Proceedings of the 10th International Students Conference "Modern Analytical Chemistry"

Prague, 22—23 September 2014

Edited by Karel Nesměrák

Charles University in Prague, Faculty of Science
Prague 2014

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Modern Analytical Chemistry (10. : 2014 : Praha, Česko)
Proceedings of the 10th International Students Conference
"Modern Analytical Chemistry" : Prague, 22–23 September 2014 /
edited by Karel Nesměrák. – 1st ed. – Prague : Charles
University in Prague, Faculty of Science, 2014. – 96 s.
ISBN 978-80-7444-030-4 (brož.)

543
▪ analytical chemistry
▪ proceedings of conferences
▪ analytická chemie
▪ sborníky konferencí

543 – Analytical chemistry [10]
543 – Analytická chemie [10]

The electronic version of the Proceedings is available at the conference webpage:
http://www.natur.cuni.cz/isc-mac/

ISBN 978-80-7444-030-4

Preface

Dear friends and colleagues,

Welcome to the 10th International Students Conference "Modern Analytical Chemistry". We are very pleased that you are participating on this platform for the presentation of your achievements in the field of analytical chemistry. The aim of the conference is to offer you the chance for improvement of the presentation skills, to provide the floor for discussion and exchange of experiences and opinions, and moreover to enhance the knowledge of English language. We hope that – thanks to you, young analytical chemists – the conference will be interesting, challenging, and successful event.

The tenth anniversary of the conference gives us the opportunity to glance back at the previous years. Beginning with only nine participants in 2004, more than 270 participants from seven countries (Austria, Czech Republic, Germany, the Netherlands, Pakistan, Poland, and Slovakia) have participated at the conference. We are very pleased that our forum has become so attractive and inspiring for young analytical chemists.

All sponsors are cordially thanked, not only for their kind financial sponsorship, but also for their continuous support and cooperation in many of our other activities.

We wish you success in the presentation of your research, vivid discussions with the audience and your colleagues, pleasant social encounters, and nice stay in the city of Prague.

Prof. RNDr. Věra Pacáková, CSc. RNDr. Karel Nesměrák, Ph.D.

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Programme

The conference is held at the Institute of Chemistry, Faculty of Science, Charles University in Prague (Hlavova 8, 128 43 Prague 2) in the main lecture hall (Brauner's Lecture Theatre). Oral presentations are fifteen minutes including discussion and speakers are asked to download their Power Point presentation on the local computer in the lecture hall before the start of the session.

Monday, September 22, 2014

9:00–9:30	**Registration of participants**
9:30–9:45	**Opening ceremony, welcoming address**
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9:45–10:00	Ston M.: *Pressure modulator development and its optimization for applications in comprehensive gas chromatography* (p. 11)
10:00–10.15	Nováková E.: *Vapour generation of selenium: comparison of existing approaches* (p. 13)
10:15–10:30	Mourão M.P.B.: *Hyphenated and comprehensive LC×GC for the identification of* Mycobacterium tuberculosis (p. 15)
10:30–10:45	Prchal V.: *Determination of nitro aromatic environmental pollutants using bismuth bulk electrode* (p. 17)
10:45–11:00	**Coffee Break**
	Session 2 ▪ chairperson: Tereza Rumlová
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11:15–11:30	Horakova E.: *Voltammetric study and determination of methyl violet 2B using a hanging mercury drop electrode* (p. 21)
11:30–11:45	Rybínová M.: *Determination of selenium using UV-photochemical volatile compounds generation in combination with QF-AAS. From the construction of the apparatus to real sample* (p. 23)
11:45–12:00	Spevak A.: *Development of extraction and HPLC methods for determination of coumarins in plant samples* (p. 27)
12:00–12:15	Machyňák Ľ.: *Determination of chromium in the waters by flow-through coulometry* (p. 28)

12:15–13:00 **Lunch**

Session 3 ▪ chairperson: Magda Staňková

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13:15–13:30 Sochr J.: *The use of boron-doped diamond electrode in the electroanalysis of stress hormones* (p. 32)

13:30–13:45 Kotora P.: *The analysis of linear and monomethylalkanes in exhaled breath samples GC techniques* (p. 34)

13:45–14:00 Rejšek J.: *Development and applications of ionization techniques in ambient mass spectrometry* (p. 37)

14:00–14:15 **Coffee Break**

Session 4 ▪ chairperson: Karel Marschner

14:15–14:30 Brama K.: *ICP-MS and SEC-ICP-MS probing of chromium and vanadium bioaccessibility by garden cress and their bioavailability for humans* (p. 39)

14:30–14:45 Poláček R.: *Fluorescence spectroscopy as a tool for determination of coumarins in melilotus officinalis by multivariate calibration* (p. 41)

14:45–15:00 Surmová S.: *Analysis of perfumes by using multi-dimensional gas chromatography* (p. 43)

15:00–15:15 Klusáčková M: *Hydrogen oxidation reaction on electrode modified by water soluble phthalocyanine* (p. 44)

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15:30–15:45 **Sponsors' Presentations**

16:00 **Get-Together Party**

Tuesday, September 23, 2014

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9:45–10:00 Truskolaska M.: *Determination of tributyltin in sediments* (p. 51)

10:00–10.15 Čížková A.: *Dispersive liquid-liquid microextraction method: application to essential oils analysis in real samples of herbal beverages* (p. 53)

10:15–10:30 Zavazalova J.: *Utilization of boron-doped diamond electrode in electroanalysis of benzophenone-3* (p. 54)

10:30–10:45 Bierhanzl V.M.: *GC-MS analysis of polar lipid headgroups* (p. 56)

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Session 6 ▪ chairperson: Eva Háková

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11:15–11:30 Hájková A.: *Voltammetric study of 2-aminofluoren-9-one using bare and DNA-modified glassy carbon electrodes* (p. 61)

11:30–11:45 Zvěřina Z.: *Laser-induced breakdown spectroscopy in analysis of liquids and solids* (p. 63)

11:45–12:00 Barcaru A.: *Retention time prediction in temperature-programmed GC×GC: modelling and error assessment* (p. 67)

12:00–12:15 Krejčová Z.: *Voltammetric behaviour and determination of toxic drug nitrofurantoin using a mercury meniscus modified silver solid amalgam electrode* (p. 69)

12:15–13:00 **Lunch**

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13:15–13:30 Marschner K.: *Arsenic speciation analysis by HPLC postcolumn hydride generation and detection by atomic fluorescence spectrometry* (p. 74)

13:30–13:45 Tůmová T.: *Analysis and characterization of antimicrobial peptides by capillary electromigration methods* (p. 78)

13:45–14:00 Rumlová T.: *Electrochemical study of 2-nitrophenol using carbon film electrode and its application to determination of model samples of drinking water* (p. 80)

14:00–14:15 **Coffee Break**

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14:30–14:45 Zlámalová M.: *Characterization of poly(methylene blue) modified graphite electrode as a sensor for hydrogen sulphide* (p. 84)

14:45–15:00 Kremr D.: *Comparison of various methods for extraction of capsaicinoids* (p. 86)

15:00–15:15 Linhart O.: *UV-photochemical cold mercury vapor generation as a derivatization step between HPLC separation and AAS detection for speciation analysis of selected mercury compounds* (p. 87)

15:15 **Closing Ceremony**

Contributions

Pressure Modulator Development and Its Optimization for Applications in Comprehensive Gas Chromatography

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Keywords
comprehensive two-dimensional gas chromatography
gas chromatography
pressure modulator
optimization

Comprehensive two-dimensional gas chromatography (GC×GC) is a modern technique that passes all sample components through two different (orthogonal) capillary columns. Separation is achieved by coupling a gas chromatography separation in the first and second column via sophisticated interface (modulator), which is a piece of hardware that transfers effluent from the exit of the primary column to the head of the secondary column as a repetitive series of external pressure pulses [1].

The main aim of this study is construction of the pressure modulator and optimization of its connection to the gas chromatograph with a flame ionization detector. Two fast two-way solenoids and one storage capillary are the key components of this modulator. The valves provide distribution of a mobile phase between two transfer lines. Each transfer line ends in one T-connector and these two connectors are separated by a storage capillary and positioned between separation columns. Storage capillary storages temporarily small volume of the effluent from the first analytical column and injects it periodically by high flow rate pulse to the second analytical column.

Optimal conditions were found for the analysis of the mixture of selected volatile solvents. The system pressure interdependences and relations between dimensions of modulator capillaries and separation columns have been evaluated with respect to the duration of the modulation period and pulse.

Acknowledgments

The study was supported by the Charles University in Prague (project GA UK No. 894513, and project SVV260084).

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Vapour Generation of Selenium: Comparison of Existing Approaches

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Keywords
chemical vapour generation
electrochemical vapour generation
interferences
photochemical vapour generation
selenium

This contribution is dealing with comparison of existing approaches to vapour generation coupled to atomic absorption spectrometry with selenium as the selected model analyte. All measurements were carried out in one laboratory thus permitting a comparison of results.

Vapour generation is an approach often coupled to element selective detection techniques with the intent to increase the sensitivity of the analyte determination into ultratrace range (sub ppb). It is commonly coupled to the atomic absorption spectrometry (AAS) as well as to the atomic emission spectrometry (ICP-OES) or atomic fluorescence spectrometry (AFS) to achieve detection limits close to those generally achieved with mass spectrometric detection (ICP-MS). Furthermore, for some elements it offers another option for speciation analysis without coupling to a separation technique. The generated volatile products of hydride forming elements are most usually binary hydrides (hence the abbreviation HG for vapour generation), however the product depends on the selected method and reagents used. Vapour generation is associated with reduced interferences due to separation of the analyte from the sample matrix, nevertheless some elements are known to negatively affect various stages of the HG-AAS determination. In general, other hydride forming elements mostly interfere in the atomization stage (gaseous phase). On the other hand, ions of transition metals are known to adsorb the analyte or its hydride or decompose the volatile hydride prior to its separation from the liquid phase [1].

Currently, three major HG approaches have been developed and studied: chemical vapour generation (CVG), electrochemical vapour generation (EcVG), and

most recently photochemical vapour generation (PVG). While the chemical vapour generation is a well-documented and established method, the alternative methods are still in the process of study. Each of these three methods has its advantages and disadvantages. Chemical vapour generation with sodium tetrahydroborate reagent is generally accepted as the most sensitive approach, however it exhibits serious liability to interferences and the reagent itself is a possible source of contamination. Electrochemical vapour generation is considered to have higher tolerance to concomitant ions in the sample, on the other hand keeping reproducible cathode surface between the measurements is not trivial [2]. The photochemical approach is promising a simpler procedure and arrangement compared to the other two methods.

The criteria for the comparison are the following: i) the performance characteristics, namely the limits of detection and determination, sensitivity, repeatability and linear dynamic range, ii) the impact caused by known interferents of the HG technique, and lastly iii) reagents and apparatus used. The greatest attention has been paid to the evaluation of interferences caused by other hydride forming elements and transition metals in the sample solution, namely As^{III}, Cu^{II}, Co^{II}, Fe^{III}, Ni^{II}, and wherever possible also by acidic anions used as reagents in the vapour generation methods (Cl^-, NO_3^-).

Acknowledgments

This work was financially supported by the Charles University in Prague (project UNCE#42, project SVV 260084/2014 and project GA UK 228214), and by the Grant Agency of the Czech Republic (Project GACR 14-23532).

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Hyphenated and Comprehensive LC×GC for the Identification of *Mycobacterium tuberculosis*

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Keywords
biomarkers
gas chromatography
liquid chromatography
Mycobacterium tuberculosis

Tuberculosis remains one of the world's most pressing public health problems. The World Health Organisation estimates a global prevalence with 14 million new cases and 1.68 million deaths each year. The majority of tuberculosis cases occur in low-income countries that have poor resources in the public health care sector. Although the disease is curable, late diagnosis has serious consequences for the patient and contributes to the increase of the epidemic [1]. Current methods for identifying the mycobacteria responsible for tuberculosis, *Mycobacterium tuberculosis*, are time-consuming, labour intensive, too expensive in terms of running costs for developing countries and lack sensitivity [2, 3]. Chromatographic methods could resolve these issues at least partially.

Several chromatography-based methods for tuberculosis diagnosis have been published in literature. An HPLC method for the identification of mycobacteria based on the mycolic acid patterns was developed by the Centre for Disease Control and Prevention (CDC) already two decades ago [4].

Recently, we have developed a fully automated GC procedure based on thermally assisted hydrolysis and methylation (THM-GC-MS) and advanced chemometrics to detect *Mycobacterium tuberculosis* [5]. Irrespective of whether LC or GC is used, due to the complexity of the samples the evaluation of potential biomarkers is extremely challenging.

The present contribution aims to combine LC and GC in parallel and in series in order to overcome the difficulties of biomarker evaluation. The in-series combination consists of the LC analysis with heart-cut or comprehensive transfer of the specific mycolic acid fractions from the LC system to the GC. In this way the strengths of the LC and the GC methods are combined resulting in better detection limits (i.e. earlier disease diagnosis) and an improved accuracy and selectivity.

Acknowledgments

Prof. Dr. Ir. Peter Schoenmakers, NanoNextNL of the government of the Netherlands and 130 partners.

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Determination of Nitro Aromatic Environmental Pollutants Using Bismuth Bulk Electrode

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Keywords
bismuth bulk electrode
differential pulse voltammetry
electrochemistry
5-nitroindazole
2,4,6-trinitrophenol

One of the challenges in modern electroanalytical chemistry is developing replacement of mercury based electrodes. Concerns about toxicity of liquid mercury have forced these traditional electrodes out of favour in modern analytical laboratories. One of the potential replacements of mercury based electrodes are bismuth based electrodes. Toxicity of bismuth is negligible compared to toxicity of mercury, while showing very similar electrochemical behaviour to mercury based electrodes. Bismuth based electrodes received a lot of attention in past fifteen years. All the important facts can be found in several reviews, most important one by Švancara et al., published in 2010 [1].

In this work, main focus was on using bismuth bulk electrode for determination of various organic pollutants of the environment. Many successful determinations of inorganic pollutants were performed using bismuth electrodes; however determinations of organic substances are rather scarce [2–4]. Determination of two organic pollutants (picric acid and 5-nitroindazole) were performed using bismuth bulk electrode. These substances are strong genotoxic agents, so development of new, cheap, and easy to perform analytical methods is more than desirable. For both compounds, newly developed methods were applied to real samples of drinking and river water. The biggest challenge when using bismuth bulk electrode can be lower repeatability, caused by passivation of the electrode surface. However, this potential problem was solved successfully by using suitable electrode pretreatment and handling. All measurements were

Table 1
Characteristics of determinations of selected nitro aromatic environmental pollutants by differential pulse voltammetry using bismuth bulk electrode.

Comp.	Matrix	Slope (nA L μmol^{-1})	Intercept (nA)	*R*	*LD* (μmol L^{-1})	*LQ* (μmol L^{-1})	*RSD* (*n* = 20) %
Picric acid	Deion. water	8.71	104.1	0.9929	0.85	2.85	2.2
	Drinking water	7.37	40.1	0.9978	1.16	3.88	6.4
	River water	7.53	17.6	0.9961	0.76	2.56	7.6
5-Nitro-indazole	Deion. water	2.92	–0.27	0.9989	0.20	0.67	3.2
	Drinking water	2.86	2.86	0.9945	0.16	0.52	5.3
	River water	2.30	–1.43	0.9963	0.32	1.08	7.6

performed using differential pulse voltammetry using three-electrode system (bismuth bulk electrode as working, Ag/AgCl 3M as reference, and platinum wire as auxiliary electrode).

