Proceedings of the 7th International Students Conference "Modern Analytical Chemistry"

Prague, 29—30 September 2011

Edited by Karel Nesměrák

Charles University in Prague, Faculty of Science
Prague 2011

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Preface

Dear friends and colleagues,

It is said that number seven is a lucky number. We hope that the upcoming 7th International Students Conference "Modern Analytical Chemistry" will also be a lucky and successful event. This year, the international aspect of the conference is enhanced, as the participants come from already four countries. We are pleased that more than twenty nine young scientists will attend the conference, will present their scientific results and will characterize the directions of their research in the field of analytical chemistry. We are convinced that the conference offers many possibilities for improving the presentation skills, provides the floor for discussion and exchange of experiences, and helps to master the English language to all the participants.

We would be unable to organize this conference without the kind financial support from our sponsors. The Zentiva, Quinta Analytica, and Shimadzu companies are cordially thanked, not only for their financial contributions on this occasion, but for their continuous support and cooperation in many of our activities.

We wish you success in the presentation of your contributions, 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.

Sponzors

The organizing committee of 7th International Students Conference “Modern Analytical Chemistry” gratefully acknowledges the generous sponsorship of following companies:

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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 Theater). Oral presentations are 20 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. The coffee breaks are held in the lecture hall.

Thursday, September 29, 2011

9:00–9:20	**Opening ceremony, welcoming address**
	Session 1 ▪ chairperson: Michal Horčičiak
9:20–9:40	Bursová M.: *Presentation and optimization of a new microextraction technique* (p. 11)
9:40–10.00	Yosypchuk O.: *Electrochemical biosensor for determination of nucleic acid bases* (p. 85)
10:00–10:20	Cífková E.: *Lipidomic analysis using off-line two-dimensional HILIC × RP-HPLC/MS* (p. 17)
10:20–10:40	Denderz N.: *Thermodynamic studies of interactions between selected anesthetics and molecularly imprinted polymers* (p. 19)
10:20–11:00	**Coffee Break**
	Session 2 ▪ chairperson: Kateřina Netušilová
11:00–11:20	Dendisová-Vyškovská M.: *Detection of biologically important substances using* in-situ *spectroelectrochemistry* (p. 22)
11:20–11:40	Franc M.: *Packing of capillary columns for HPLC* (p. 23)
11:40–12:00	Horčičiak M.: *Determination of glyphosate in drinking waters on an electrophoretic chip* (p. 28)
12:00–12:20	Jánošková N.: *Discrimination of botanical origin of honeys based on their GC×GC* (p. 31)
12:20–13:20	**Lunch**

Session 3 ▪ chairperson: Stefan Wilhelm

13:20–13:40 Kačeriaková D.: *Study of the mechanistic aspects of separation of enantiomers of alcohols on the 6-tert-butyldimethyl-silyl-2,3-di-alkyl α- and β-cyclodextrin type stationary phases* (p. 34)

13:40–14:00 Kaftan F.: *Imaging mass spectrometry of cuticular lipids of* Drosophila melanogaster (p. 37)

14:00–14:20 Karásek J.: *Construction of miniaturised detection cell for voltammetric determination of various analytes at soil extracts* (p. 39)

14:20–14:40 Kozlík P.: *Comparison of HPLC and capillary liquid chromatography with tandem mass spectrometric detection for analysis of estrogen pollutants* (p. 42)

14:40–15:00 Krasulová J.: *GC-MS analysis of termite defensive compounds secreted from their frontal glands* (p. 46)

15:00–15:20 **Coffee Break**

Session 4 ▪ chairperson: Darina Kačeriaková

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15:40–16:00 Míková R.: *Methodology of lipid analysis of vernix caseosa using MALDI-TOF MS* (p. 57)

16:00–16:20 Netušilová K.: *Lipidomic profiling of patients with cardiovascular diseases using GC/FID and HPLC/MS* (p. 60)

16:20–16:40 Palatzky P.: *Application of novel capillary probes for the investigation of electro-chemically assisted injection (EAI) using a scanning electrochemical microscope for EAI cell development* (p. 61)

16:40–17:00 **Sponsors' presentations**

19:00 **Conference Dinner**

Friday, September 30, 2011

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9:00–9:20 Ráczová J.: *Anion-exchange chromatography in combination with stepwise gradient for characterization of humic substances in an alkaline medium* (p. 63)

9:20–9:40 Sobolčiak P.: *Pulsed laser technique in conjunction with size exclusion chromatography as tool for determination propagation rate coefficient of free-radical polymerization of zwitterionic monomers* (p. 68)

9:40–10:00 Szultka M.: *SPME-LC/MS^n for the analysis of selected biologically active compounds* (p. 70)

10:00–10:20 Troška P.: *The use of miniaturized capillary electrophoresis in monitoring of some neurological diseases* (p. 73)

10:20–10:40 Tylová T.: *UHPLC-DAD-ToF-MS: A useful tool for chromatographic fingerprinting of fungal extracelullar metabolites* (p. 76)

10:40–11:00 **Coffee Break**

Session 6 ▪ chairperson: Tomáš Křížek

11:00–11:20 Wilhelm S.: *Magnetic and upconverting luminescent core/shell nanoparticles for sensor applications* (p. 79)

11:20–11:40 Wranová K.: *Determination of platinum on different concentration levels by inductively coupled plasma mass spectrometry* (p. 81)

11:40–12:00 Buszewska M.: *The influence of different methods of preservation on the content of bioactive compounds in blueberried honeysuckle juice* (p. 15)

12:00–12:20 Zábranská M.: *Analysis of plant membrane lipids by RP-HPLC/-HR-ESI-MS* (p. 87)

12:20–13:20 **Lunch**

Session 7 ▪ chairperson: Petr Kozlík

13:20–13:40 Křížek T.: *Electrophoretic approaches to analysis of saccharides for enzyme kinetics studies of hexosaminidases* (p. 50)

13:40–14:00 Dinisová P.: *LC/MS separation of natural antioxidants in herbs and honey extracts* (p. 91)

14:00–14:20 Fryš O.: *Utilization of modern extraction methods for analysis of propellant components* (p. 93)

14:20–14:40 **Closing Address**

Contributions

Presentation and optimization of a new microextraction technique

Miroslava Bursová, Radomír Čabala

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Keywords
GC-MS
liquid phase microextraction
response surface method

The objective of the presented work is to present and chemometrically optimize new liquid-liquid microextraction technique. The microextraction method followed by GC-MS was used for determination of selected environmental pollutants in aqueous samples.

