, is Solid Phase Microextraction (SPME). SPME is widely applied in analytical practice because of its undoubted advantages: simplicity of operation, short extraction time, solvent-free nature of the separation process, the possibility of automation, and easy coupling with measuring instruments. However, SPME is not devoid of some drawbacks like poor selectivity and the limited choice of polar commercially available fibre coatings as well as low affinity for polar compounds. The latter is particularly important in the case of isolation of polar analytes from samples with a polar matrix (like water).

One of the concepts for overcoming these problems is to separate the polar retention medium from the sample by means of a impermeable membrane, in our studies out so far, this system has been implemented in SPME. The main reason for application of membrane is to avoid mixing of extraction and sample phases. An analyte needs to be only very slightly soluble for this sorption system to effectively isolate it from an aqueous medium. The practical aspects of the M-SPME approach create new opportunities in SPME, such as the use of new classes of polymers, which, due to their solubility in water, have so far been rejected from these applications.

The idea of this technique with the first results have been published on Euroanalysis 2009, presented results concern application of novel double layer sorbent system for the isolation and preconcentration of organic pollutants from water samples using determination by gas chromatography. The results show that extraction of fenols and volatile organic compounds from standard aqueous solutions using M- SPME is several times more efficient than with commercially available polar fibers and show a high extraction recovery from the headspace of the most polar compounds in the mixture. Results of preliminary studies indicate that Membrane-SPME may become a powerful tool for the determination of polar organic compounds in water samples.

MAGNETIC SEPARATION AS A NEW METHOD EXTRACTION OF LOW MOLECULAR WEIGHT SUBSTANCES FROM HUMAN BIOLIQUIDS

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New classes of doping substances such as selective androgen receptor modulators (SARM) and agonists of peroxisome proliferator-activated receptor δ (PPARδ) have been recently developed. SARMs influence the androgen receptors responsible for muscle growth. They are intended to have the same kind of effects as androgenic drugs like anabolic steroids but are much more selective in their action. PPARδ increase endurance by changing metabolic processes in muscle tissue. PPARδ and SARMs belong to the new doping classes that have been under the investigation for a few years. They are considered as emerging potential doping substances and are prohibited by the World Anti-Doping Agency.

A new method of extraction of these doping substances from urine was proposed. The method is based on application of C18-modified ferromagnetic micro-particles for sample preparation followed by liquid chromatography–tandem mass spectrometry.

Sample preparation using magnetic separation is easier and faster than solid phase (SPE) or liquid-liquid extraction (LLE). Sample preparation time is 5 minutes only. A suspension of ferromagnetic micro-particles is added to 1 ml of urine sample, mixture is mixed thoroughly, aqueous phase is discarded using a magnetic separator, and micro-particles are washed out by deionized water and eluted by an organic solvent.

It was found that optimal amount of the sorbent is 200 μl of micro-particles solution with the concentration of 1.25 mg/ml (sorbent mass 0.25 mg). In this case the highest extraction recovery and lowest ion-suppression effects were observed. The comparison of elution solvents has demonstrated that desorption of substances is the same for methanol and acetonitrile. Methanol was selected because acetonitrile resulted in the tailing of chromatographic peak fronts.

Our experiments demonstrate that magnetic separation gives promising results. The recovery of doping substances is close to 100%. Ion-suppression effects are negligible. The limit of detection for PPARδ and SARMs is 0.02-0.33 ng.

DETERMINATION OF TRACE CADMIUM IN WATER SAMPLES BY USING IONIC LIQUID-BASED SINGLE DROP MICROEXTRACTION-ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Determination of cadmium at trace levels is very important for monitoring environmental pollution because it is classified as toxic metal even at very low concentrations [1]. A new method based on single drop microextraction (SDME) combined with graphite furnace atomic absorption spectroscopy (GF-AAS) was developed to determine the trace amounts of cadmium in water samples. In the present work, the ionic liquid 1-hexyl-3-methylimidazolium hexafluorophosphate ($HMIMPF_6$) was used as a solvent for SDME of cadmium as ammonium pyrroldinedithiocarbamate (APDC) complex. The extracted complex was directly injected into the graphite furnace. The density of ionic liquid-phase is greater than that of aqueous solution. It is not possible to suspend a microdrop of $HMIMPF_6$ from the tip of needle because it is easily released. In order to avoid this problem, mixture of $HMIMPF_6$ and di-isobutyl ketone (DIBK) was used as microdrop. One of the important factors in SDME is the volume of the microdrop. The effect of drop volume on the extraction efficiency was studied in the range of 3–15 μL. Optimisation of variables such as type of ionic liquid and other solvents, pH of the sample, concentration of APDC, stirring rate and extraction time were studied, and the optimized procedure was applied to determine cadmium in aqueous samples.

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NEW APPROACH FOR ACETALDEHYDE DETERMINATION IN WINES

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The purpose of this study was to develop a rapid and easy extraction procedure for HPLC-UV analysis of free and total acetaldehyde in wine by gas-diffusion microextraction (GDME). GDME is an innovative technique that combines the advantages of membrane aided gas-diffusion with microextraction concepts. Volatile and semi-volatile compounds released by the sample can permeate the membrane pores and are collected by an adequate solution. The experimental simplicity of the sample preparation process is the main advantage of this extraction system [1,2].

Acetaldehyde (or ethanal) is the main aldehyde found in wine and has significant sensory properties that can impart pleasant or unpleasant notes to different products. It is formed during fermentation as an intermediate product of the ethanol's formation from sugars and its content in wine mainly depends on the aeration. Also, ethanol's oxidation can increase the acetaldehyde's concentration during storage. Sulphur dioxide (SO_2) is a widespread additive used in wine making due to its antiseptic and antioxidant properties. Also, its high capacity in binding to carbonyl compounds becomes very useful in wine's aroma regulation. That is where the concept of free, combined and total SO_2 comes from. To the sensorial quality of the product, free forms of the compounds are the major contributors. However, due to many chemical alterations that can occur during storage it is important to know the total content of flavoured compounds for long-term wine quality prediction.

The methodology is based on the extraction of acetaldehyde from the sample through the membrane, and its entrapment by an appropriate solution placed in the other side of the extraction module. Such solution is 2,4-dinitrophenylhydrazine that reacts with aldehydes originating 2,4-dinitrophenylhydrazones, detected by UV spectrophotometry [3]. Several extraction parameters were studied: temperature, time, membrane reproducibility and SO_2 influence. Finally, free and total acetaldehyde was quantified in various wine samples.

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