New methods for electrochemical determination of the picric acid and 5-nitroindazole were successfully developed and tested. Calibration curves were examined in concentration ranges from 1 μmol L^{-1} to 100 μmol L^{-1} with limits of quantification in micromolar and submicromolar region for all sample matrices. All results for both compounds are summarized in Table 1.

Acknowledgments

Financial support by Grant Agency of the Czech Republic (project P206/12/G151), and Charles University in Prague (SVV260084) is gratefully acknowledged.

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Hyphenation of Ion Chromatography and Capillary Electrophoresis

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Keywords
capillary electrophoresis
hyphenation
ion chromatography
mass spectrometry
two-dimensional separation

The separation and analysis of samples containing numerous different analytes is a challenging task. The separation efficiency of a single separation technique such as chromatography or electrophoresis is not sufficient enough to resolve all components in complex biological and environmental samples [1]. In contrast to one dimensional separation techniques, the peak capacity of multidimensional separation systems is much higher rendering them more suitable for analysis of complex samples [2]. These systems preserve the separation throughout their whole subsystems, which preferably possess orthogonal separation mechanisms. The most important techniques include comprehensive hyphenation of gas chromatographic systems (GC×GC), liquid chromatographic systems (LC×LC), or the hyphenation with electrophoretic systems [2–5].

We present the hyphenation of the two most important techniques in instrumental ion analysis, namely ion chromatography (IC) and capillary electrophoresis (CE). Both techniques are based on completely different separation mechanism providing high orthogonality. They became technically compatible as modern IC instruments work in capillary dimensions [6] and their flow rates resemble the flow rates of CE (low μL min^{-1} range). Moreover, instrumental ion suppressors remove highly concentrated eluents such as potassium hydroxide [7]. Due to this, only analytes in pure water are subsequently transferred to CE annihilating any matrix effects. Finally, the time ranges of both techniques are compatible to each other as we (and others) demonstrated that very fast CE separations (in the range of seconds) are possible [8, 9]. Both IC and CE were linked by a transfer capillary leading to a modulator, which submits the IC effluent to the CE cell (Fig. 1). The modulator was developed from the setup of capillary

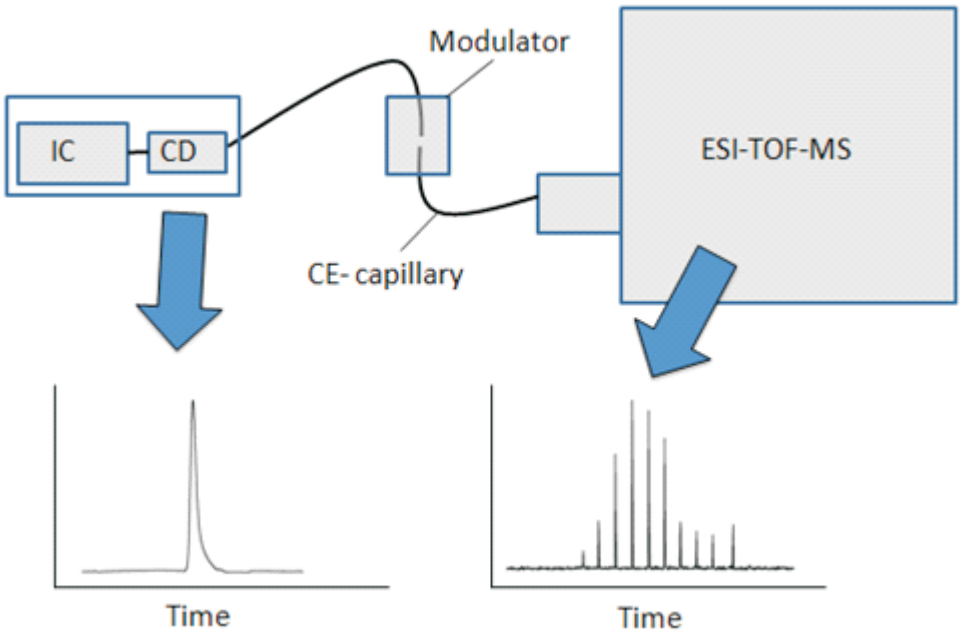

Fig. 1. Scheme of the hyphenation of ion chromatography (IC) with conductivity detection (CD), capillary electrophoresis (CE), and high resolution time-of-flight mass spectrometry with electrospray ionization (ESI-TOF-MS).

batch injection, which we described in previous works [10]. Capillary batch injection enables the injection of small discrete sample volumes from the transfer capillary into the separation capillary. High resolution time-of-flight mass spectrometry (MS) with electrospray ionization was used for detection. The IC×CE-MS system was evaluated by analysis of a model system of nucleotides and their corresponding cyclic derivates to investigate the performance of the resulting hyphenation approach.

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Voltammetric Study and Determination of Methyl Violet 2B Using a Hanging Mercury Drop Electrode

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Keywords
determination
hanging mercury drop electrode
methyl violet 2B
triphenylmethane dyes
voltammetry

There is an ever increasing demand for determination and monitoring of trace amounts of hazardous compounds in the environmental and clinical samples. Many sensitive chromatographic methods for the determination of hazardous compounds in air, water, and biological matrices have been developed [1], but polarographic and voltammetric methods offer less expensive and competitive alternatives [2].

Methyl violet 2B belongs to the group of triphenylmethane dyes. They are used in paper dyeing, as inks, pH indicators [3] and in medical sciences [4]. Because they have many negative effects [5–9], study of their biological effects [10, 11] and monitoring of the occurrence in biological matrices [8, 12] and environment [13] is important. Triphenylmethane dyes are electrochemically reducible [14], so it is possible to determine them using electrochemical methods.

In this work, DC voltammetry (DCV), differential pulse voltammetry (DPV), adsorptive stripping DPV (AsSDPV), and cyclic voltammetry at a hanging mercury drop electrode (HMDE) were used to study the voltammetric behavior of methyl violet 2B, and DCV, DPV and AdSDPV were used for the development of sensitive methods for its determination in water solutions. The optimum pH for the determination of methyl violet 2B was sought (at concentration of 10^{-4} mol L^{-1}) in the pH range of 2.0–12.0 of the Britton-Robinson buffer solution, and it was found to be pH = 4.0 for all the techniques examined. The dependence of the wave/peak current on the methyl violet 2B concentration was measured for DCV in the

concentration range of 2–100 μmol L^{-1} with the limit of quantification of 0.65 μmol L–1, for DPV in the concentration range of 0.1–100 μmol L^{-1} with the limit of quantification of 0.05 μmol L^{-1} and for AdSDPV in the concentration range of 0.02–0.2 μmol L^{-1} with the limit of quantification of 0.01 μmol L^{-1}.

The applicability of the newly developed methods was successfully verified on model samples of drinking and river water. In addition, on the basis of the results obtained using cyclic voltammetry, the mechanism of the electrochemical reduction of methyl violet 2B on the HMDE was predicted.

Using UV-Vis spectrophotometry, the stability of the methyl violet 2B stock solution was monitored and the acidity dissociation constant of methyl violet 2B was determined. Moreover, it was possible to determine methyl violet 2B spectrophotometrically in the concentration range of 0.5–50 μmol L^{-1} with the limit of quantification of 0.5 μmol L^{-1}, which confirmed that the differential pulse-based voltammetric methods (DPV and AdSDPV) developed in this work can be considered as more sensitive alternative for the determination of methyl violet 2B.

It was shown that voltammetric methods at the HMDE are suitable for the determination of submicromolar concentrations of methyl violet 2B in simple environmental matrices drinking and river water. This work can also lay out the foundation for the use of other electrodes, e.g., silver solid amalgam electrodes, which represent a non-toxic and mechanically robust alternative to the traditional mercury electrodes [15].

Acknowledgments

This research was carried out within the framework of the Specific University Research (SVV260084). Financial support from the Grant Agency of the Czech Republic (Project P206/12/G151) is gratefully acknowledged.

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Determination of Selenium Using UV-photochemical Volatile Compounds Generation in Combination with QF-AAS. From the Construction of the Apparatus to Real Sample

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Keywords
food supplements
QF-AAS
selenium
UV-photochemical generation
water samples

Presented study was focused on the determination of selenium in aqueous medium using UV-photochemical generation of its volatile compounds (UV-PVG). Atomic absorption spectrometry with the externally heated quartz furnace atomizer (QF-AAS) was utilized for the detection.

UV-photochemical generation of its volatile compounds represents an interesting alternative to the user's favorite chemical generation with borohydride (most often $NaBH_4$). It is a dynamically developing technology in the field of analytical chemistry that can attract attention due to its ease of instrumentation or the chemicals used. Also high sensitivity and low detection limits can be achieved with this technique. Compared to chemical generation mentioned above, this approach to volatile compounds generation eliminates the difficulties associated with the reducing agent and its instability or limited purity. Furthermore, UV-photochemical generation increases resistance to some interferents.

As the name of the technique implies, during UV-photochemical generation, the conversion of nonvolatile precursors (Se(IV)) from the condensed phase to volatile species occurs under the action of UV irradiation. The presence of low molecular weight organic acids is also crucial requirement [1]. Formic acid and

acetic acid were chosen in this work. Reaction mechanism remains the subject of discussion because of the complicated nature of photoreactions [2].

Selenium was selected as a model analyte for the study. It is one of the representatives of typical hydride forming elements and it is also interesting in terms of importance for the human body. Depending on the intake level it is either essential or toxic and the boundary distinguishing these two states is relatively narrow. Therefore, it is necessary to pay close attention to the determination of this element on ultra-trace range. For a better idea, in the Czech Republic, 0.055 mg is the recommended daily dose of this element [3]. At the same time it can be found in the literature that the oral exposure of 0.853 mg/day denotes no observed adverse effect level (NOAEL) while 1.261 mg/day represents the lowest concentration of a substance discovered by observation that causes an adverse effect on the body as compared with the control group (LOAEL) [4].

Several targets have been demanded for the study. First, it was necessary to assemble the apparatus in a flow mode. Attention was paid especially to the main component of the system, the volatile compounds generator; reaction tubes made of different materials, with different sizes/inner diameters have been used. From all tested arrangements a PTFE tubing (1.4 mm o.d./1.0 mm i.d.) wrapped around low-pressure Hg UV lamp (20 W, 253.7 nm) was the best. Optimum experimental conditions for generation were found after the completion of the system. Following key parameters were optimized: length of the reaction coil, the sample flow rate, the carrier gas flow rate (argon) as well as the auxiliary hydrogen flow rate (necessary for atomization), the concentration and type of organic acid or the concentration of additives that improve the analytical response (especially nitric acid).

In a further step, basic characteristics were determined. To be specific, with the instrumental set-up and under the optimum analytical conditions detection limits range from 25 to 45 ng L^{-1} of Se(IV); exact values vary depending on whether the additive nitric acid was used. Repeatability of 1.5 % (RSD, n = 10) was achieved by UV-PVG-QF-AAS. Sensitivity or linear dynamic range was also found. The accuracy of the method was validated by determining Se(IV) in certified reference material with good agreement between certified and our achieved value. Further, interference study was also done.

Analysis of real samples was carried out to demonstrate the performance of the technique. The method of standard addition was used for the evaluation. Initially, the content of selenium in food supplements freely available in pharmacy was determined. The tablets were dissolved in deionized water, nitric acid or hydrochloric acid, and subjected to ultrasound (ultrasonic bath for 30 min). Insoluble part of the tablet was then separated by filtration and only the remaining filtrate was used for the analysis. More than 80% of the declared amount of selenium was obtained for all tested preparations.

Based on the encouraging results, selenium content in water samples has been examined. Tap water, groundwater and bottled mineral water were analyzed.

Even though it was assumed that the selenium content in these real samples will be low, values close to the limit of quantification were achieved by the technique of UV-PVG/QF-AAS.

Acknowledgments

This work was financially supported by the Charles University in Prague project SVV 260084/2014, project UNCE#42 and GAUK 228214.

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Development of Extraction and HPLC Methods for Determination of Coumarins in Plant Samples

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Keywords
coumarins
HPLC
plant samples
solvent extraction

Coumarins are a group of flavour substances occurring in the free form or glycosidically linked compounds. Coumarin is used in certain perfumes and fabric conditioners. It has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned as a flavouring food additive, due to concerns regarding its hepatotoxicity in animal models [1]. Therefore of this it is very important to develop methods of extraction and determination of coumarin and its derivatives in real samples.

Aim of this work was to develop the method for analysis of medical plant samples (lavender, chamomile, melilotus officinalis, and cinnamon). The work was also orientated on development of method for solvent extraction of coumarins from plant samples. Solvent extraction procedures with stirring-assisted extraction and ultrasonic-assisted extraction were investigated to extract coumarins from dried plant samples. The experiment were performed by using water (23 °C), water (90 °C) and the mixture of methanol/water (1/1, v/v, 23 °C) as extraction solvents. For all solvents the extraction time was 60 minutes. The best results were obtained employing the mixture of methanol/water (1/1, v/v).

The HPLC method was used for analysis of extracts. The gradient HPLC method was optimized and a baseline resolution was obtained for the investigated compounds. Two stationary phases of C18 and Phenyl-hexyl type were compared for the separation of coumarins. The mixture of 0.3% acetic acid/acetonitrile (9/1, v/v) and 100 % acetonitrile with gradient elution was as mobile phase used. The good selectivity was ensured by the use of a spectrophotometric detector. The

group of compounds (esculine, daphnetin, umbelliferone, 4-methylumbelliferone, scoparone, and herniarin) was detected at wavelength 323 nm and wavelength 280 nm was suitable for the coumarin and 4-hydroxycoumarin. The limits of detection ranged from 0.1 to 0.7 g/mL and limits of quantification from 0.3 to 2.0 g/mL. Qualitative analysis was performed by comparing the retention factors and UV spectra of individual peaks of extract components with those of the standards analysed under the same conditions. In the tested plant extracts was found out the presence of three coumarins: umbelliferone, coumarin, and herniarin.

Acknowledgments

This research was supported by Grant VEGA nr. 1/0499/14, and Grant supporting young researchers, Slovak University of Technology in Bratislava (nr. 1361).

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Determination of Chromium in the Waters by Flow-Through Coulometry

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Keywords
chromium
flow-through coulometry
water

In natural environment, chromium occurs in two thermodynamically stable oxidation states, Cr(VI) and Cr(III). Its toxicity strongly depends on its oxidation state; Cr(VI) is an inhaled carcinogen, toxic to humans and other mammals, while Cr(III) at trace levels is an essential mineral supplement [1]. The speciation of chromium determines not only its ecological impact, but also its mobility and transport behaviour in the environment. High concentrations of chromium and related compounds have been found in polluted soil and water bodies due to its extensive use in dyeing, leather tanning and electroplating industries [2]. Studies related to reduction and migration of Cr(VI), distribution of Cr(III) between inorganic and organic compounds, and remediation of contaminated environment are currently underway. Accurate and precise analyses of chromium speciation in environment samples is therefore required [3].

In this work the determination of chromium in the water has been investigated by flow-through coulometry. The measurements were done on an electochemical flow system EcaFlow® 150 GLP manufactured by Istran, s.r.o., Bratislava. The three-electrode flow cell consisted of the working electrode, the auxiliary platinum electrode and the Ag/AgCl reference electrode which was separated from the flowing solution by a membrane. As a working electrode we used a reticulated vitreous carbon (RVC) for determination of Cr(VI) and total chromium and for determination of Cr(III) we used a gold wire electrode. Carrier electrolyte used for determination of Cr(VI) and total chromium was 0.1 mol L^{-1} HCl and for Cr(III) it was a solution of 0.1 mol L^{-1} Na_2HPO_4 with addition of 0.04 mol L^{-1} HCl. In the first step we focussed on the optimization of the working parameters for the electrochemical determination of Cr(VI) by flow-through coulometry and in the next step on validation of this analytical procedure. Finally

we investigated the possibility of a direct electrochemical oxidation of Cr(III) to Cr(VI).