The new microextraction technique belongs to large group of liquid-liquid microextraction technique [1] and its novelty is represented by the application of a special bell-shaped plastic extender which allows application of very small volume of the extracting solvent, typically 50–300 μL. An intense mixing of the aqueous sample creates distinct vortex on which surface the extraction solvent floats without escaping from the interior of bell-shaped plastic extender. After the extraction, bell-shaped plastic extender provides an easy and almost complete withdrawal of the extraction solvent. The great advantage of this method is a possibility of a selection from a wide polarity range of organic solvents lighter than water according to the polarity and solubility of analytes. The subsequent analyses of extracts can be performed by many suitable instrumental methods, e.g., GC, GC-MS or HPLC methods.

The new extraction procedure was optimized with help of statistical response surface method. The method fits experimental data with a polynomial equation, which should describe the dependence of the selected response of an experimental system on the experimental parameters [2]. In this case, the selected experimental parameters were: type and volume of extraction solvent, effect of salt addition, stirring rate, diameter of extraction vial, extraction time, shape of extender, and flushing of extender with acetone before microextraction. The sum of the relative peak areas of all analytes was used as the analytical response.

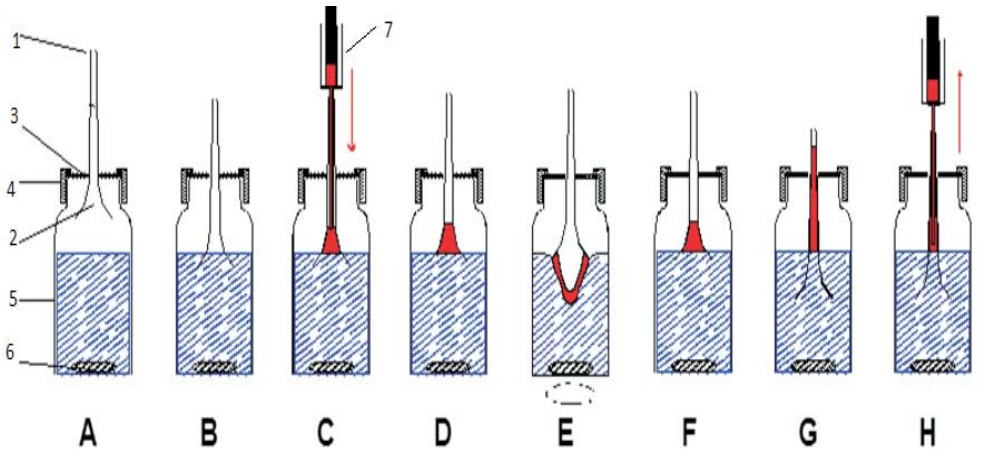

Fig. 1. Microextraction procedure with bell-shaped plastic extender: (1) extender, (2) funnel, (3) septum, (4) cap, (5) glass vial with water sample, (6) stir bar, (7) microsyringe with extraction solvent.

Experimental

Chemicals. Toluene, ethylbenzene, mesitylene, phenol, nitrobenzene, octanol, naphthalene and dimethylphthalate were used as the analytes. Stock solutions of individual compounds in methanol (1 mg mL^{-1}) were diluted to aqueous working solutions. Heptane and *tert*-butyl acetate were used as the extraction solvents. Methylhexadecanoate was used as the internal standard in extraction solvents at concentration of 100.2 μg mL^{-1}.

Microextraction procedure. A 10 mL of the aqueous sample spiked with the analytes at level of 0.1 μg mL^{-1} were placed in a 16 mL glass vial and stir bar was added. The vial was placed on the magnetic stirrer and bell-shaped plastic extender was fixed in the septum of the glass vial cap (Fig. 1A). Before extraction, the lower end of bell-shaped plastic extender was immersed in the sample so that the level of the liquid had reached the middle of the extender (Fig. 1B). A 150 μL of *tert*-butyl acetate as extraction solvent containing methylhexadecanoate as internal standard was dispensed into bell-shaped plastic extender by 250 μL glass microsyringe (Fig. 1C). The stirring rate was set at max. 1000 rpm to create a stable vortex in the sample (Fig. 1E). Extraction time was set to 24 min and after the extraction the extender containing the extraction solvent was immersed about 0.5 cm deeper into the sample (Fig. 1G) to push the solvent into the top part of the extender where it can be easily withdrawn by microsyringe for further analysis (Fig. 1H). The organic solvent was transferred into the glass cone-shaped vial and the analytes determined by GC-MS. The new microextraction technique is being applied for the patent at the moment.

GC Instrumentation. GC-MS analyses were performed on a GC 17A-GCMS-QP 5050A instrument (Shimadzu) equiped with DB-5ms capillary column (32 m × × 0.25 mm ID, 0.25 μm; Agilent Technologies). Helium (99.999 %) was used as carrier gas at linear flow velocity of 40 cm s^{-1}. MS interface temperature was 275 °C, injector

Table 1
The experimental parameters and their levels in the Plackett-Burman design.

Parameter	Low (–1)	High (+1)
Stirring rate [rpm]	700	950
Volume of extraction solvent [μL]	150	250
Extraction solvent	heptane	*tert*-butylacetate
Amount of added NaCl [g]	0.5	3
Extraction time [min]	8	20
Type of extraction extender	A	B
Diameter of vial [cm]	1.9	2.7
Plug of extraction extender	no	yes
Flushing of extender by acetone	no	yes

Table 2
The experimental parameters, their levels and star points in the central composite design.

Parameter	–α (–1.63)	–1	0	+1	+α (+1.63)
Extraxtion time [min]	4	8	14	20	24
Stirring rate [rpm]	690	750	850	950	1013
Amount of added NaCl [g]	0.00	0.50	1.75	3.00	3.50

temperature 250 °C, and the temperature program was: 50 °C for 5 min, at 30 °C min^{-1} to 250 °C and 3 min isothermal. Total analysis time was 14.67 min. GC Solutions program (Shimadzu) was used for acquisition and data evaluation. The data and experimental design were processed by statistical program Minitab 16 (Minitab Inc., State College PA, USA).

Results

Nine experimental parameters were selected in order to obtain the optimum conditions for the microextraction procedure and their low and high values are listed in Table 1. The Plackett-Burman design was used for screening of these variables [3]. The overall design matrix contained 12 runs in duplicate. The analysis of variance was used to evaluate the data and statistically significant effects were determined using *t*-test (95% probability). According to *t*-test, the stirring rate was the most significant variable followed by the effect of salt addition. The extraction time was on the border of 95% confidence level and was intentionally added to list of significant parameters that were used for central composite design [2]. *Tert*-butyl acetate was chosen as extraction solvent.