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0797-11.

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Vernix caseosa and its Newly Discovered Nonpolar Lipids

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Keywords
2D-offline chromatography
mass spectrometry
lipids
vernix caseosa

Vernix caseosa is a multicomponent mixture, which is consisted mainly of water (80%) and then of proteins and lipids in the same amount. This unique human material begins to be formed in the third trimester of pregnancy and is present on the skin of newborns after delivery [1, 2]. The lipids of vernix caseosa are classified as barrier lipids (cholesterol, free fatty acids, phospholipids, ceramides) and lipids originated from fetal sebaceous glands. Nonpolar lipids such as sterol esters, wax esters and triacylglycerols are dominant components of vernix caseosa [2, 3].

The aim of this work is a description of newly, so far undescribed nonpolar lipids, which are components of vernix caseosa. Firstly it was pre-separated 4.7 grams of lipid isolated from vernix caseosa (the same part from boys and girls) by column chromatography with silica gel and we got 30 mostly nonpolar lipids fractions. Information about elemental composition was obtained by analysis of these factions by high resolution ESI-MS (orbitrap). From this measurement we discovered that acquired fractions contain lipids with up to eight oxygens. Afterwards 2D-offline chromatography was used so that the fractions could be described in detail. In the first step the selected fractions were separated by HILIC chromatography and sub-fractions were collected. This step was followed by separation of fractions using RP-HPLC-MS^2. By this measurement we obtained detailed information about each class of lipid. Our results were also supported by IR and NMR measurement.

Acknowledgments

This work was financial supported by the Czech Science Foundation (Project No. P206/12/0750) and Charles University in Prague (Project SVV 260084).

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The Use of Boron-Doped Diamond Electrode in the Electroanalysis of Stress Hormones

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Keywords
determination
differential pulse voltammetry
epinephrine
human urine

Epinephrine (also known as adrenaline) is stress hormone and neurotransmitter, which belongs to the group of catecholamines. Its variable concentration levels in body fluids reflect symptoms of different diseases, e.g. Parkinson's and Alzheimer's disease, stress, dysfunction of thyroid or presence of kidney tumors [1]. Therefore, detection and quantification of epinephrine in biological samples is actually of great importance. It is usually determined by chromatographic [2] and spectral [3] methods. Concerning the electrochemical methods, boron-doped diamond electrode recently represents a perspective non-toxic electrode material characterized by unique properties such as the widest known potential range, low background and high resistance against passivation [4].

The voltammetric study was performed in strongly acidic medium (in 0.5 mol L^{-1} $HClO_4$) in order to obtain the best electrochemical oxidation performance of epinephrine on the boron-doped diamond electrode. Oxidation and reduction peak at +0.6 V and –0.1 V vs. Ag/AgCl, respectively, indicate the quasi-reversible behaviour with diffusion controlled process (Fig. 1). For the determination purposes, the operation parameters of two sensitive voltammetric techniques (differential pulse, and square-wave voltammetry) were optimized: modulation amplitude of 200 mV (differential pulse voltammetry), amplitude of 100 mV and frequency of 50 Hz (square-wave voltammetry). According to the values of calibration curves slopes, differential pulse voltammetry was shown to be the more sensitive technique (2.6-fold higher than square-wave voltammetry) with the detection limit of 0.48×10^{-6} mol L^{-1}. The proposed method utilizing

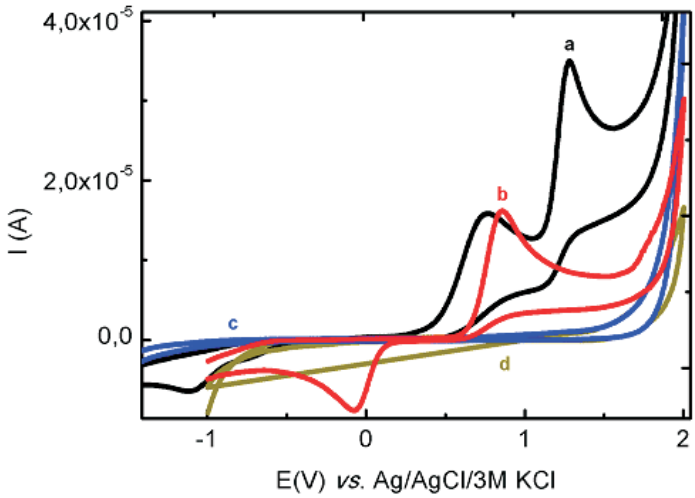

Fig. 1. Cyclic voltammogramms of epinephrine measured at scan rate of 100 mV s^{-1} in: a) acetate buffer solution, b) 0.5 mol L^{-1} perchloric acid. Curve c) and d) are cyclic voltammogramms for pure acid and buffer solution, respectively.

differential pulse voltammetry technique was applied to the determination of epinephrine in spiked human urine samples with recovery values in the range of 100.8–102.8% demonstrating the good accuracy. Interference study was also examined and it was found that most components of urine have no major effect on the epinephrine oxidation signal. Thus, boron-doped diamond electrode may be considered as an alternative electrode material for the determination of various biologically active compounds important in the field of clinical and pharmaceutical analysis.

Acknowledgments

This work was supported by the Grant Agency of the Slovak Republic (grant No. 1/0051/13), and the Slovak Research and Development Agency under the Contract No. APVV-0797-11.

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The Analysis of Linear and Monomethylalkanes in Exhaled Breath Samples GC Techniques

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Keywords
exhaled breath
inside needle capillary adsorption trap
methylalkanes
solid-phase extraction

Analysis of the composition of exhaled breath may provide insight into a variety of biochemical processes in healthy and diseased human body. Breath composition comprises volatile and non-volatile substances [1], which together form the 'exhaled metabolome' large number of compounds that can be combined with various diseases. Alkanes in exhaled breath have been proposed as endogenous marker compounds [1, 2]. Their analysis has medical importance, because it can lead to noninvasive clinical trials for cancer, oxidative stress, and rejection of the transplanted heart. In the study of the relationship of age and the presence in the breath methylalkanes [3], twenty two different C6–C17 normal and monomethylalkanes have been proposed as markers of oxidative stress of the compound, which is considered to be a pathological mechanism of aging and certain diseases. Phillips et al. [4] imply that oxidative stress degrades membrane polyunsaturated fatty acids, and thus produces *n*-alkanes and methylalkanes that are excreted in expired air, and that may vary depending upon the extent of oxidative stress. Phillips et al. [5] propose the following compounds as markers for lung cancer: butane, 3-methyltridecane, 7-methyltridecane, 4-methyloctane, 3-methyl heptane, 2-methylhexane, pentane, and 5-methyldecane. As markers for the detection of heart transplant rejection suggested 2-methylpropane, 5-methyloctadecane, 6-methyloctadecane, 2-methylpentadecane, octane, 2-methylheptane, 3-methylundecane, 2-methyloctadecane, and 2-methylhexadecane. In addition to endogenously produced volatile organic compounds are used for breath testing ^{13}C and ^{14}C-labeled precursor compounds. An example is the ^{13}C-labeled urea, which is used in assays for the detection of *Helicobacter pylori* bacterial infections

associated with gastric ulcers. Other examples of precursors are ^{14}C-aminopyridine and ethanol testing for impairment of liver function and ^{13}C-dextromethorphan bromide testing for CYP2D6 activity [6].

Sample preparation is the basis of chemical analysis. Concentration is a critical step when volatile organic compounds, which we want to determine the concentration levels ppb or ppt. Needle trap devices are a promising tool for robust and reproducible sample processing, which combines the benefits of the solid-phase extraction and solid-phase microextraction techniques [7]. One of the biggest advantages is that no additional equipment, as opposed to the heated GC injector, is not required [8]. Some applications packed monolayer sorbents such as Carboxen and divinylbenzene, have been described in the environmental monitoring (e.g., analysis of BTEX or higher alkanes). For the analysis of complex samples containing compounds with a wide range of polarity and volatility, single-bed needle trap devices are insufficient, and it is therefore necessary multibed needle trap device. Trefz et al. recently managed usability multibed needle trap device and expansive flow technique in clinical breath analysis [9].

Inside needle capillary adsorption trap is a needle trap device. His new treatment has been developed by Kubinec et al. [10]. This device is more robust than most needle gillnets equipment or solid-phase microextraction fibers and gives comparable results [10]. The aim of this work was the development of a new inside needle capillary adsorption trap device allows analysis of non-polar compounds with a wide range of volatility. Linear and monomethylalkanes were selected for this study because they are considered exhalation markers of various diseases. Many articles focus on the analysis of the volatiles in breathing out using a variety of analytical methods, in which the GC-MS and GC-FID become widely used. However, there are only a limited number of articles on GC×GC analysis of samples of exhaled breath [11]. Separation and identification monomethylalkanes in a wide range of carbon atoms is problematic due to the narrow and storage multicomponentity isomers of methyl branching near the middle of the carbon chain. Bottlenecks are the lack of standard reference materials and poor reproducibility of published data retention.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the contract no. APVV-0840-11, APVV-0416-10, APVV-0061-11, APVV-0415-11, and APVV-0665-10, also by ITMS 26240220071 and ITMS 26240220007 supported by the Research & Development Operational Programme funded by the ERDF and by Comenius University in Bratislava (grant nos. UK/84/2014).

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Development and Applications of Ionization Techniques in Ambient Mass Spectrometry

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Keywords
ambient mass spectrometry
desorption atmospheric pressure photoionization
desorption electrospray ionization

Ionization in ambient mass spectrometry carries out outside the machine in an open atmosphere. Nowadays, we recognize about thirty ionization techniques in the ambient mass spectrometry. They provide rapid analysis with no or only minimum sample preparation.

Desorption electrospray ionization [1] and desorption atmospheric pressure photoionization [2] are the ionization techniques examined in this study. These methods employ solvent spray for desorption and ionization of analytes from the solid surfaces.

Techniques were investigated in three research areas. First application was TLC/MS, where the appropriate conditions for desorption electrospray ionization analysis of different lipids representing different lipid classes after the separation on the TLC plate were found. Desorption atmospheric pressure photoionization was utilized for the detection of some lipid components of the vernix caseosa extract after the separation on the TLC plate.

Second application was the development of non-invasive imaging technique suitable for the examination of biological samples. Examined model objects were two insect species, i.e. soldiers of the termite *Prorhinotermes simplex* and adult stink bugs *Graphosoma lineatum*. Desorption atmospheric pressure photoionization in the negative ion mode was used to map the spatial distribution of (*E*)-1-nitropentadec-1-ene, which is the biosynthesized defensive compound, on the body surface of *Prorhinotermes simplex* soldier. Opening of the frontal gland was localized. Desorption atmospheric pressure photoionization in the positive

ion mode was used to track the spatial distribution of selected unsaturated aldehydes (previously described as the predominant components of the defensive secretion [3]) on the body surface of *Graphosoma lineatum*. Opening of the metathoracic scent glands was localized in the posterior part of the thorax.

Last application was the use of desorption electrospray ionization for analysis of endogenous steroids, e.g. dehydroepiandrosterone sulfate, dehydroepiandrosteron glucuronide and androsteron glucuronide, and their changes in the urine samples in the course of pregnancy.

Acknowledgments

The work was supported by Internal support program for projects of international collaboration AS CR M200551204, by Specific university research (Project SVV260084, Charles University in Prague) and by project GACR P206/12/0750.

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ICP-MS and SEC-ICP-MS Probing of Chromium and Vanadium Bioaccessibility by Garden Cress and Their Bioavailability for Humans

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Keywords
bioavailability
elemental speciation
hyphenated techniques

Vanadium and chromium are transition metals that exist in environment in several oxidation states; the most common states of vanadium are V(IV) and V(V) and of chromium Cr(III) and Cr(VI). The main anthropogenic sources of these metals are fossil fuels and steel, glass, and pigment industry [1, 2]. Chromium and vanadium, depending on oxidation state, are considered to be toxic and carcinogenic for living organisms or essential microelements important for proper glucose metabolism in human organisms. Because of that they became a quite popular component of dietary supplements [3].

Elemental supplementation with chemical components, especially when misused as a suitable 'short-cut' to balanced diet, may cause unnecessary changes in metal homeostasis and lead to various unspecific side effects [4]. In this context the functional food based on micronutrients, such as chromium and vanadium, merits attention [3]. The role of such food may play yeast or plants such as *Lepidium sativum* L. (garden cress), a subject of presented study. In comparison to knowledge about role of chromium and vanadium in animals, there is a small information about their function or toxicity in plants [5, 6]. However, the bioligands synthesized by plants to bind chromium or vanadium can influence significantly the effectiveness of human diet supplementation by these elements. In aim to produce more sufficient pharmaceutical or para-pharmaceutical specimens for diabetes speciation analysis for chromium and vanadium was carried out in garden cress depending on the type of growth medium.

The preliminary investigation of metals bioaccumulation by plants was carried out by mass spectrometer with inductively coupled plasma (ICP-MS). Analysis of chromium and vanadium by means of ICP-MS has significantly hindrances caused by spectral interferences. The main sources of isobaric multi-elemental interferences are argon (the plasma gas), and components of the complex biological matrices, such as chlorine, oxygen, sulphur, carbon [7, 8] or isobaric isotopes of other elements. The mathematical correction was used to compensate $ArCl^+$ molecular ion interference on chromium signal. Complexes of chromium and vanadium were investigated by size exclusion chromatography (SEC) coupled to isotope specific ICP-MS due to gentle steric interactions and high complex recovery from stationary phase. Due to that SEC-ICP-MS is considered as an important approach for screening of metal complexes in biological samples. Although SEC resolution is not good enough to separate of individual compounds, when hyphenated to ICP-MS it enables to estimate size of isolated compounds and to indicate the potential bioligands in complexes with metal.

Chromium and vanadium were found to show antagonistic character to magnesium and iron ions during hydroponic growth. Additionally, lower accumulation of both metals was observed when medium rich in other metal ions was used (5.63 μg of Cr g^{-1} of dry material) in contrast to chromium and vanadium prepared in deionised water (11.52 μg of Cr g^{-1} of dry material). Chromatograms of chromium and vanadium were rich in peaks corresponding to complexes with investigated metal ions, which were transferred to low molecular weight complexes already via in vitro peptic digestion simulating stomach digestion. The results were compared with chromatograms obtained for chromium(III) picolinate, which is typically present in para-pharmaceutical products.

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Fluorescence Spectroscopy as a Tool for Determination of Coumarins in *Melilotus officinalis* by Multivariate Calibration

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Keywords
coumarins
multivariate calibration
synchronous fluorescence spectrometry

Coumarin is a natural substance found in many plants, as the tonka bean, woodruff, vanilla grass, cassia cinnamon and especially in sweet clover (*Melilotus officinalis*). Also it is found in fruit such as strawberry, cherry, and raspberry. Coumarins are used as a fixative in perfumes, additives for paints and spray and food flavoring. They have great applicability in anticoagulant drugs, which alter the kinetics of blood coagulation. The plants containing coumarin converts into 4-hydroxycoumarin and subsequently condenses on dicoumarol (3,3'-methylene-bis-(4'-hydroxycoumarin)). This conversion is caused by an action of several mold and fungi, including *Penicillium nigricans, Penicillium jensi, Aspergillus fumigatus, Fusarium* and *Mucor* [1]. European committee established the maximum content of coumarin in foods on 2 mg/kg and in cosmetics products, where its concentration limits are 0.001% in leave-on and 0.01% in rinse-off products. The presence of dicoumarol in all products is prohibited by European directive [2, 3].