The resulting significant factors were used in the central composite design for investigation of their interaction and their examined levels are listed in Table 2. The overall design matrix consists of 20 runs (8-factorials, 6-central and 6-star experiments)

[4]. The results were evaluated by ANOVA test and a second-order polynomial regression model was used to calculate the response surface

$$response = 10.5563 + 2.7296\,x_1 + 2.8515\,x_2 + 2.0241\,x_1^2 \quad (1)$$

where x_1 is stirring rate and x_2 extraction time.

Applying the desirability function, Minitab 6 software located the optimal conditions as follows: the extraction time 24 min, stirring rate 1013 rpm and no addition of sodium chloride. At these conditions, the model reached very good agreement with the experiments because the system predicted response value was 27.48 whereas experimental response value was 27.71 (100.8% of the predicted value).

Conclusion

This work presented new kind of liquid-liquid microextraction technique and successfully demonstrated the easy application of chemometric software for its fast and reliable optimization.

Acknowledgments

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The influence of different methods of preservation on the content of bioactive compounds in blueberried honeysuckle juice

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Keywords
antioxiadant activity
blueberried honeysuckle
preservation
polyphenols

The past two decades mark the growing understanding of the role of diet in combating civilization diseases. One of factors with demonstrated preventive potency is diet rich in fruits and vegetables. Both fruits and vegetables are the unrivalled source of compounds with antioxidant properties in diet of Poles. Due to climatic conditions and consumer preferences, the richest source of compounds exhibiting the highest antioxidant potential are berries. However, in the case of berries are seasonal fruits, it is necessary to use methods of preservation, in order to ensure their availability throughout the year. The content of secondary metabolites, including antioxidants is strongly influenced by both storage conditions of raw material and physicochemical parameters used during processing. Therefore, the methods of preservation are sought that ensure microbiologically safe products containing high levels of bioactive phytochemicals.

Our study was carried out for juices obtained from blueberried honeysuckle (*Lonicera caerulea* L. var. *edulis*), the fruit regarded as containing components particularly beneficial for human health. The purpose of this research was to determine the effect of different food preservation technologies on the content of bioactive compounds find in this berry. The following ways of processing were tested: microwave treatment, high pressure treatment, pasteurization, sterilization, long and short heating at 100 ºC; to determine which of these technologies causes, the lowest degradation of

valuable substances. The impact of technological process on the profile of bioactive compounds found in juice from blueberried honeysuckle was monitored by TLC technique. Qualitative and quantitative analysis of juice samples was performed using high performance liquid chromatography (HPLC-DAD). Total antioxidant activity was determined using spectrophotometric tests.

Based on the obtained results, it can be concluded that the sterilization process and long heating caused the greatest loss in the concentration of anthocyanins. In the case of certain types of preservation, the changes in composition of phenolic compounds were observed including the formation of new compounds with antioxidant properties. It can be concluded that the microwave treatment, high pressure treatment and short heating are the best methods that can be used in food preservation. The results of these methods were reproducible. Our results enable the optimal use of the fusing process, a condition for maximum stability of phenolic compounds, and thus reinforce the wholesomeness of their daily diet.

Lipidomic analysis using off-line two-dimensional HILIC × RP-HPLC/MS

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Keywords
hydrophilic interaction liquid chromatography
lipidomics
lipids
phospholipids
two-dimensional liquid chromatography

Lipids are important components in all biological tissues having many essential functions associated with the proper function of organisms. Lipidomics contributes towards the understanding how lipids function in a biological system and for the elucidation of the mechanism of lipid-based diseases, i.e., obesity, atherosclerosis, cancer, cardiovascular problems, etc. For this purpose, the lipidomic analysis using off-line two-dimensional coupling of hydrophilic interaction liquid chromatography (HILIC) and reversed phase high-performance liquid chromatography (RP-HPLC) mode with mass spectrometry detection was optimized [1]. In the first dimension, total liquid extracts were fractioned using the HILIC separation into individual lipid classes. Chromato-graphic conditions have been carefully optimized to achieve the best separation of the maximum number of lipid classes. Optimized HILIC separation enables the fractio-nation of nineteen lipid classes and three regioisomeric pairs that cover a wide range of polarities. The fractions of individual lipid classes were collected and separated using RP-HPLC in the second dimension. Chromatographic conditions for polar lipids were optimized to achieve the highest number of separated species. The fractions of non-polar lipid were analyzed using previously developed [2, 3] non-aqueous RP-HPLC. Lipids were separated into individual lipid species according to the acyl lengths and number of double bonds. Individual lipid species, their fatty acid composition and position of fatty acyls on the glycerol skeleton was identified using mass spectrometry. Electrospray was used for the identification of polar lipids and atmospheric pressure chemical ionization for non-polar lipids. Off-line coupling of

HILIC and RP-HPLC modes enables the separation of extremely high number of lipid species.

Acknowledgments

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Thermodynamic studies of interactions between selected anesthetics and molecularly imprinted polymers

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Keywords
liquid chromatography
molecularly imprinted polymers
solid phase extraction
van't Hoff equation

Presently the molecularly imprinting is one of the most developing method in sample preparation due to its usefulness in a wide range of applications. The large interest of molecularly imprinted polymers has progressed mainly in chemistry and biology. Molecularly imprinted polymers are synthetic and highly crosslinked polymers prepared in presence of specific analyte, called template [1, 2]. In the presence of the template there are creating special binding sites, tailor-made by the copolymerization of functional and crosslinking monomers. After a polymerization the print molecule is removed leaving three-dimensional cavities. These binding sites are complementary to the template and may recognize only one structure or group of structures on which was designed [3].

Molecularly imprinted polymers possess many advantages – they are very selective and sensitive materials, they have highly mechanical strength, durability to heat, pressure or aggressive chemicals (such concentrate bases, acids or organic solvents), their preparation is simply and cheap. However, the lack of universal method for molecularly imprinted polymers preparation, their insolubility and presence of non-imprinted cavities makes problems [4–7].

Molecularly imprinted polymers have an application as sorbents, stationary phases, synthetic receptors or drug delivery systems in liquid chromatography, solid-phase extraction, solid-phase microextraction, capillary electrophoresis, capillary

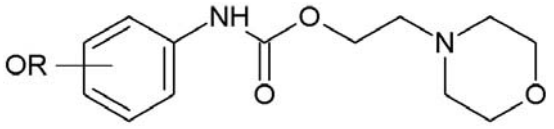
electrochromatography or in chemical sensors. They are using to separation, extraction, catalysis or adsorption of drugs, biomolecules and metals from different complex matrices [8–12].

In order to fully cognition of interactions occurring between the target molecule and the molecularly imprinted polymer the thermodynamic studies are needed. Calculated values of entropy and enthalpy should explain types of binding mechanisms which are taking place during the sorption processes on the molecularly imprinted polymer. Thanks to HPLC analysis of target molecules – potential local anesthetics – morpholinoethyl esters of alkoxysubstituated phenylcarbamic acids (Fig. 1) in different temperatures using molecularly imprinted polymers as stationary phases and calculation of van't Hoff plots the investigation of mentioned interactions is possible. The van't Hoff plots were calculated using following equation

$$\ln k = -\frac{\Delta H^{\circ}}{\boldsymbol{R}T} + \frac{\Delta S^{\circ}}{T} + \ln \varphi \qquad (1)$$

where k is retention factor for the solute, ΔH° is the standard partial molar enthalpy of transfer, ΔS° is the standard partial molar entropy of transfer, $\boldsymbol{R}$ is the gas constant, T is the absolute temperature and φ is the phase ratio (the volume of the stationary phase, V_S, divided by the volume of the stationary phase, V_M) [13].

The aim of presented work was the synthesis of suitable molecularly imprinted polymer with morpholinoethyl ester of methoxysubstituated phenylcarbamic acid as a template, study of the temperature influence on molecularly imprinted polymers binding properties in different solvents, calculation of changes of enthalpy and entropy during sorption processes and statistical evaluation of obtained results.



R = 2 –CH_3, 3 –CH_3, 4 –CH_3,
2 –$C_{10}H_{21}$, 3 –$C_{10}H_{21}$, 4 –$C_{10}H_{21}$

Fig. 1. Structure of morpholinoethyl esters of alkoxysubstituated phenylcarbamic acids used as templates and target molecules.

Acknowledgments

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Detection of biologically important substances using *in-situ* spectroelectrochemistry

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Keywords
copper substrates
in-situ spectroelectrochemistry
surface-enhanced Raman scattering

Surface-enhanced Raman scattering (SERS) spectroscopy is a technique suitable for detection of low amount of various analytes. An effect of surface-enhanced signal was discovered in 70s of 20th century. The effect was observed by Fleischmann *et al.* in 1974 [1] on rough silver electrode without any suggestions on the mechanisms which caused the extremely intense signal. Nowadays it is accepted that the Raman scattering is enhanced by two mechanisms [2]: *(i)* electromagnetic, which is based on local plasmon resonance on "rough" surface of metal and *(ii)* chemical/charge-transfer mechanism based on formation of "surface complex". Surface-enhanced Raman scattering spectroscopy is a detection technique suitable for analysis of molecules with different functional groups adsorbed on specially prepared metallic (nano)materials [3]. The most used substrates are based on silver [4], gold [5] or copper [6, 7]. To achieve a high contribution of electromagnetic mechanism [8] is necessary to prepare appropriate morphology of the individual surface [9]. The surfaces have to be roughened in nanometer to micrometer range [10] considering the wavelength of radiation used for SERS excitation. Several different experimental techniques for generation of SERS substrates have been developed recently. For example the roughness can be achieved by electrochemical roughening of metal electrodes. Copper is less used material for SERS spectroscopy in comparison with silver and gold, but we have demonstrated that copper substrates can exhibit similar enhancement factor [11]. From the chemical point of view copper is more reactive than the other SERS active metals. Thus chemisorption is more probable on copper for a broad range of analytes. In

fact SERS spectroscopy on silver and/or gold has been applied for various analytes as biologically important substances either phytogenic [12] ones or pharmacological compounds as analgesics [13]. Generally, the analytes can be adsorbed to copper with appropriate morphology and several studies of interactions [14] and sorption of a few substances on copper surfaces have occurred [15,16]. Intensity of bands with respect to used potential during oxidation-reduction cycles has been studied on copper surfaces rarely [17, 18]. Furthermore, we should notice that in-situ measurements allow studying of processes in interface liquid/surface performed on silver and platinum [19, 20].

To study *in-situ* processes on copper substrates we designed a special spectro-electrochemical cell. It allows changing of applied potential in three-electrode arrangement and measurement of SERS spectra for individual analytes adsorbed onto copper surface of target/electrode. Both the contribution of electromagnetic mechanism to the SERS enhancement and the adsorption of the species depend on the potential applied. We can propose that analytes with different functional groups adsorb onto the surface in different ways. In this study we show for several compounds that intensities of analytes bands in SERS spectra depend on applied potentials.

The special spectroelectrochemical cell (Fig. 1) was designed and made at our department. The Teflon cell contained three-electrode arrangement. The working electrode was copper coated platinum target, the auxiliary electrode was platinum plate and the cell was equipped by salt bridge for any referent electrode. All potentials were reported with respect to a saturated argentochloride electrode.

Fig. 1. Special spectroelectrochemical cell.

First the platinum target was coated by copper in two-electrode arrangement from ammoniac bath containing $[Cu(NH_3)_4]^{2-}$ ions with a counter sequence from 10 mA to 50 mA with step 10 min. Morphology of surface was modified by oxidation-reduction cycles in the cell in 0.1 M KCl from –1000 mV to 0 mV (scan rate 20 mV s^{-1}).

During *in-situ* spectroelectrochemical measurement the electrolyte (aqueous solution KCl) contained dissolved analyte (β-carotene, chlorophyll, lutein, vitamins B, *etc.*) at low concentration and spectra of the analytes were recorded.