The aim of present work is to develop rapid, simple and low cost screening method for simultaneous determination of coumarin, 4-hydroxycoumarin and dicoumarol in herbal tea by synchronous fluorescence spectroscopy and multivariate calibration using partial least square methods (PLS).

In synchronous fluorescence spectroscopy the optimization of suitable $\Delta\lambda$ is needed for the best analytes separation. Each analyt was measured at different $\Delta\lambda$ from 10 to 100 nm, the optimal resolution of λ_{max} values were obtained at

$\Delta\lambda$ = 80 and 90 nm and those values were used for future experiments. For multivariate analysis, two different sets (i.e. calibration and prediction sets) of real samples were prepared. The four-factor at three-level experimental design of orthogonal arrays OA9 [4] was exploited to create calibration and prediction sets. The quantitative deter-minations of predictive characteristics were compared between the two PLS models: PLS1 model determines the concentration of components in analytes individually and PLS2 model calculates the concentration of components in analytes simultaneously.

Acknowledgments

This work was supported by the Competence Center for SMART Technologies for Electronics and Informatics Systems and Services, ITMS 26240220072, funded by the Research&Development Operational Programme from the ERDF. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0797-11.

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Analysis Of Perfumes By Using Multi-Dimensional Gas Chromatography

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Keywords
essential oils
heart-cut
multi-dimensional gas chromatography
perfumes

Nowadays, perfumes are produced from natural or synthetic substances. The most of perfumes contain a lot of compounds. This research deals with the analysis of volatile organic compounds in natural perfumes such as Praga Alchymica, which is produced by Rafaella. Rose Taif and Rose Josefina are additional samples. There are the starting materials for the manufacture of perfumes.

For analysis of volatile substances headspace and gas chromatography-mass spectrometry (GC/MS) have been used as these are frequently used methods for parfume analysis. The results indicate that nature of pure perfume like Rose Natura and Rose Taif has different compounds without 1-linalool as both of them have different smells. Moreover, on the aforementioned basis such information can be used for determination of basic compounds in mixed perfume like Rose Josefina. The same substances from Rose Taif and other aromas were measured in this parfume. Praga Alchymica was mixed from Rose nature and unknown flavor. This research also proved that for small amount of samples it is better to use GC/MS compared to headspace GC.

Research will also entertain using "heart-cut" MDGC system with using chiral columns in the second dimension. In contrast with conventional GC/MS, heart-cut (H/C) MDGC instrumentation enables the transfer of selected bands of overlapping compounds from a primary (1D) to a secondary (2D) column, connected by means of an interface (either a switching valve or a Deans switch).

Hydrogen Oxidation Reaction on Electrode Modified by Water Soluble Phthalocyanine

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Keywords
hydrogen oxidation
water soluble phthalocyanine

Hydrogen sensors are of increasing importance due to ensuring the safety people wherever hydrogen is produced, stored, transported, and used. Many different hydrogen sensor technologies have been reported in depending on various applications [1, 2]. It is well known that phthalocyanine are unique macromolecular complexes that catalyze many target species. The rich and reversible redox behavior of metallophthalocyanines allows them to serve as mediators in many electron transfer reactions [3, 4].

Our interest has been focused on water-soluble complex *N,N´,N´´,N´´´*-tetramethyltetra-3,4-pyridinoporphyrazinocobalt, simply called CoTmt-3,4-ppa, which forms insoluble electrically conducting films on the graphite electrode surface. Adsorption, electrochemical deposition, and spincoating on basal plane of highly ordered pyrolytic graphite (HOPG) electrode were employed as deposition techniques and characterized by cyclic voltammetry. The electrochemical behavior of modified electrode was completed by in situ backscattering VIS spectroscopy and ex situ atomic force microscopy.

We have studied the electrocatalytic oxidation of hydrogen on two different electrodes including annealed gold (Au111) and highly ordered pyrolytic graphite (HOPG), both modified by layer CoTmt-3,4-ppa and utilized this compound in construction of electrochemical gas sensor. Electrocatalytic activity of CoTmt-3,4--ppa to hydrogen was measured by cyclic voltammetry and potentiometric in aqueous buffer phosphate solution as an electrolyte at laboratory temperature. We report on the interaction of hydrogen with reduced form of the CoTmt-3,4-ppa

complex involving metal centre and ligand catalytic activity. During cyclic voltammetry on modified electrode surface anodic peaks of hydrogen evolution were observed in the potential range corresponding to redox couple of the complex CoTmt-3,4-ppa. Influence of electrode type, deposition methods, thickness of the deposit, and pH were observed. The electrocatalytic results were completed by atomic force microscopy which allow to get better understanding of process mechanism and change film nanomorphology respectively.

Acknowledgments

This work was supported by the research project grant project SVV260084 of Charles University in Prague.

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Characterization of Polymethacrylate--Based Monolithic Stationary Phases

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Keywords
capillary polymethacrylate monolithic columns
capilary liquid chromatography
hydrophilic interaction chromatography
monolithic stationary phases
phenolic compounds

Liquid chromatography is the most commonly used separation technique. Currently, the most widely used chromatographic systems are reversed-phase chromatography and hydrophilic interaction chromatography. The first one uses non-polar stationary phase and polar organic or water-organic mobile phase. Hydrophilic interaction chromatography utilized of a polar stationary phase and highly organic mobile phases. Generally, capillary liquid chromatography columns are packed with spherical particles that can be totally porous, with porous layer or non-porous. Alternatively, monolithic stationary phases can be used. Monolithic capillary columns are prepared in fused silica capillary by radical polymerization of polymerization mixture, containing crosslinking monomer, functional monomer, porogenic solvents, and thermal initiator of polymerization reaction.

Various crosslinking monomers were used for the preparation of capillary monolithic columns suitable for the hydrophilic interaction chromatography. It was synthesized seven different monolithic column with *N,N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine as a zwitterionic functional monomer and various crosslinking monomers with different lengths of alkyl chain between two methacrylate units (ethylene-, tetramethylene-, and hexamethylene dimethacrylate); with oxyethylene chain (dioxyethylene dimethacrylate); with three acrylate units (pentaerythritole triacrylate); and with aromatic units in their structure (bisphenol A- and bisphenol A glycerolate dimethacrylate). 1-propanol, 1,4-butanediol, and water were used as porogenic solvents and azobisizobutyronitrile was used as thermal initiator of polymerization reaction.

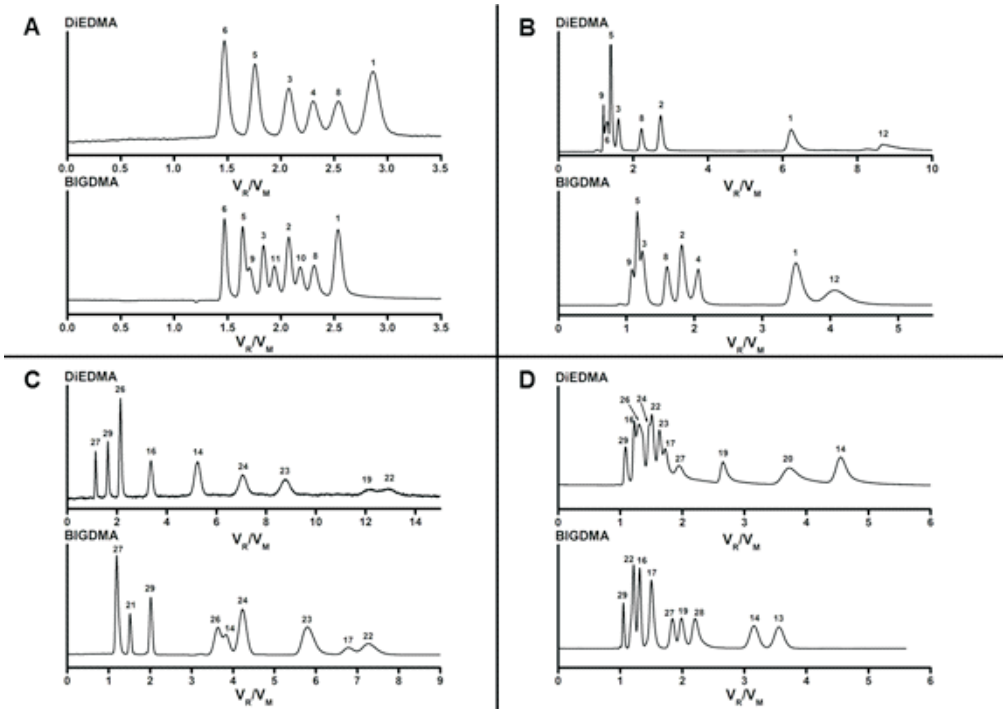

Fig. 1. Separation of phenolic acids and flavones in hydrophilic interaction chromatography and RP mode, UV detection at 214nm.

(A) Phenolic acids: 40% ACN/60% 10 mM NH_4Ac, DiEDMA: l = 168 mm, F_m = 6.3 µl/min, p = 19.6 MPa, BIGDMA: l = 183 mm, F_m = 3.3 µL/min, p = 6.3 MPa.

(B) Phenolic acids: 85% ACN/15% 10 mM NH_4Ac, DiEDMA: l = 179 mm, F_m = 2.5 µL/min, p = 2.8 MPa, BIGDMA: l = 132 mm, F_m = 5.8 µL/min, p = 3.7 MPa.

(C) Flavones: 40% ACN/60% 10 mM NH_4Ac, DiEDMA: l = 168 mm, F_m = 5.5 µL/min, p = 17.1 MPa, BIGDMA: l = 123 mm, F_m = 3.1 µL/min, p = 4.4 MPa.

(D) Flavones: 80% ACN/20% 10 mM NH_4Ac, DiEDMA: l = 179 mm, F_m = 2.0 µL/min, p = 3.2 MPa, BIGDMA: l = 123 mm, F_m = 2.1 µL/min, p = 1.7 MPa.

Analytes: (1) gallic acid, (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (4) salicylic acid, (5) vanillic acid, (6) syringic acid, (7) hydroxyphenylacetic acid, (8) caffeic acid, (9) sinapic acid, (10) *p*-coumaric acid, (11) ferullic acid, (12) chlorogenic acid, (13) (–)epicatechine, (14) (+)catechine, (15) flavone, (16) 7-hydroxyflavone, (17) apigenine, (18) lutheoline, (19) quercetine, (20) rutine, (21) naringine, (22) biochanin A, (23) naringenine, (24) hesperetine, (25) hesperidine, (26) 4-hydroxycoumarine, (27) esculine, (28) morine, (29) vanillin.

To investigate the effects of crosslinking monomers on the pore size distribution of prepared column, the size-exclusion curve of several polystyrene standards and toluene was measured. Further, the effect of the applied crosslinking monomers on the efficiency of prepared columns using van Deemter plots was also determined. The dioxyethylene dimethacrylate and bisphenol A glycerolate dimethacrylate columns showed approximately 70 000 theoretical plates/m for toluene at minimum of van Deemter plot, which is one

of the highest column efficiency achieved at polymer-based monolithic stationary phases. These columns provide good run-to-run and batch-to-batch reproducibility of the elution volumes. The relative standard deviations in ten repeated runs (run-to-run reproducibility) were lower than 0.4% for both types of columns and for batch-to-batch repeatability were lower than 0.6% for dioxyethylene dimethacrylate columns and lower than 1.3% for bisphenol A glycerolate dimethacrylate columns.

The prepared columns provided a dual retention mechanism and were used for isocratic separation of model mixture of phenolic compounds both in acetonitrile--rich mobile phases in HILIC mode as well as in the mobile phases with higher concentrations of water in the reversed-phase mode (Fig. 1).

The effect of type of crosslinking monomer on the separation power and efficiency of prepared columns has been explored. The efficiency of prepared columns strongly depends on the applied crosslinking monomer. Columns prepared with dioxyethylene dimethacrylate and bisphenol A glycerolate dimethacrylate crosslinkers provide the highest column efficiency (H = 16.5 μm), long-term stability and excellent reproducibility. Optimized monolithic columns have been used for separations of polar compounds such as phthalates or barbiturates. Further, prepared columns can be applied in the first dimension of the comprehensive two-dimensional liquid chromatography for the separation of complex mixture of phenolic compounds.

Acknowledgments

The financial support of GACR project P206/12/0398 is gratefully acknowledged.

Direct Silylation Method for Aqueous Samples for Gas Chromatography

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Keywords
aqueous samples
hexamethyldisilazane
GC-MS/MS
metabolites
N,O-bis-trimethylsilyl-trifluoroacetamide
trimethylsilylation

Nowadays, the analytical methods with a minimal number of operations and processes used in the sample preparation are preferred. These methods allow the shortening of sample preparation time, reducing losses of volatile analytes, and reducing the risk of sample contaminations. For the gas chromatographic analysis of metabolites, the traditional procedures are based on complex sample preparation and derivatization and only a few methods allow the simultaneous derivatization of all polar functional groups. Therefore, the development of a simple method enabling the simultaneous derivatization of different polar groups in the sample is needed.

The most common derivatization method performed before GC analysis is silylation. Trimethylsilylation is the most commonly used silylation method, as trimethylsilyl derivates have suitable thermal and chemical stability as well as high volatility. They can be easily prepared and have excellent chromatographic properties. But the main disadvantage of silylation reagents is their susceptibility to hydrolysis.

A new methodology of silylation was developed. The active hydrogen in polar group was replaced with trimethylsilyl group $-Si(CH_3)_3$ and formed a high volatile and stable trimetylsilyl derivatives. Two silylation reagents were used. The first was hexamethyldisilazane, weakly reactive, suitable for derivatization of easily silylable hydroxyl groups. The second, *N,O*-bis-trimethylsilyl-trifluoroacetamide is one of the most universal silylation reagents able to silylize most functional groups, including amino groups. But in the same time, it is so reactive that it could cause decomposition of analysed compounds (e.g., glucose).

The process of silylation with two different silylation agents in two separate steps has several advantages. This two-step silylation method was used for the analysis of metabolic products (glucose, lactate, alanine, glycerol, succinate) of protozoa *Trypanosoma brucei* in aqueous samples using GC-MS/MS.

In the first step, water is removed from the sample by reaction with less reactive reagent hexamethyldisilazane. At the same time, hydroxyl groups are silylized under mild reaction conditions. For example, hydroxyl functional groups of glucose are silylized with hexamethyldisilazane, but on the other hand, silylation of glucose directly with *N,O*-bis-trimethylsilyl-trifluoroacetamide created derivates of glucose decomposition.

In the second step, when there is no water left in the sample and moreover glucose is in a form of stable penta-trimethylsilyl derivate, it is possible to use more expensive and more reactive reagent *N,O*-bis-trimethylsilyl-trifluoroacetamide. In this step, silylation of amino groups (which were not fully silylized by less reactive hexamethyldisilazane) is completed. For investigated metabolites, this method was examined with one-step silylation (with hexamethyldisilazane or only with *N,O*-bis-trimethylsilyl-trifluoroacetamide) to confirm that the method is the best choice for analysis of selected groups of analytes.

An amount of 600 µL of hexamethyldisilazane-acetonitrile mixture (1:1, v/v) was added as a silylation agent to 20 µL of aqueous sample and 2 µL of trifluoroacetic acid was added as a catalyst. The reaction mixture was heated to 50 °C for 30 min at 650 rpm in a tempered shaker. Vials were open during this step to ensure the escape of the ammonia gas produced in the reaction of hexamethyldisilazane and water from the sample. In the second step, 400 µL of pure *N,O*-bis-trimethylsilyl-trifluoroacetamide was added to the mixture and it was heated to 80 °C for 30 min in a closed vial.