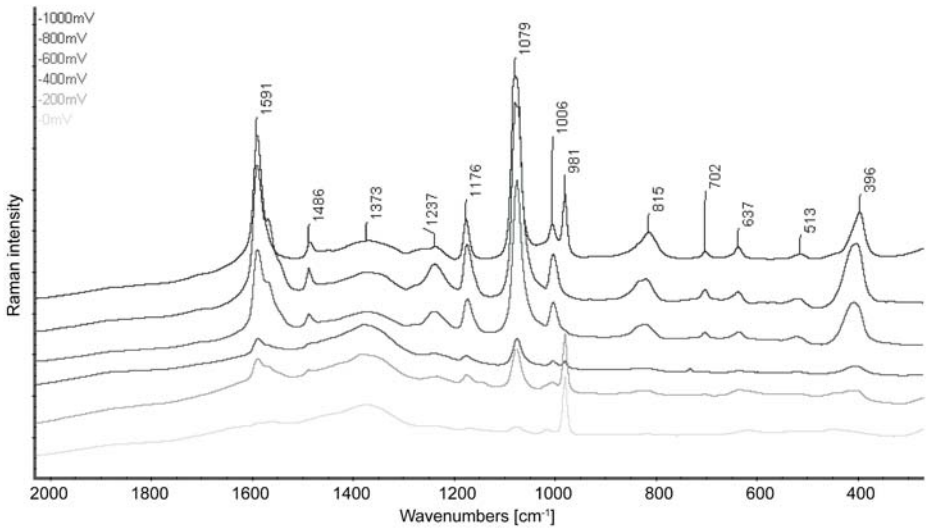

Fig. 2. *In-situ* surface-enhanced Raman scattering spectra of 4-aminobenzenethiol at increasing negative potential.

The Raman probe of Raman spectrometer Dimension P2 ($\lambda_{exc.}$ = 785 nm) was connected to the cell. The laser beam irradiated the surface of target through glass window and it was focused using a micrometre positioning device. The potential was changed using potentiostat accordance with parameters during oxidation-reduction cycles. The SERS spectra were accumulated for each applied potential.

Intensities of analytes bands depend significantly on applied potential. Generally intensity of bands increases with increasing value of negative potential (Fig. 2). But acetaminophen does not adsorb onto the surface at increasing negative potential. It is observed that if analyte contains good adsorbing functional group (as thiol) irreversible chemisorption is occurred. The reversibility of adsorption cycling the potential indicates physisorption.

As summarized combination of SERS spectroscopy and electrochemistry enables variation of applied potential and measurement of Raman spectra depending up potential values. Enhancement conditions change with variation of potential and the sorption of individual analyte differs for different potentials. We can detect substances which are adsorbed on metal surface by both chemisorption and physisorption. To tune the adsorption of analytes we have developed instrumentation which enables combination of Raman spectroscopy with potentiostatic technique. We demonstrate that intensities of Raman bands depend on applied potential. At the present time we record in a few minutes reliably high-quality SERS spectra at bulk concentration to 10^{-5} mol L^{-1}.

Acknowledgments

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Packing of capillary columns for HPLC

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Keywords
capillary chromatography
column packing

Capillary chromatography is a widely used method because of its many advantages. Among them are its environmental friendliness due to low solvent consumption, small amounts of samples required and also its easy coupling with advanced detection methods, such as mass spectrometry. A broad selection of commercial columns is available today, but there are still some benefits in packing of one's own columns. Self packed column can be customized to the given separation problem and the packing process is quite simple and cheap.

There are several techniques that can be employed to prepare a capillary column. Very popular these days are monolithic columns. Their bed is made of a single block of polymere, whose properties are determined by the exact composition of the polymerization mixture. Monolithic bed does not require any frits, as it is bound to the column wall [1]. Therefore it is usually used for capillary electrochromatography, where a frit's dead volume could worsen the separation efficiency. Short piece of monolith, imobilized in the capillary, can also act as an inlet or outlet frit for other type of column [2].

Conventional particle stationary phase beds can be prepared by three similar techniques based on filtration process. Stationary phase particles are pushed into an empty column by packing media under high pressure and retained inside by the column's outlet frit. Packing media can be gas, liquid or supercritical fluid carbon dioxide. The easiest of these methods is packing using liquid, usually called slurry packing, because the stationary phase particles are in the form of slurry [3]. Although slurry packing requires only an isocratic pump and some kind of slurry reservoir, the resulting columns are of high quality and slurry packing is even used for the preparation of HPLC chips [4]. In spite of the simplicity of the setup, there is a number of parameters that can influence the resulting column performance. While several parameters, for instance solvents used [5], have already been studied, most of them, such as packing pressure or

use of ultrasound, are usually selected by trial and error and their exact influence is uncertain.

There are several other types of stationary phases, for instance gel phases like Sephadex. New possibilities emerges with self assembly molecules. They are monomers which can reversibly polymerize into a gel-like substance [6, 7]. As they can be prepared with basically any function group required, they have the potential of becoming next step in tailor made column preparation. Currently, their usefulness as a stationary phase for capillary columns is being evaluated.

Acknowledgments

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Determination of glyphosate in drinking waters on an electrophoretic chip

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Keywords
chip electrophoresis
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glyphosate
pesticide

The aim of this work was an optimization of electrophoresis separation conditions for determination of the most widely used pesticide, glyphosate in drinking waters using a column-coupling chip with the conductivity detection. Capillary zone electrophoresis separations (CZE) on-line combined with isotachophoresis (ITP) sample pre-treatment were performed in a hydrodynamically closed separation system with suppressed electroosmotic flow on the column-coupling chip. Isotachophoresis performed in the first separation channel was used as a very effective concentration and injection technique for fast CZE resolution and detection of glyphosate performed in the second separation channel. The column-coupling chip was provided with two injection channels of 0.9 a 9.9 μL volumes while total volume of both ITP and CZE separation channels was 9.3 μL. The column-coupling chip with enhanced sample loadability (a 9.9 μL volume of the injected sample) was used to reach extremely low limit of detection for glyphosate following the US Environmental Protection Agency, which determined the maximum concentration level of glyphosate in drinking waters to a 700 μg/L concentration [1].

Isotachophoresis separations were performed at low pH (pH of leading electrolyte was 3.2) and provided very favorable sample clean-up, whereas CZE separations performed at higher pH (pH of background electrolyte was 6.1) afforded quick resolution and detection of glyphosate on the column-coupling chip. Isotachophoresis – capillary zone electrophoresis analysis of model and real samples (spiked drinking waters) allowed very good short-term (one day) and long-term (five days) repeatabilities of qualitative (0.2–3.2% RSD of migration time) and quantitative (0.6–6.9% RSD of peak

areas) parameters for glyphosate. Limit of detection for glyphosate was estimated at 20 μg/L concentration on column-coupling chip using a 0.9 μL volume of the injection channel. Recoveries of glyphosate in water samples spiked with 100–700 μg/L concentrations of the analyte varied in the range 102–106%. Degassing (by ultrasound) and proper dilution (1:1 in terminating electrolyte solution) of drinking waters were the only pre-treatment steps before the ITP-CZE analysis.

An influence of higher sample injection volume on ITP-CZE separation parameters of glyphosate in drinking waters performed on the column-coupling chip with enhanced sample loadability (volume of injected sample was almost the same as the volume of the separation channels on the column-coupling chip) was monitored. Injection of a 9.9 μL volume of the sample on the column-coupling chip did not cause decreasing the separation efficiency and/or a loss of resolution of glyphosate. In addition, its migration position stayed relatively clean in CZE step of ITP-CZE combination (Fig. 1). These indicate a very effective sample clean-up of isotachophoresis step in ITP-CZE combination on the column-coupling chip. The estimated limit of detection for glyphosate was 2.7 μg/L using a 9.9 μL volume of injected sample. Recoveries of glyphosate in spiked water samples (10–100 μg/L) were in the range of 99–119%.

The developed isotachophoresis – capillary zone electrophoresis method is very useful for simple and, at the same time, fast determination of glyphosate in drinking

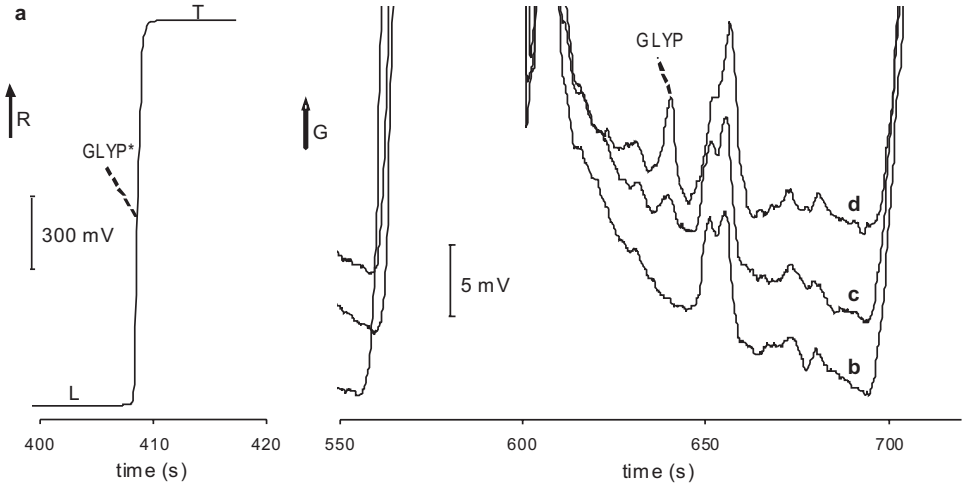

Fig. 1. Isotachophoresis – capillary zone electrophoresis separations of glyphosate in spiked drinking water on the column-coupling chip. The (a) isotachophoresis, and (b, c, d) capillary zone electrophoresis stages of ITP-CZE combination were performed on the column-coupling chip with conductivity detection. A 9.9 μL volume of the sample was injected into the chip containing (a, b) tap water diluted 1:1 with 50% terminating electrolyte solution; (c) the same as in (b) with addition of 10 μg/L glyphosate; (d) the same as in (b) with addition of 50 μg/L glyphosate. Driving current was stabilized at a 20 μA in both isotachophoresis and capillary zone electrophoresis channels. GLYP* – a migration position of glyphosate in the isotachophoresis stack; G – conductance.

waters (ca. 10 minutes total analysis time) without additional time-consuming sample pre-treatment. Simultaneously, the column-coupling chip with enhanced sample loadability allowed determining ca. 100-times lower concentration of glyphosate in drinking waters as proposed by regulation of US Environmental Protection Agency.

Acknowledgments

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Discrimination of botanical origin of honeys based on their GC×GC

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Keywords
comprehensive gas chromatography
honey
volatile organic compounds

Honey is produced by honey bees from nectar of plants, as well as from honey dew. Some of the components (carbohydrates, water, traces of organic acids, enzymes, amino acids, pigments, pollen and wax) are created during maturation process, some are added by the bees and some of them are derived from the plants. Honey from the same regions can vary due to seasonal climatic variations or due to different geographical conditions. In addition to the definition of honey according to the *Codex alimentarius* (1981), there are also other definitions in the regulations of various countries and also within the EU. Various physical types of honey (pressed, centrifuged, drained) and their forms (comb, chunk, crystallized or granulated, creamed, heat processed) are available on the market. Raw honey contains extraneous matter, such as pollen, traces of wax, variable amounts of sugar-tolerant yeasts, and probably crystals of dextrose hydrate. The most honey undergoes crystallisation in time unless some preventive actions are applied. Thus the processing of honey includes controlled heating to destroy yeasts and dissolve dextrose crystals, combined with fine straining or pressure filtration. Honey is usually warmed to a temperature of 32–40°C in order to decrease its viscosity, which facilitates extraction, straining or filtration. This temperature is similar to that in beehives and does not affect the honey quality during the relatively short processing period. However, some honeys are heated to higher temperatures followed by liquefaction or pasteurization [1].

Except of major constituents each type of honey contains also minor components that usually come from given flowers used in honey production. Thus we can expect different composition of compounds that are responsible for characteristic aroma perception of various flowers. The aroma profile is one of the most typical features that

influences overall organoleptic quality and consequently also botanical origin of honey. It is well known that aroma profiles can serve as a 'fingerprint' of the product, and could be used for estimation of botanical origin of honeys and also their quality. Rape together with sunflower is one of the most common agricultural plants in Slovak Republic and honey from it is very popular. The second most frequently honey that occurs on market is made from acacia. The rape and acacia are blossoming in the same time, so often nectar collected by bees is mixed together that produces mixed so called multifloral honey. In this work a volatile organic compounds from twenty acacia honey and five rapeseed honey samples from various European countries (Slovakia, Italy, Ukraine, Czech Republic, Hungary, Croatia, Poland and Germany) were extracted by SPME. A comprehensive gas chromatographic separation system LECO PEGASUS IV equipped with TOF MS detector was used in those experiments. Volatile organic compounds were extracted using solid phase microextraction with DVB/CAR/PDMS fibre using Gerstel MPS2 autosampler.

In all honeys samples were analyzed following compounds: hydrocarbons; alcohols; aldehydes; ketones; acids; methyl, ethyl esters; ethyl esters; terpenes; benzene derivatives; heteroatom compounds. Using GC×GC-TOF-MS, we found differences in the composition of rapeseed and acacia honey. The acacia honey, we set compounds 3-heptanone, 3,4-dimethyl-3-hexanol, 3-ethyl-3-heptanol, (*E*)-2-hexen-1-ol, dihydrolinalool, 1-methylpyrrolidinone, (*E,E*)-2,4-*d*ecadienal. This compounds are analyzed only samples acacia honeys. We are analyzed compounds which absent in samples acacia honeys: 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, 2-butoxyethanol, acetic acid, 2-methylpropanoic acid, 2,2-dimethylpropanoic acid, carvone, 1-phenylethanol, hexanoic acid, guaiacol, 2-ethylhexanoic acid, octanoic acid, eugenol, *p-v*inylguaiacol.