Acknowledgments

This publication is the result of the project implementation: ITMS 26240220071, and ITMS 26240220007 supported by the Research & Development Operational Programme funded by the ERDF. Work was also supported by the Slovak Research and Development Agency under the contract numbers APVV-0840-11, APVV-0416-10, APVV-0061-11, APVV-0415-11, and APVV-0665-10.

Determination of Tributyltin in Sediments

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Keywords
optical emission spectrometry
solid phase microextraction (SPME)
tributyltin

Organotins, in particular tributyltin, are considered as hazardous chemicals due to their deteriorating effects on marine organisms. Actually, organotin compounds are highly toxic both to marine organisms and humans. In the environment, organotin compounds may be partially degraded to inorganic tin by various chemical and biological processes. An understanding of the toxic effects of these compounds requires the identification of all species in the environment. Consequently, the analytical methods should be capable of distinguishing between organotin compounds and be sensitive enough to detect them at trace levels [1].

The most common analytical technique used for the determination of tin species is gas chromatography employing a variety of different detectors, for example flame photometric detector, atomic absorption spectrometry or mass spectrometry [2].

The aim of this study was to develop the analytical procedure to carry on the selective determination of tributyltin in soils, sediments and fish tissues. The method consists of acid or alkaline extraction of the sample and solid-phase microextraction (SPME) coupled with plasma optical emission spectrometry (OES) [3–4]. The SPME-TD-MIP-OES method was validated against several certified reference materials with values assigned for tributyltin.

Tributyltin may be extracted from sediments by shaking with hydrochloric acid solution of 3 mol L^{-1}. Next, tributyltin is released from the sample solution in the form of volatile tributyltin chloride [5], then the analyte is collected from the headspace with SPME. The detection limit by this method is of 90 μg L^{-1}.

Another approach for selective determination of tributyltin is based on the use of masking agents, namely ethylenediaminetetraacetic acid and diphenylcarbazone, which form non-volatile complexes with the mono- and dibutyltin,

respectively [6]. The following step of the analytical procedure is ethylation of tributyltin using sodium tetraethylborate under optimized experimental conditions, including the pH value and sodium tetraethylborate concentration. Also, the pH value of the complexation reaction and the concentration of both EDTA and diphenylcarbazone should be optimized. Another important parameters of the analytical procedure, like the analyte extraction time and desorption temperature have also been considered. As a result, a very low detection limit of 0.25 μg L^{-1} has been achieved. The proposed method has been successfully used for the determination of tributyltin in a number of soil and sediment certified reference materials.

Acknowledgments

This project is financed in the framework of grant entitled 'Production and attestation of new types of reference materials crucial for achieving European accreditation for Polish industrial laboratories' attributed by the National Center for Research and Development

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Dispersive Liquid-Liquid Microextraction Method: Application to Essential Oils Analysis in Real Samples of Herbal Beverages

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Keywords
dispersive liquid-liquid microextraction
essential oils
herbal tea

This work deals with the optimisation and the application of two microextraction methods for isolation of essentials oils in herbal teas. These two approaches are Dispersive Liquid-Liquid Microextraction (DLLME) and Dispersive Liquid-Liquid Microextraction Based on the Solidification of Floating Organic Drop (DLLME-SFO).

Based on the preliminary experiments the solvent systems (i.e. extraction and dispersive solvents) were selected. The optimisations of individual experimental conditions for both mentioned methods were performed with the use of orthogonal central composite design (CCD). Namely, volumes of both extraction and dispersive solvents, and sonication time, were optimised. Evaluation of optimal parameters was realised by the response surface modelling (RSM) approach where the desirability of individual parameters were tested.

The optimised procedures were applied for the extraction of essential oils from real herbal tea samples. The analytical separation was performed with the usage of gas chromatography with the flame ionization detector. The results obtained by these methods were compared. Moreover, both tested extraction methods were compared to each other as such. This comparison included the method applicability, compatibility with the analysis of essential oils, and consumption of environmentally not-friendly chemicals.

Utilization of Boron-Doped Diamond Electrode in Electroanalysis of Benzophenone-3

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Keywords
benzophenone-3
boron-doped diamond electrode
cyclic voltammetry
differential pulse voltammetry
surfactant

Benzophenone-3 (2-hydroxy-4-methoxybenzophenone) is an organic compound used as UV filter in many cosmetic preparations [1]. Wide use of these products can lead to contamination of environment by benzophenone-3. As an endocrine disruptor, benzophenone-3 can negatively influence living organisms by disturbing its hormonal equilibrium. Therefore, it is convenient to develop methods for its determination.

Benzophenone-3 is electrochemically active compound, thus it can be easily determined by modern voltammetric techniques [2, 3]. Boron-doped diamond film is favourite electrode material with properties such as wide potential window, mechanical and chemical stability, low residual current and biocompatibility [4, 5].

The aim of this study was to find suitable conditions for voltammetric determination of benzophenone-3 at boron-doped diamond electrode and to confirm the possibility of use of boron-doped diamond electrode for determination of studied analyte in the presence of surfactant.

Two types of boron-doped diamond electrodes were used as working electrode. For voltammetric determination of benzophenone-3 in the absence of surfactant was used boron-doped diamond film deposited at silicon wafer placed in teflon electrode body (constructed in our laboratory [6], further marked as

BDD_A). Geometric area of this electrode was 10.2 mm^2. Boron-doped diamond films were prepared by microwave plasma-assisted chemical vapour deposition on silicon wafers of mixtures containing 99.0% H_2/1.0% CH_4 and trimethylboron gas with variable B/C ratio in the gas phase 500, 1000, 2000, 4000, and 8000 ppm at Institute of Physics of the ASCR, v. v. i. in Department of Functional Materials. For voltammetric determination of benzophenone-3 in the presence of surfactant was used commercially available boron-doped diamond electrode with diameter of 3.0 mm (area 7.1 mm^2, Windsor Scientific, UK; further marked as BDD_B).

Voltammetric behaviour of benzophenone-3 was investigated in anodic region using cyclic and differential pulse voltammetry at boron-doped diamond electrodes BDD_A. Optimum conditions for the determinations of studied analyte were estimated based on the influence of pH on the voltammograms in Britton--Robinson buffer with pH values ranging from 2.0 to 12.0. Under the optimized conditions, the analytical parameters of benzophenone-3 were determined at all boron-doped diamond electrodes BDD_A.

Further, the enhancement of voltammetric signal of benzophenone-3 in the presence of cationic surfactant cetyltrimethylammonium bromide was confirmed at BDD_B. Further investigation, such as an optimization of differential pulse voltammetric determination, will be the subject of additional study.

Acknowledgments

The research was financially supported by the Grant Agency of the Charles University in Prague (Project GAUK 684213), and Charles University in Prague (project SVV260084).

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GC-MS Analysis of Polar Lipid Headgroups

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Keywords
GC-MS
phospholipids
phosphates
silanization
trimethylsilanization

A fast, instrumental, sensitive, selective and robust GC-MS method has been developed for analysis of phosphorylated compounds that are product of phospholipids hydrolysis. Phosphoserine, phosphoethanolamine phosphoglycerol, and inorganic phosphate are derivatives of phosphoric acid and they are bound at *sn*-3 position on glycerol chain in phospholipids. These phosphoesters provides hydrophilic properties of phospholipids and they are classified into classes according their phosphoester groups. Nevertheless none of these phosphoesters is nonpolar enough to avoid derivatisation for efficient GC analysis [1]. The method consists of two-stage trimethylsilylation procedure with two agents of different reactivity. In the first step, hexamethyldisilazane reacts with hydroxyl, carboxylic and phosphate groups, whereas less reactive groups (mostly amine) are trimethylsilylated in the next step with more reactive agent bis(trimethylsilyl)trifluoroacetamide. This agent is too reactive for hydroxyl groups so it cannot be used as a single agent because the reaction proceeds too vigorously and the reproducibility of measurements is insufficient [2]. Trimethyilsilylated phosphoesters have been identified according to their retention times and mass spectra. All measurements were performed on DB-5 column with temperature gradient program. Gas chromatograph was provided with EI-MS detection, so the identification was carried out by comparison with spectra databases or previously published mass spectra. Major fragments have been evaluated for all the polar analytes the best suitable one was chosen for any single compound to be used for scan mode analysis to lower the limit of detection

and quantification (e.g. trimethylsilyphosphate 5 μg mL^{-1}). The repeatability and precision of procedure was measured and evaluated for future standard use. Also probable structures of major or specific fragments were specified and structures of fragments of resembling compounds were compared between each other. Suitability of this method has been tested on lysed cytoplasmic membrane of *Bacillus subtilis*, which is a microbe cultivated and researched for a production of new antibiotics [3]. Some analytes has been found in that lysate, others compounds, the amines, were not founded probably due to so strong conditions of hydrolysis of bacterial lysate. Next some fatty acids were trimethylsililated and analyzed together with trimethylsilylated phosphoesters, however, their structures are similar so they cannot be identified by mass spectra only. Therefore, retention indices must have been additionally calculated for these fatty acids. The measurements were performed in SIM mode with particular fragments in selected time windows, similarly as the analysis of polar headgroups to allow determination of fatty acids and polar parts of phospholipids in one run. The major future challenge is actually a development of hydrolysis that is soft enough to avoid a cleavage of phosphoester bonds and would allow a comparison with previously published composition of microbe membranes [4].

Acknowledgments

Project was supported by Specific University Research (SVV260084)

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Ion Chromatography As a Tool for Sample Preparation in the Investigation of Second Messenger Molecules

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Keywords
cyclic nucleotides
ion chromatography
sample preparation
second messenger
urine

The majority of prescription drugs are targeting the G protein coupled receptors [1] since they are involved in many important physiological processes such as heart rate regulation [2] or proliferative regulation [3]. These ligand activated receptors trigger intracellular reactions which consume or produce so called second messengers such as cyclic nucleotides [4]. These substances give information about the impact of pharmaceuticals targeting the G protein coupled receptors and therefore their concentration changes are of interest. In urine samples their concentration is very small in relation to the huge amount of chloride, which is a hassle to many direct analytical approaches. Hence, an additional sample preparation step is needed. Except for sample-taking, this is the most error-prone and, clearly, the most time consuming step within the analytical process. Thus, it has to be an easy, reproducible, automated, and fast procedure. We realized this with an ion chromatography system, which is a suitable method since modern ion chromatography instruments possess a suppressor whose effluent consists only of the analytes in pure water. Also, the anion exchange column separates five of the six investigated nucleotides/cyclic nucleotides from chloride (Fig. 1) when using a hydroxide gradient elution [5] in less than ten minutes. Unfortunately, the most commonly used detector in ion chromatography, viz. the conductivity detector, is not appropriate for detecting such low concentrations. For this reason, different 'heart cuts' are performed to collect regions of interest. These fractions can be concentrated, which makes them

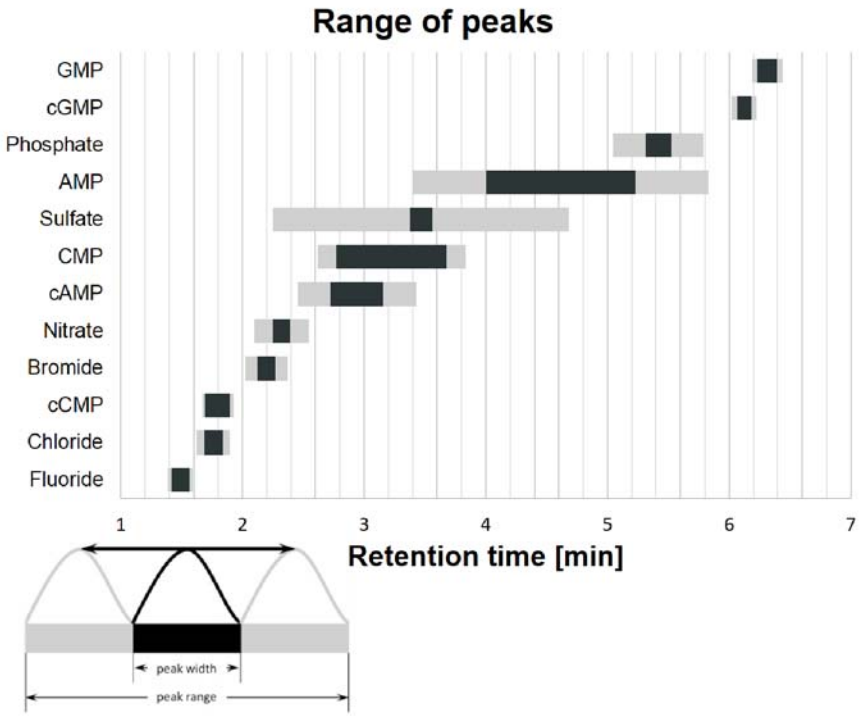

Fig. 1. Ion chromatography of some nucleotides/cyclic nucleotides in a matrix of standard anions.

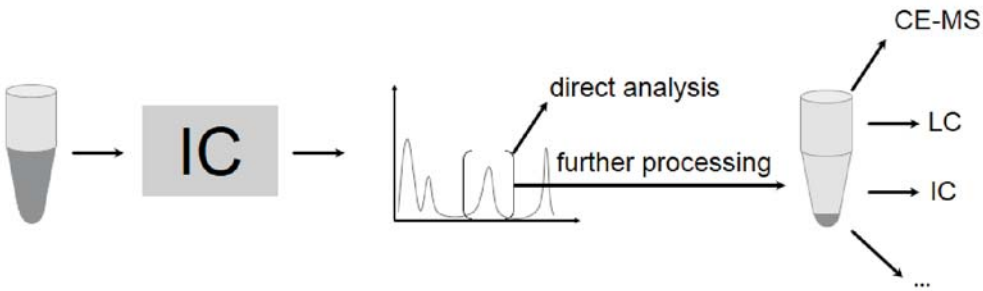

Fig. 2. Schematic overview of the analysis.

accessible for either direct analysis using mass spectrometry, or further separation, if needed, with a second separation technique such as fast capillary electrophoresis coupled to mass spectrometry (Fig. 2).

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Voltammetric Study of 2-Aminofluoren-9-one Using Bare and DNA-Modified Glassy Carbon Electrodes

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Keywords
2-aminofluoren-9-one
determination
DNA
glassy carbon electrode
voltammetry

Polycyclic aromatic hydrocarbons with oxo and/or amino functional group are well-known environmental chemical carcinogens and/or mutagens frequently contaminating water, soil and sediments [1, 2]. Therefore, analytics and monitoring of environmental pollutants belong among the most dynamically developing branches of chemical analysis nowadays [3]. Electrochemical techniques have merits of simplicity, rapidity, high sensitivity, good compatibility with biological samples, and they are inexpensive from the point of view of both investment and running costs [1].

2-Aminofluoren-9-one is a well-known hazardous substance with genotoxic effects containing oxo and amino functional groups. In the environment, its occurrence is associated mainly with the processing and purification of natural gas in gas refineries and with the combustion processes [4, 5].

In this contribution, the voltammetric behavior of 2-aminofluoren-9-one was investigated at a bare glassy carbon electrode as a function of the pH to provide an overall information regarding electrochemical transformations of 2-aminofluoren-9-one in the positive potential region where the anodic oxidation of the amino group occurs. The optimal medium for its direct current voltammetric (DCV) and differential pulse voltammetric (DPV) determination was a mixture of methanol–Britton-Robinson buffer of pH = 8.0 (1:9, v/v). Under the optimal conditions found, the calibration curves were measured for both DCV and DPV in the concentration range from 0.4 to 100 μmol L^{-1}, with the limits of quantification

of 0.8 and 0.6 μmol L^{-1}, respectively. The practical applicability of the newly developed voltammetric methods was verified on the direct determination of 2-aminofluoren-9-one in model samples of drinking and river water. The results of the determination of 2-aminofluoren-9-one at the bare glassy carbon electrode using the anodic oxidation of the amino group are comparable to those obtained by the same voltammetric methods using the cathodic reduction of the oxo group at the glassy carbon, mercury [4], and silver solid amalgam [5] electrodes.