The obtained two dimensional chromatograms were treated by statistical package included in ChromaTof software to compare honey samples based on presence/absence of particular peaks and their areas. Finally, a statistical compare was utilised to define the volatile organic compounds profile with potentially significant class differences between acacia and rapeseed honeys. The statistical compare operation is based on calculation of Fisher ratios [2] which calculate difference of the analyte means from different classes divided by the difference of the analyte variance between different classes. The numerical value of the Fisher Ratio is related to the degree of variance by the size of the number. The higher the Fisher Ratio numerical value is, the greater the class variance is for a parti-cular compound. The volatile organic compounds were ordered according to calculated fisher ratios. This dataset serves as an input for additional PCA and LDA analysis that allows us to distinguish honey samples based on their botanical origin.

Acknowledgments

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Study of the mechanistic aspects of separation of enantiomers of alcohols on the 6-*tert*-butyldimethyl-silyl-2,3-di-alkyl α- and ß-cyclodextrin type stationary phases

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Keywords
alcohols
capillary gas chromatography
cyclodextrines
diols

The world around us is chiral, although it may not be seen at first sight. Chirality can be find not only in molecules but also in macroscopic objects. Just look at own hands, according to which chirality is named. A large number of compounds that make up the living world are chiral. Without these substances living organisms could not exist, and also metabolic processes could not run. Very important chiral compounds are carbohydrates. Carbohydrates with water make up almost 90% of the volume of honey, one of the most important natural products. The remaining 10% is a colorful variety of organic compounds which are also inseparable part 1-alcohols and 2-alcohols that create a distinctive taste and aroma of natural products. Poor handling of honey, e.g., storage of honey in the broad containers, or its overheating, can cause a lost of characteristic flavor, due to volatility of alcohols. Although enantiomers, thus also enantiomers of 2-alcohols and 3-alcohols, have the same physical and chemical properties in the presence of other chiral substances may react differently. This is the assumption on the distribution of a mixture of enantiomers individual stereoisomers. This feature can also cause different biological activities of individual enantiomers and their different behavior in the environment.

In this work, the separation of enantiomers of alcohol, diols on the 6-*tert*-butyldimethylsilyl-2,3-di-alkyl α- and β-cyclodextrins stationary phases has been studied.

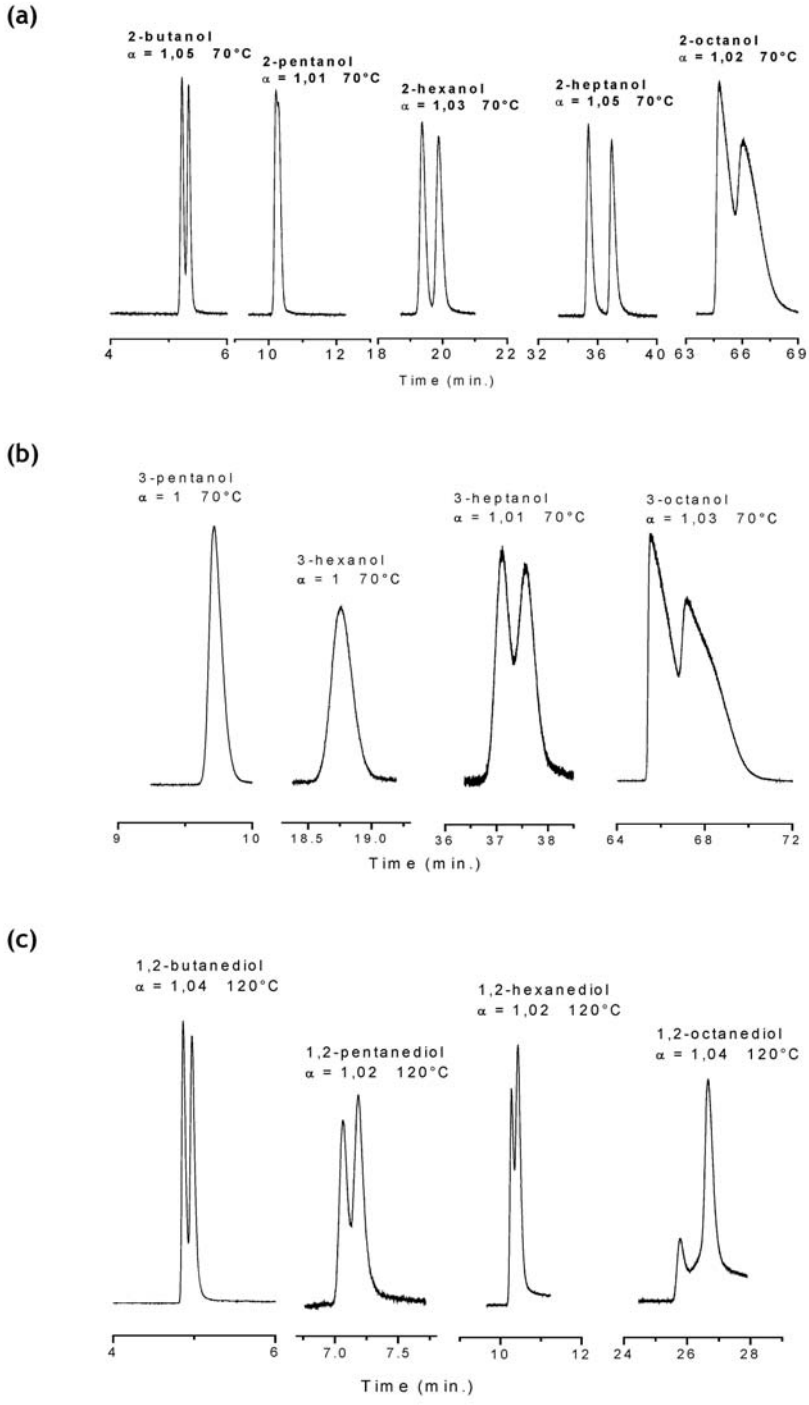

Fig. 1. Separation of enantiomers of (a) 2-alcohols, (b) 3-alcohols, and (c) 1,2-diols (C) on 6-*tert*-butyl-dimethylsilyl-2,3-di-ethyl-β-cyclodextrin type stationary phases.