The effects of genotoxic substances in living organisms may range from mild discomfort to serious diseases such as cancer. Therefore, investigation of both *in vivo* and *in vitro* interactions between DNA and various xenobiotic compounds should be raised to the highest priority [6, 7]. The interaction between 2-aminofluoren-9-one and double-stranded DNA (ds-DNA) was investigated in this work by square wave voltammetry at the DNA-modified glassy carbon electrode (ds-DNA/GCE). Square wave voltammetry was carried out to monitor the changes in the oxidation signal intensity of guanine and adenine moieties before and after interaction with 2-aminofluoren-9-one. Onto the glassy carbon electrode surface, ds-DNA from salmon sperm was adsorbed, and the ds-DNA/GCE was incubated for various times and in various concentrations of 2-aminofluoren-9-one. The predominant interaction observed during this experiment was the intercalation of 2-aminofluoren-9-one between the DNA base pairs.

Acknowledgments

This research was carried out in the framework of the Specific University Research (SVV 2014-260084). Financial support from the Grant Agency of the Charles University in Prague (Project GAUK 430214/2014/B-CH/PrF) and from the Grant Agency of the Czech Republic (Project P206/12/G151) is gratefully acknowledged.

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Laser-Induced Breakdown Spectroscopy in Analysis of Liquids and Solids

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Keywords
laser ablation
LIBS
liquids
melts

Laser-Induced Breakdown Spectrometry (LIBS) is a type of atomic emission spectrometry which uses a focused highly energetic laser pulse as the excitation source [1]. LIBS is based on the detection of photons emitted by the atomized sample constituents in the laser-induced plasma created during laser ablation. The basic principle consists in the interaction of a focused laser beam with a sample surface which results in laser-induced micro plasma creation. In principle, any physical state (solid [2, 3], liquid [4–6] or gas [7, 8]) can be analyzed by LIBS with low detection limits for most elements [9]. The laser-induced micro plasma emits radiation which includes analytical information about analyzed sample. This information can be used for qualitative and quantitative purposes. LIBS enables analysis of solid samples with minimal or no sample preparation.

This work was divided into several parts. The aim of the whole work was the construction of the new special setup which allows the analysis of liquids, including in particular finding a suitable geometry for the analysis of liquids and melts which are placed in an goldsmith furnace.

The first part was focused on a construction of new experimental setup for analysis of liquids by the LIBS. We prepared two arrangements for forming laser--induced micro plasma; first one was constructed observing laser-induced micro plasma on the liquid surface and the second one on dropplets. In case of producing laser-induced micro plasma on the liquid surface the laser beam was tilted at an angle of 70° to the liquid surface due to waves which were created at an angle greater than 70° and the reproducibility of laser-induced micro plasma signal is getting worse. It was tested setup for the analysis of liquids in the flow through the

capillaries and ablation of droplets but this arrangement did not provide reproducible laser-induced micro plasma. All measurements were performed with aqueous solutions of $FeCl_3$ and steel sample in air under atmospheric pressure.

The second part this work was focused on an upgrade of this setup for analysis of melts by means of LIBS. Influence of temperature of the sample on laser-induced micro plasma intensities was observed. For this purpose samples of pure metals (Bi, Ga, Pb, In, Sn, Zn) and glass and their melts were analyzed.

The next aim of this part was to construct a new compact experimental setup with unvarying optical path for analysis of melt by means of LIBS and to observe an influence of surrounding gas (air, He, and Ar) on time resolved laser-induced micro plasma emission. The whole study was performed using glass samples. In particular using of argon as inert gas should improve the signal of emission lines.

For the laser-induced micro plasma production the Nd:YAG laser (Quantel, France) operated at fundamental wavelenght of 1064 nm and frequency 1 Hz was used. Set-up for measurement of glasses and metals is shown on Fig. 1. Radiation was transported into monochromator of the spectrometer TRIAX 320 (Jobin Yvon, France) by means of 3 meters long optic fiber system. It goes into spectrometer through the entrance slit with the width of 50 μm and it was dispersed by a 2400 grooves mm^{-1} grating. Dispersed radiation was detected by the ICC detector (Jobin Yvon, France) for a record of emission spectrum. Laser power was optimized to 1.4 W. The power of the laser pulse was monitored by an energy meter (Nova-Ophir, Optronics). For experiments with liquids the laser beam was focused by glass lens with focal distance of 50 mm.

For experiments with melts the laser beam was focused by quartz lens with focal distance 100 and 120 mm, respectively. Measurements of melts were performed using the pure metals which were melted in goldsmith furnace. The analysis of glass was performed on glass standards for XRF spectroscopy. Glass samples and samples of metals were placed into a goldsmith furnace (0412G, Clasic, Czech Republic) with a programmable temperature regulator (Clare 4.0, Clasic, Czech Republic). The glass samples were melted at temperature of 900 °C. In case of solids the temperature of metals was about 50–100 °C above melting point of analyzed metal. In case of observing time behavior of laser-induced micro plasma signal the photomultiplier R928 with gated assembly C1392 (Hamamatsu, Japan) was used for detection of selected emission line and it was recorded by oscilloscope TDS 2024C (Tektronix, USA). The time behavior of sodium atomic emission line (588.99 nm) in three different ambient atmospheres (air, He, Ar) is shown in Fig. 2. We can see that argon had a positive effect on the intensity and shape of the emission lines. The plasma emission was acquired 0.5 μs after the laser pulse and the integration time of the emission signal was 10 μs. After optimization were measured full spectrum for each sample of the glass, metals in the range of 200–600 nm and were identified spectral lines of elements.

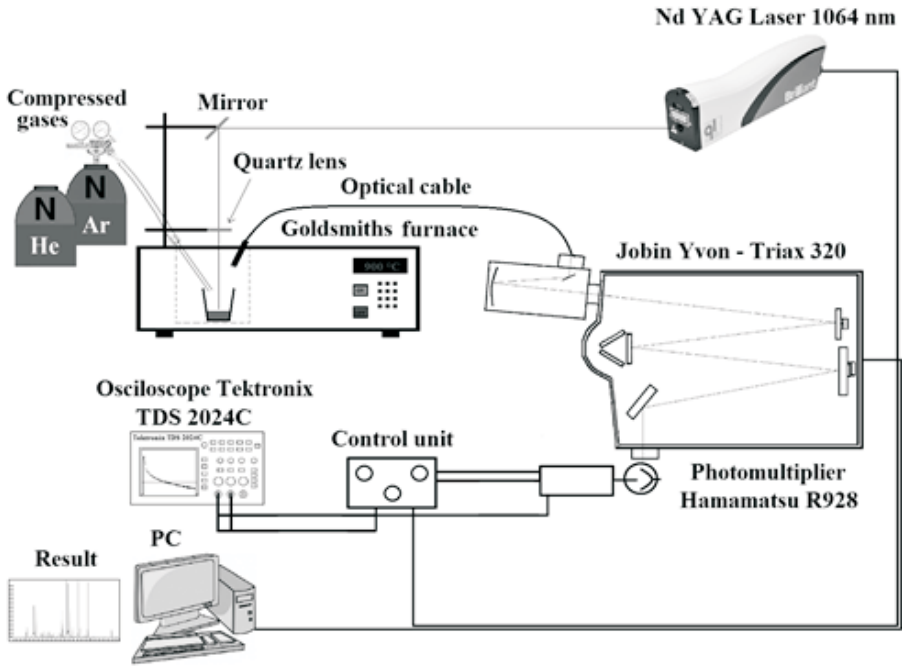

Fig. 1. Scheme of experimental setup for analysis of melts.

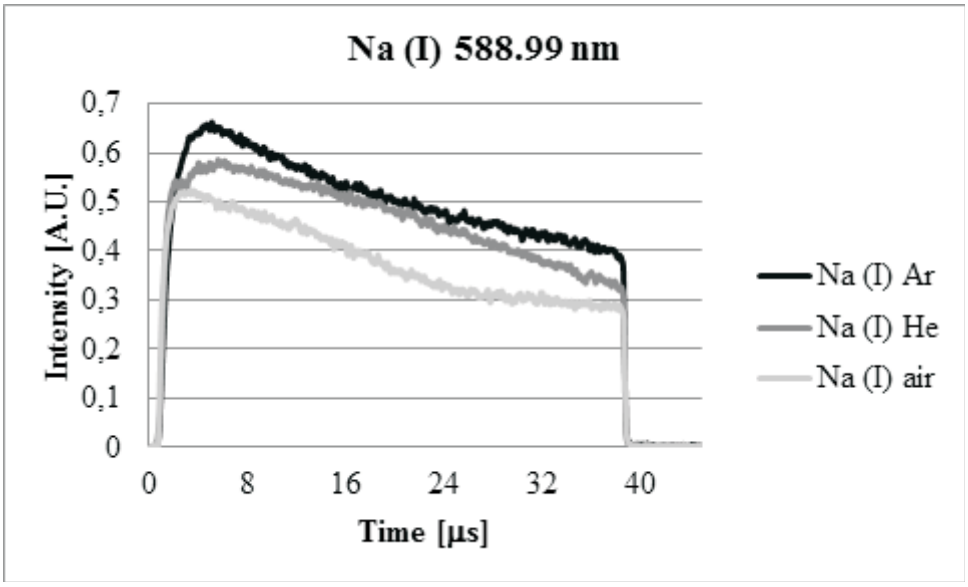

Fig. 2. Plasma extinction of sodium atomic emission line 588.99 nm in three different ambient atmospheres (air, He, Ar).

All results obtained by new designed experimental setup will be discussed in detail in oral presentation on European Symposium on Atomic Spectrometry ESAS 2014 & 15th Czech-Slovak Spectroscopic Conference.

Acknowledgments

This work was supported by the project 'CEITEC – Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund, and Student Project Grant at MU (specific research, rector's programme) – Category A (MUNI/A/0992/2009).

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Retention Time Prediction in Temperature-Programmed GC×GC: Modelling and Error Assessment

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Keywords
error assessment
gas chromatography
Monte-Carlo
model
prediction

As the comprehensive analysis of complex materials demand a high peak capacity, their chromatographic separation cannot be achieved using one-dimensional chromatography and two-dimensional chromatography offers a key advantage. The importance of modelling retention time and band broadening in GC×GC has become a point of interest in the last decade [1–7]. However, modelling two-dimensional, temperature programmed gas chromatography is complex due to the large number of parameters involved and the non-linear behaviour of some of them.

In this paper we present a model relating experimental factors (column lengths, diameters and thickness, modulation times, pressures and temperature programs) with the retention and band broadening. Unfortunately, an analytical solution to calculate the retention in temperature programmed GC×GC is impossible, making thus necessary to perform a numerical integration. In this paper we present a programmed physical model of GC×GC, capable of predicting with a high accuracy retention times in both dimensions, given different conditions. Our model is based on Poisseuile gas flow through two capillary columns described in work of Beens et al. [1] which describes better the interdependence of the conditions between the connected columns. Once fitted (e.g. calibrated), the model is used to make predictions, which are always subjected to error. In this way, the prediction can result rather in a probability distribution of (predicted) retention

times than in a fixed (most likely) value. One of the most common problems that can occur when fitting unknown parameters using experimental data is the overfitting. In our case, this problem is translated in terms of using the same conditions for both validation and training set. In order to avoid the overfitting problem, the Monte-Carlo sampling combined with cross-validation technique is used [8]. Another technique of error assessment used in this article is the use of error propagation using Jacobians. This method is based on estimation of the accuracy of the model by the partial derivatives of the retention time prediction with respect to the fitted parameters (in this case entropy and enthalpy for each component) in a set of given conditions. By treating the predictions of the model in terms of intervals rather than precise values, it is possible to considerably increase the robustness [9] of any optimization algorithm.

Acknowledgments

Dr. Gabriel Vivó-Truyols, Prof. Dr. Hans-Gerd Janssen, Prof Dr. Peter Schoenmakers.

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Voltammetric Behaviour and Determination of Nitrofurantoin Using a Mercury Meniscus Modified Silver Solid Amalgam Electrode

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Keywords
determination
drinking and river water
mercury meniscus modified silver solid amalgam electrode
nitrofurantoin
voltammetry

Because of the difficulty of predicting the collective effects of the increasing number of pollutants in receiving ecosystems, there is a need for screening methods in environmental monitoring. The implementation of safety programs calls for environmental analysis comprising of two parts: i) screening methods based on highly-throughput analysis and capable of continuous field monitoring on-line with low costs, ii) and analysis of the positive samples with confirmatory more sophisticate and more expensive analytical techniques [1].

For determination of such compounds, which undergo electrochemical oxidation or reduction, voltammetric methods can be used. The main advantages of voltammetric methods are short time of analysis, low cost of assays, possibility of portability and real-time measurements [2].

In this study, nitrofurantoin was chosen because of its potentially damaging environmental effects, particularly for water ecosystems [3]. It belongs to the group of nitrofuran derivatives [4], and is often used in the treatment of urinary tract infections [5].

A sensitive and reproducible procedure has been developed for determination of nitrofurantoin in Britton-Robinson buffer using differential pulse voltammetry (DPV) and direct current voltammetry (DCV) at a mercury meniscus modified silver solid amalgam electrode as a working electrode. The optimum pH for the

determination of nitrofurantoin was sought (c(nitrofurantoin) = 1×10^{-4} mol L^{-1}) in the pH range of 1.0–13.0 of buffer, pH = 7.0 was selected as an optimal medium for both methods. Nitrofurantoin gave at mercury meniscus modified silver solid amalgam electrode one to three waves/peaks, which are attributed to the reduction of nitro group, depending on buffer pH. Other experimental parameter for the drug assays was the aqueous–methanolic ratio (9:1). In order to reduce influence of an electrode passivation, the suitable regeneration potentials, based on 150-times switching the electrode potential from 0 mV to 900 mV, were established.

The optimum conditions have been found for nitrofurantoin determination in concentration ranges from 6×10^{-6} to 1×10^{-4} mol L^{-1} using the DCV technique and from 2×10^{-7} to 1×10^{-4} mol L^{-1} using the DPV technique, both in the medium Britton-Robinson buffer–methanol (9:1). The attained limit of quantification of nitrofurantoin was 1.6×10^{-6} mol L^{-1} for DCV and 8.1×10^{-8} mol L^{-1} for DPV.

The practical applicability of the newly developed DPV methodology was verified for the direct determination of nitrofurantoin in model samples of drinking and river water in the concentration range from 4×10^{-7} to 1×10^{-5} mol L^{-1}.

Acknowledgments

This research was carried out within the framework of the project of the Specific University Research (SVV 2014). It was financially supported by The Grant Agency of the Czech Republic (Project P206/12/G151), and the Charles University Grant Agency (Project 140214/2014/-B-CH/PrF).

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Miniaturization of Asymmetrical Flow Field Flow Fractionation Channel for Separation of Macromolecules

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Keywords
flow field-flow fractionation
macromolecules
point of care diagnostic test

Asymmetrical flow field-flow fractionation is a versatile flow assisted sepa-ration technique [1]. The separation of sample components depends on the interaction with a cross-flow, which is perpendicular to the axial flow in a channel (Fig. 1). The axial flow is laminar, and therefore has parabolic velocity profile, with the highest flow velocity in the middle and a lower velocity close to the channel walls. Due to the molecular diffusion, the sample components take different positions across the parabolic velocity profile under influence of a cross-flow. As a result, the sample components eluate with different velocities, which leads to a separation of the sample components.

Thanks to the fractionation channel design, this method is suitable for a wide range of analytes: macromolecules, nano-sized and micron-sized analytes. The channel does not contain any stationary phase, and consequently the sample components are not affected by a mechanical or shear stress. As a result the analytes are separated without a modification of their native structure.

Some samples such as proteins are available only in a limited volume. That brings difficulties with the detection, since the used detection method might not be sufficiently sensitive. The solution for this situation could be the miniaturization of the asymmetrical flow field-flow fractionation channel. There are several evidences in the literature [2–4] that prove the practicality of the miniaturization of the asymmetrical flow field-flow fractionation systems. The miniaturization of the asymmetrical flow field-flow fractionation channel

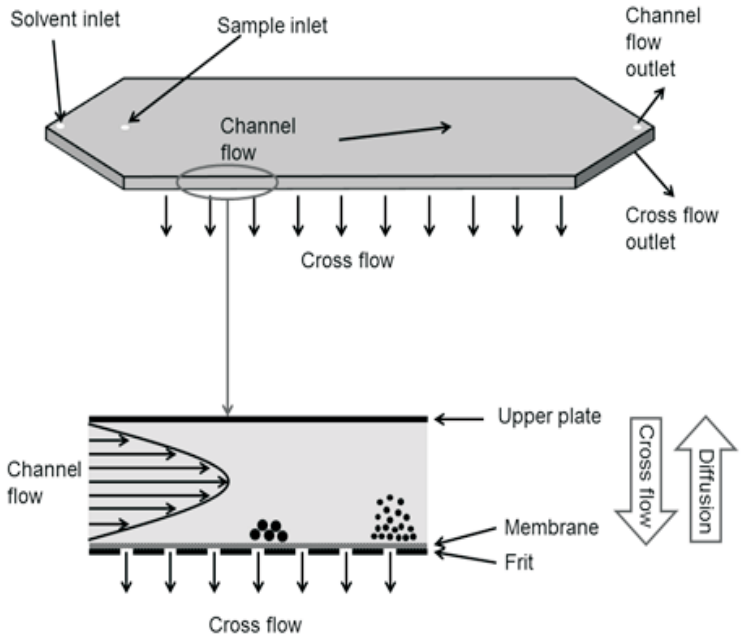

Fig. 1. A schematic diagram of the asymmetrical flow field-flow fractionation technique. Upper part of the figure shows the fractionation channel. The bottom pert of the figure shows the principle of the asymmetrical flow field-flow fractionation separation.

dimensions improves the detectability of the sample, and reduces the total analysis time and the carrier liquid consumption. Nevertheless, a careful trade-off between the analysis time and the resolution needs to be made.

Asymmetrical flow field-flow fractionation, in its miniaturized version, is well suited for the separation of macromolecules (such as lipoproteins) for a point of care diagnostic test. An instrument for a point of care diagnostic test should be able to handle a very small volume of unprocessed samples, it should have a short analysis time, a small size, and it should be safe and easy to use.

The most successful examples of point of care diagnostic tests include pregnancy test and glucose test. Point of care diagnostic tests have the advantage of providing results fast and near the patient. Although, point of care diagnostic tests can be administered by a medical professional, point of care diagnostic devices are also widely self-administered, making patients more responsible for managing their own condition. Empowering individuals to do their own tests can improve patient adherence to diagnosis and treatment regimes.

The purpose of this study is to demonstrate the potential of a miniaturized asymmetrical flow field-flow fractionation technique for a point of care diagnostic

device. There are many asymmetrical flow field-flow fractionation parameters that depend significantly on the channel dimensions, such as a system size, an injection volume, a carrier liquid consumption, a separation speed or a resolution. And especially the system size, the injection volume and the carrier liquid consumption would benefit greatly from the channel miniaturization. A miniaturized symmetrical flow field-flow fractionation channel shows promising results for a future production of a point of care diagnostic test for the analysis of macromolecules in body fluids. This study focuses on the analysis of markers for cardiovascular diseases in blood samples, namely high-density and low-density lipoproteins.

Acknowledgments

This work is supported by NanoNextNL, a micro and nanotechnology consortium of the Government of the Netherlands and 130 partners.

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Arsenic Speciation Analysis by HPLC Postcolumn Hydride Generation and Detection by Atomic Fluorescence Spectrometry

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Keywords
arsenic
atomic fluorescence spectrometry
HPLC
postcolumn hydride generation
speciation analysis

The occurrence of arsenic in water and foodstuff has received worldwide concern in recent years due to its chronic toxicity associated with skin and bladder cancer. Inorganic arsenic species (arsenite (iAs^{III}) and arsenate (iAs^{V})) are the most toxic species that can be found in the environment. Biomethylation is the major human metabolic pathway for inorganic arsenic yielding other species: monomethyl arsenate (MMA^{V}) or dimethyl arsenate (DMA^{V}). Therefore, the speciation analysis of arsenic metabolites is essential to a better understanding of arsenic metabolism and health effects arising from higher levels of those arsenic species in the human tissues [1].

Several methods for speciation analysis of arsenic were described [2], most of them are based on chromatographic separation by means of HPLC. To improve detection limits of the most toxic arsenic species hydride generation can be incorporated between HPLC and detector since all these species are "hydride--active" and can be converted to their corresponding volatile arsines by the reaction with tetrahydroborate. The advantage of hydride generation step is separation of an analyte from a sample matrix and much higher transport and overall introduction efficiency to the detector in comparison to nebulization techniques. This allows to replace inductively coupled plasma mass spectrometry

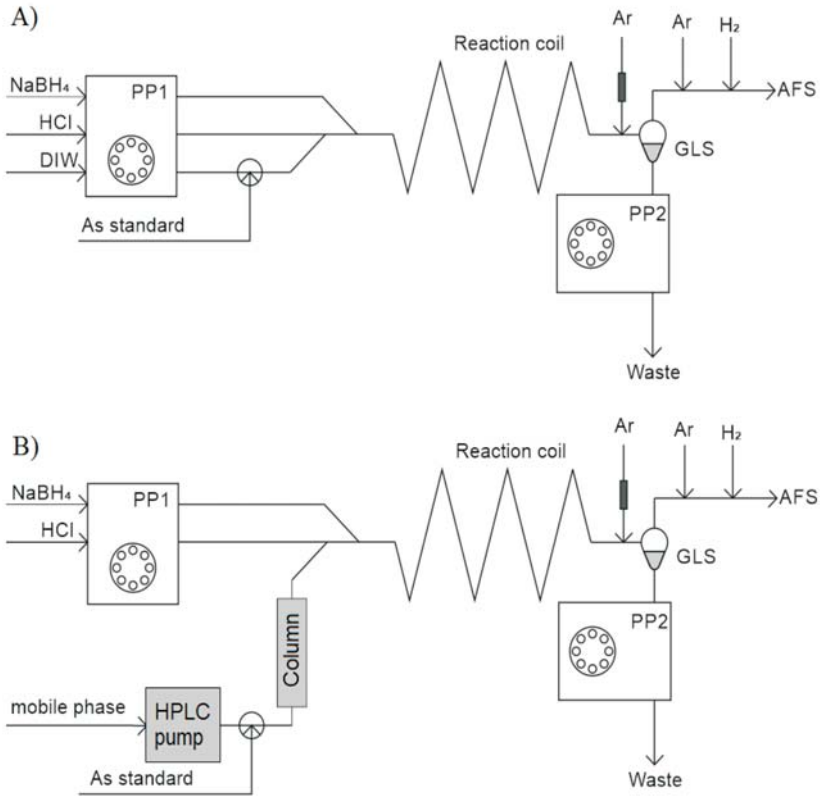

Fig. 1. Scheme of A) flow injection-hydride generation-atomic florescence system, B) HPLC-hydride generation-atomic florescence system (PP1, PP2 are peristaltic pumps and GLS is gas-liquid separator).

detector with substantially cheaper atomic absorption or atomic fluorescence detectors. Up to now, one of the disadvantages was the different sensitivity for each arsenic species due to different efficiency of hydride generation from hydrochloric acid medium.

The aim of this contribution is to present a new method of hydride generation that enables to generate arsines from iAs^{III}, iAs^{V}, MMA^{V} and DMA^{V} in a flow injection mode with the same efficiency and in the next step connection of this hydride generator with HPLC column. The system consisted of a peristaltic pump which pumped reagents: hydrochlorid acid, sodium tetrahydroborate, and deionized water that was a carrier into which the arsenic standard was injected by an injection valve (Fig. 1a). The reagents are mixed in the reaction coil and after the reaction coil argon chemifold is introduced. The reaction mixture then continues to the gas-liquid separator where arsines are evolved to the gaseous phase and transported by the argon to the detector. The remaining liquid phase is

pumped out from the gas-liquid separator to the waste by a second peristaltic pump. For a sensitive detection a laboratory assembled atomic fluorescence instrument was utilized with a miniature diffusion flame atomizer. When the HPLC-hydride generation-atomic fluorescence measurements were performed the channel for deionized water (and injection valve) was replaced by the HPLC apparatus (Agilent) with an autosampler (Fig. 1b).

Efficiency of hydride generation of arsenic species is known to be dependent on concentration of sodium tetrahydroborate and length (or volume) of the reaction coil and strongly dependent on concentration of hydrochloric acid. Generation efficiency of inorganic arsenic increases with higher concentration of hydrochloric acid. In contrast to MMA^V and DMA^V that have maximum generating efficiency at lower concentration of hydrochloric acid [3, 4]. The highest generating efficiencies for all four arsenic species in the flow injection mode were reached for concentration of hydrochloric acid of 2 mol dm^{-3}. At this concentration the relative signals (always compared to iAs^{III} that is easily generated in a broad range of pH) were approximately 60±1%, 90±2% and 56±1% for iAs^V, MMA^V and DMA^V. Another factor which affects the efficiency of hydride generation is the reaction time that is proportional to the volume of the reaction coil. It was found out that extending of the reaction coil leads to increase in generation

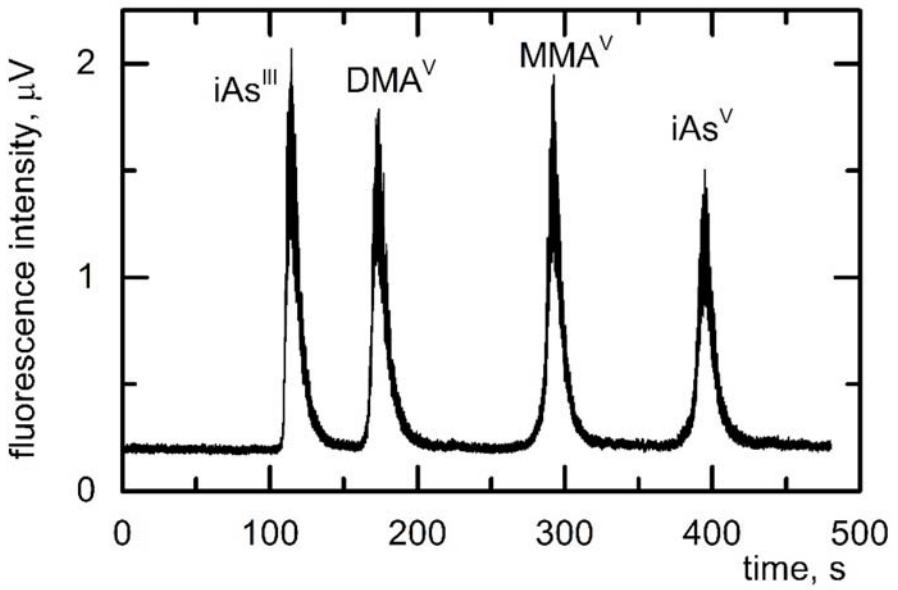

Fig. 2. Typical chromatogram of a mixture containing arsenite (iAs^{III}), dimethyl arsenate (DMA^V), monomethyl arsenate (MMA^V), and arsenate (iAs^V), in deionized water obtained by the HPLC-hydride generation-atomic florescence spectrometry. Separation was carried out on an ODS-3 column with a mobile phase containing 4.7×10^{-3} mol dm^{-3} tetrabutylammonium hydroxide, 2×10^{-3} mol dm^{-3} malonic acid, and 4% methanol (v/v). Concentration of arsenic species 10 ng mL^{-1}.

efficiency. For the reaction coil of the total volume of 10.4 mL generation efficiency reached approximately 90±1%, 102±3% and 84±1% for iAs^V, MMA^V, and DMA^V, respectively. Finally, by increasing the concentration of sodium tetrahydroborate to 2.5% (m/v) generation efficiencies reached 100% for all species: namely 98±3% for iAs^V, 100±3% for MMA^V, and 98±3% for, DMA^V. Subsequently, it was verified that the composition of the mobile phase (4.7×10^{-3} mol dm^{-3} tetrabutylammonium hydroxide, 2×10^{-3} mol dm^{-3} malonic acid, and 4% methanol (v/v)) does not affect the efficiency of hydride generation.

For real application of these results that have never been seriously reported in literature up to now, the modified hydride generator with a long reaction coil was coupled to the output of the HPLC to generate arsines postcolumn, i.e. after separation of arsenic species (Fig. 1b). The typical separation of all four arsenic species on the column is shown in Fig. 2. Since the sensitivity for all four arsenic species was comparable as well, it is possible now to use a single species standardization. This means that the calibration curve can be constructed only by means of one standard (e.g. DMA^V which is the most stable species of an analyte) and this calibration curve can be used for quantification of all other arsenic species. The advantage of this approach lies in shortening of the entire analysis because DMA^V is eluted within 3 minutes and thus it is not necessary to wait for the elution of all arsenic standards from the column.

Acknowledgments

This work was supported by GA CR (grant No. P206/14-23532S), Institute of Analytical Chemistry of the ASCR, v. v. i. (project no. RVO: 68081715), and by Charles University in Prague (project SVV260084).

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Analysis and Characterization of Antimicrobial Peptides by Capillary Electromigration Methods

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Keywords
antimicrobial peptides
capillary electrophoresis
coating
physico-chemical parameters

Antimicrobial peptides are extensively investigated compounds with the potential to replace conventional antibiotics [1]. Antimicrobial peptides have been isolated from various species, e.g. from insects, frogs or mammals. At our Institute, novel antimicrobial peptides from the bee *Halictus sexcinctus* were recently isolated and characterized. These peptides and their analogues, named halictines, are composed of 10 to 12 amino acid residues with frequent occurrence of basic amino acids (His, Lys, Arg); their secondary structure is alpha-helix [2]. For their quantitative and qualitative analysis, separation and characterization, high-performance capillary electromigration methods (zone electrophoresis, affinity electrophoresis, isotachophoresis and micellar electrokinetic chromatography) have been applied. These methods possess a great potential for analysis and physic-chemical and biochemical characterization of peptides [3]. In addition to purity control of synthetic or isolated *a*ntimicrobial peptides, they have been employed also for determination of important physico-chemical parameters of halictines, such as effective and ionic mobilities, effective charges, Stokes radii, isoelectric points, acid dissociation constants of their ionogenic groups and association (binding) constants of their complexes. These measurements were perfomed in a series of the background electrolytes within a wide pH range using fused silica capillaries coated with physically adsorbed polybrene or covalently attached hydroxypropylcellulose to suppress sorption of basic peptides to the inner capillary wall [4, 5].

Acknowledgments

The work was supported by the Czech Science Foundation (projects nos. P206/12/0453, 13-17224S), and by the ASCR (Research Project RVO 61388963).

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Electrochemical Study of 2-Nitrophenol Using Carbon Film Electrode and its Application to Determination of Model Samples of Drinking Water

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Keywords
carbon film electrode
electrochemistry
2-nitrophenol
voltammetry

This study is focused on the application of a carbon film electrode for the development of voltammetric methods for the determination of submicromolar concentrations of biologically active compounds detrimental to the environment (2-nitrophenol was chosen as a model substance). The advantages of carbon film electrode are primarily its wide potential window in cathodic region, high sensitivity, and low noise of measurements [1]. Another important advantage of carbon film electrode is also non-toxicity compared to mercury electrodes.

2-Nitrophenol is well known for its pesticide effects and also as potential carcinogen, teratogen and mutagen [2]. The determination of 2-nitrophenol is based on cathodic reduction of present nitrogroup. For testing of optimal conditions, modern electroanalytical methods as direct current voltammetry (DCV) and differential pulse voltammetry (DPV) were chosen. During the optimization the electrochemical behaviour of 2-nitrophenol in different pH media and under/with various regeneration potentials were carried out. The highest and the best developed peak was obtained using Britton-Robinson buffer of pH = 5.0 (DCV) and of pH = 6.0 (DPV). The best repeatability was carried out with regeneration potentials E_{in} = 0 mV; E_{fin} = 0 mV. Using these optimized conditions, the repeatability and reproducibility of measurements were tested. The calibration curves of 2-nitrophenol in deionized water were measured in

concentration rage from 2×10^{-6} to 1×10^{-4} mol L^{-1} for both methods, DCV and DPV. Limit of quantification (*LOQ*) for DCV was 1.2×10^{-6} mol L^{-1} and for DPV 2.0×10^{-6} mol L^{-1}. Application of both methods were tested in the same range in model samples of drinking water with *LOQ* = 3×10^{-7} mol L^{-1} for DCV and *LOQ* = = 1×10^{-6} mol L^{-1} for DPV.

In this work the applicability of carbon film electrode for the determination of submicromolar concentration of 2-nitrophenol in model samples of drinking water has been proven.

Acknowledgments

This research was carried out in the framework of specific university research of the Charles University in Prague (Project SVV 2014), and (of) the project Open Science III. (reg. n. CZ.1.07/-2.3.00/35.0023) supplied by Academy of Science of Czech Republic. The Grant Agency of the Charles University (Project GAUK 468214/2014/B-CH/PrF), and the Grant Agency of the Czech Republic (Project P206/12/G151) are gratefully acknowledged.

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Determination of Oxalic and Citric Acid in Chromium(III)-Containing Industrial Solutions by Capillary Zone Electrophoresis

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Keywords
capillary electrophoresis
indirect UV detection
industrial solutions organic acids
sample pre-treatment

Development of industrial technologies using nontoxic, environmentally-friendly substances is a hot topic nowadays. Small carboxylic acids are important component of various surface finishing baths, their usage as complexing and pH-stabilizing agents significantly improves the effectiveness of the technological process [1]. This work deals with the development and optimization of conditions of pre-treatment of two industrial surface finishing baths containing chromium(III) ions and oxalic and citric acid and their electrophoretic analysis [2]. Some model mixtures containing known amounts of components of industrial solutions have been made for simulation of complex matrices of the real samples. Prior to analysis a sample pre-treatment consisting of different dilution and addition of fluoride, hydroxide or EDTA anions as suitable agent releasing acid out of the stable chromium complex were studied. Determination of organic anions was accomplished by indirect UV detection at 350 nm with a reference at 230 nm [3]. A commercially available background electrolyte, pH = 5.7, was used for separation of analytes. The most appropriate pre-treatment to release acids have been achieved by precipitation of chromium(III) hydroxide. The method of standard additions was used for the quantification. The content of oxalate and citrate in the real samples was calculated as 98.29% (S.D. = 6.99%) and 97.53% (S.D. = 2.78%), respectively, of declared amount. Satisfactory repeat-abilities were obtained for both analytes with R.S.D. values (n = 5) for migration times lower than

0.51%, R.S.D. for peak areas of oxalic acid was 5.15% and 2.95% in case of citric acid. Total analysis time less than 6 minutes was achieved. This simple inexpensive method is suitable for rapid routine determination of citric and oxalic acid in chromium(III)-based solutions.

Acknowledgments

This work was financially supported by Charles University in Prague, project SVV 2014-260084.

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Characterization of Poly(Methylene Blue) Modified Graphite Electrode as a Sensor for Hydrogen Sulphide

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Keywords
hydrogen sulphide
methylene blue
sensor

In recent years, hydrogen sulphide has been extensively studied as the human endogenous messenger molecule of gas with auspicious therapeutic effects [1]. It has a physiologic gasotransmitter function comparable to NO and CO [2]. Due to very low physiological concentrations and potential therapeutic concentrations, there has been increasing need for more appropriate analytical methods with emphasis on their sensitivity and selectivity.

Chemically modified electrodes were found to be advantageous for a wide range of applications including electroanalytical studies and analyzes. In general, the electrode surface modification can improve the electrochemical response to analyt by mediating or catalyzing its charge transfer reaction, protecting the surface against passivation and by changing the electrochemical reaction mechanism respectively. Utilizing electrodes with immobilized suitable chemical mediators can solve some problems with bare solid electrodes such as insufficient sensitivity and selectivity, slow electron transfer, current and potential oscillations, or high overpotential.

Electropolymerization is an effective way of surface modification. Electrosynthesized polymers exhibit some unique behavior that the corresponding monomers do not always display. In the past few years, surface-modified electrodes based on the electropolymerization of various phenoxazine and phenothiazine derivatives have been reported in the literature [3, 4].

Methylene blue is a water-soluble phenothiazine cationic dye. Electrochemical polymerization of monomer methylene blue leads to immobilization of a conductive polymer film on the electrode surface. The electrocatalytic activity of poly(methylene) blue in presence of some biologically active compounds has been already reported in several studies [5–8]. It was observed that the rate of the polymerization increased with increasing pH indicating that basic solutions are the optimal media for the polymerization of methylene blue [5]. Thanks to the strong adhesion of the polymer to the electrode surface this system exhibits good stability. It has been already published that electrochemical properties of resulting film are dependent on the electrode substrate [9].

The aim of our work is devising new system which combines auspicious electrochemical properties of poly(methylene) blue and advantages of using highly oriented pyrolytic graphite (HOPG). For the first time poly(methylene blue) film was deposited on the HOPG basal plane substrate. We tried to define electrochemical behaviour during electropolymerization and electrochemical properties of resulting HOPG-poly(methylene blue) electrode. Modified electrode exhibits electrocatalytic activity towards sulfhydryl group. Our findings will be utilized for development of new potentiometric or amperometric sensor.

Acknowledgments

This work was supported by grant project SVV260084 of Charles University in Prague.

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Comparison of Various Methods for Extraction of Capsaicinoids

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Keywords
capsaicinoid
extraction
HPLC

Capsaicinoids as *N*-vanillylamides of fatty acids are a group of pungent chemical analogues that occur in chili peppers, fruits of plants belonging to genus *Capsicum*. Capsaicinoids are insoluble in water but willingly soluble in fat or some organic solvents. The aim of this work has been a comparison of three conventional extraction methods and a new developed 'green' method for extraction of capsaicinoids from chilli peppers. The conventional methods were: two types of ultrasonic assisted extraction (extraction in an ultrasonic bath, and extraction with an ultrasonic probe), and Soxhlet extraction. The new method was pressurised hot water extraction. Pressurised hot water is a 'green' alternative to methanol that is commonly used for extraction of these alkaloids. At elevated temperature, the viscosity and surface tension of water decreases, while diffusivity and solubility of the capsaicinoids increases. Separation, identification and quantification of four capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and nonivamide) were performed using reversed phase high performance liquid chromatography with mass spectrometry.

UV-Photochemical Cold Mercury Vapor Generation as a Derivatization Step Between HPLC Separation and AAS Detection for Speciation Analysis of Selected Mercury Compounds

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Keywords
AAS
cold vapor generation
HPLC
mercury
speciation analysis

The aim of the presented work was the development of an analytical method for ultra-trace determination of several mercury compounds in ground water samples.

Mercury occurs in various forms in the environment. It can be present in a metallic form or its vapor, as well as in the form of inorganic (mercurous and mercuric) and organic mercury compounds [1, 2]. Mercury and its compounds belong to the most toxic substances. Each oxidation state and each organic species have characteristic toxic effects. No other metal better illustrates the diversity of effect caused by different chemical species than mercury does [3]. Due to the different toxicity of mercury species, it is insufficient to know only total mercury content in a sample. Therefore, it is necessary to carry out speciation analysis. Only from the knowledge of each mercury species concentration, the level of toxicity of the sample can be determined. Mercury is (together with cadmium) one of only two elements which are known because of high atomic vapor pressure at room temperature. This fact resulted into the name of generated metallic vapor for analytical purposes which is called 'cold vapor'. Other metals produce their vapor at much higher temperature [4, 5]. UV-photochemical cold mercury vapor generation is a quite new analytical technique which provides an attractive

alternative approach to common chemical or electrochemical mercury cold vapor generation.

Mercury chloride (Hg^{+II}), methylmercury chloride ($MeHg^{+I}$), ethylmercury chloride ($EtHg^{+I}$), and phenylmercury chloride ($PhHg^{+I}$) have been chosen as model compounds. UV-photochemical cold mercury vapor generation was used as a derivatization step between high performance liquid chromatography on reverse phases (RP-HPLC) and atomic absorption spectrometry with quartz detection tube.

Cold mercury vapor was successfully generated from all the selected mercury species under UV-irradiation in presence of low molecular weight organic acid and 2-mercaptoethanol. Both of these two chemicals were therefore added into the mobile phase.

In the first part of the project, the main attention was paid to the construction of the UV-photochemical generator. The UV-photochemical generator was assembled by attaching the reaction coil on the surface of low pressure Hg lamp (20 W, 254 nm). The reaction coil was constructed from PTFE tube (1 m × 1 mm ID × 2 mm OD) which was wrapped around the UV lamp. PTFE tubes of different diameters and lengths were tested. The UV-photochemical generator was tested in the flow injection mode. Following key parameters were optimized: the mobile phase flow rate, composition of mobile phase (concentration of buffer, pH, organic phase content, and concentration of 2-mercaptoethanol), the carrier gas flow rate and the sampling loop volume.

In the next part of this project, the UV-photochemical generator was used as a postcolumn derivatization unit for speciation analysis of a mixture of selected mercury compounds (Fig. 1). Separation of mercury species was realized by RP-HPLC. Not optimum but compromise values had to be chosen in order to achieve sufficient resolution within the separation of mercury species on one hand and a comparable efficiency of cold mercury vapor generation from all the four species on the other hand. Following key parameters were optimized in this step: the mobile phase flow rate, the carrier gas (argon) flow rate, the concentrations of acetic acid, 2-mercaptoethanol, and ethanol in the mobile phase, pH of mobile phase, and the column temperature.

The attained results indicate that presence of 2-mercaptoethanol in the mobile phase is of significant importance in the UV-photochemical generation of cold mercury vapor as well as in the separation process. It was concluded that ethanol is the most appropriate organic solvent in the mobile phase for this speciation analysis.

Although future investigations are still needed, the results show that the UV-photochemical cold mercury vapor generation is a promising alternative to the traditional chemical generation because of its simplicity, green profile and cost effectiveness.

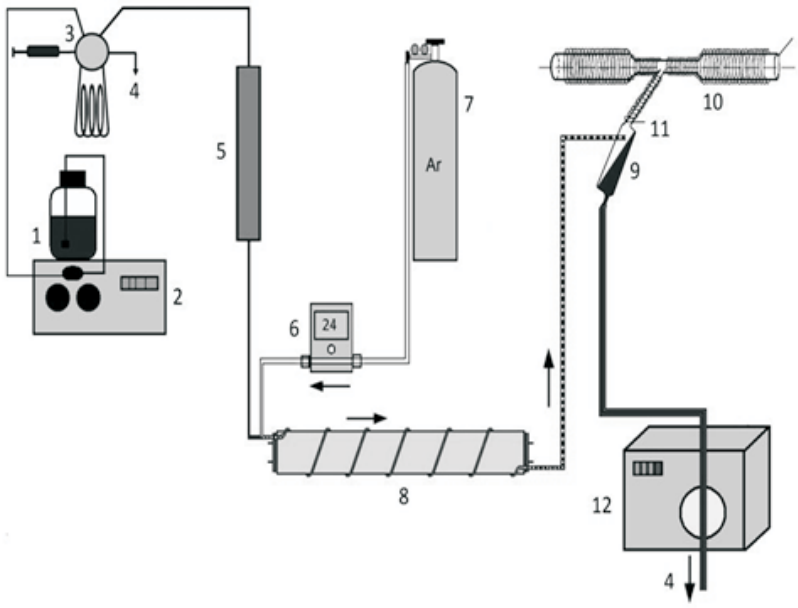

Fig. 1. The instrumental set-up: (1) mobile phase, (2) HPLC pump, (3) injection loop, (4) waste, (5) HPLC column, (6) gas flow meter, (7) carrier gas, (8) UV-photoreactor, (9) gas-liquid separator, (10) quartz detection tube, (11) resistance heating, (12) peristalic pump.

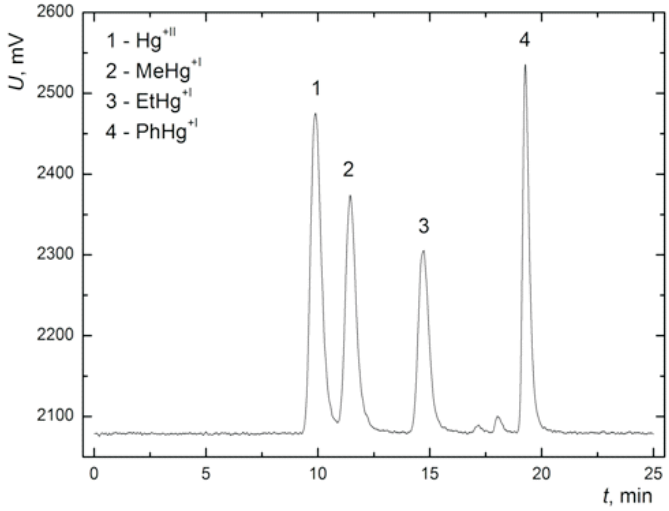

Fig. 2. Typical chromatogram of four mercury species using system of RP-HPLC with UV-photochemical cold mercury vapor generation and quartz detection tube AAS. Experimental conditions: $c(Hg^{+II}) = c(MeHg^{+I}) = c(EtHg^{+I}) = c(PhHg^{+I}) = 500$ µg L^{-1}; v(Ar) = 150 mL min^{-1}; v(MPh) = = 0.175 mL min^{-1}, V(sampl. loop) = 50 µL, c(EtOH) = 40 % (v/v), t = 40 °C, c(buffer) = 20 mmol L^{-1}, pH = 4.75, c(ME) = 0.1 %, l(PTFE reactor) = 1 m.

Acknowledgments

This work was financially supported by the Charles University in Prague (Project UNCE#42, GAUK 152214 and project SVV 260084), and by the Grant Agency of the Czech Republic (Project GACR 14-23532S).

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Proceedings of the 10th International Students Conference "Modern Analytical Chemistry"

Edited by Karel Nesměrák.
Published by Charles University in Prague, Faculty of Science.
Prague 2014.
1st edition – 96 pages – Number of copies: 70

ISBN 978-80-7444-030-4

ISBN 978-80-7444-030-4