The mechanistic aspects of separation of enantiomers of alcohols and the chiral recognition process on the two cyclodextrin type stationary phases were investigated using gas chromatography. The chiral recognition process on cyclodextrin stationary phases is mainly influenced by the steric parameters of stationary phases and the structure of enantiomers.

Substitution of hydrogen atoms in the –OH group bound to 6 carbon atoms *tert*--butyldimethylsilyl group in cyclodextrin molecule significantly alter its conformation, which contributes to enantioselectivity of such derivatives. Additionally, in this also the effect of structure of linear alcohols with –OH group attached on 2nd or 3rd carbon atom (C4–C9), and 1,2-diols (C4–C8) on the resolution of their enantiomers on two β- and γ-cyclodextrin derivatives was studied in details. In performed experiments the effect of prolonging the alkyl chain in 2- and 3-alcohols while simultaneously their retention was compared to non-chiral 1-alcohols. Enantiomer separation of 2-alcohols were also compared with the separation of enantiomers of chiral 1,2/diols, thus evaluating the effect of introducing additional –OH group on the quality of enantiomeric separations was also evaluated. It was found that the *tert*-butyldimethylsilyl groups in the 6-position of the cyclodextrins influences of cyclodextrin enantioseparation abilities. Superior resolution of studied enantimers was achieved on β-cyclodextrin columns. Finally, the effect of cyclodextrin cavity size on resolution of 2- and 3- alcohols was also studied. The thermodynamic data characterizing overall and enantioselective interactions of the enantiomers with chiral stationary phases and enthalpic--entropic compensation were used to gain more detailed insight into the mechanistic aspects of enantio separation on modified cyclodextrins.

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Imaging mass spectrometry of cuticular lipids of *Drosophila melanogaster*

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Keywords
cuticular lipids
Drosophila melanogaster
lithium 2,5-dihydroxybenzoate matrix
MALDI imaging

Cuticle of most insects, including *Drosophila melanogaster*, has a lot of functions, owing to a thin layer of wax on its surface. One of its functions is mechanical protection against external environment. Cuticle also protects insect against pathogens, UV radiation or can regulate water intake and loss. Cuticular lipids consisting mainly of hydrocarbons and wax esters [1]. Partly there are aldehydes and ketones, alcohols or acids [2, 3]. Proportional incidence and diversity of cuticular lipids are characteristic for the species. Many of these substances are biologically active, it means involved in chemical communication [4].

The spatial distribution of cuticular lipids on the surface (head, thorax, abdomen and wings) of *Drosophila melanogaster* flies has been imaged and studied using MALDI--TOF mass spectrometry and scanning electron microscopy. The experiments were performed both in LDI-TOF and MALDI-TOF mode. In the case of MALDI-TOF experiments the lithium 2,5-dihydroxybenzoate matrix [5] was sprayed on samples using commercial airbrush. Based on scanning electron microscopy images, it was confirmed that the deposits of lithium 2,5-dihydroxybenzoate matrix were homogenous and the salt was form into clusters of crystals (50–100 μm), which were separated from each other by uncovered cuticle surface (approx. 20 μm). The experiments were carried out with intact six days old *Drosophila melanogaster* imagoes.

Attention was paid to female pheromones (*Z,Z*) 7,11-heptacosadiene, (*Z,Z*) 7,11-nonacosadiene and male anti-attractant 11-*cis*-vaccenyl acetate. In addition, distributions of triacylglycerols and other lipids were studied. Special metal MALDI targets were designed for a successful MALDI Imaging of 3-D biological objects . The first one was manufactured with simple cuttings, whereas the second one contained sophisticated profiled males and females cells. These cells ensured correct spatial orientation and fixation of fruit flies in correct height level on the plate, what is crucial for successful imaging of 3-D biological object.

Acknowledgments

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Construction of miniaturised detection cell for voltammetric determination of various analytes at soil extracts

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Keywords
amalgam electrode
miniaturised cell
1-nitropyrene
voltammetry

Detection cell was constructed for achieve portable device for measurements at field research. Also great contribution of this is option of voltammetric determination of various analytes at very small volume of sample which opens wide use for this cell at bioanalytical experiments [1].

As a model analyte 1- nitropyrene was used at arrangement with meniscus-modified silver solid amalgam electrode [2]. At previous studies were determinated nitrated polycyclic aromatic compounds under the various conditions like differrent construction or material of working electrodes and differencies at base electrolytes [2–4]. Therefore no further optimization of this determination was necessary. The only exception was the removal of oxygen, which can not be performed as usual because highly volatile sample in small volume evaporates immediately during standard process of oxygen removal.

Working electrode was placed together with reference (Ag/AgCl/3 mol L^{-1} KCl) and platinum wire auxiliary electrode in miniaturized voltammetric cell, using 120 μL of base electrolyte for measurements. The oxygen was removed from measured solutions by addition of saturated solution of sodium sulphite. Base electrolyte was composed of methanol and Britton Robinson buffer pH =12,0 in the ratio of 7:3.Optimalization of determination was confirmed for DC voltammetry and differential pulse voltammetry. Limits of quantification of 1-nitropyrene were comparable of about 4×10^{-6} mol L^{-1} in

both cases. The methods showed reproducible results with relative standard deviation <3.65% (n = 10).

Procedure mentioned above was the first experiment only. Aim of this study is to built device suitable for field work and for small amounts of biological samples. This is object of future research. Aim of this will be determination of metals which has vital function for microorganisms at soil, potentially for determination of products of secondary metabolism of soil microbes in microcosms and also at field research, too.

Construction of cell was based at vial for HPLC samples where samples is to be placed together with electrodes. Various electrodes can be used at these configuration but under the condition that they are thin enough to fit properly to measuring cell. Working and auxiliary electrode usually poses no problem with their proportions because they can be easily miniaturized. Main problem with miniaturization is with reference electrode that usually can not be miniaturized enough. It is the main reason why least reached quantity of measuring volume was 120 μL.

Vial with electrodes was places into the standard voltammetric cell which fits to Eco-Tribo polarograph at the pilot study. For field use special carrier made of glass will be made. Whole construction is shown in Fig. 1. There is vial with electrodes placed inside where the measure is taken in the middle of the bottom of the cell. Fig. 2 shows how whole apparatus looks like at experimental condition during the pilot studies. After optimization of all factors there will be special glass tube made and vial will be placed inside in removable settings.

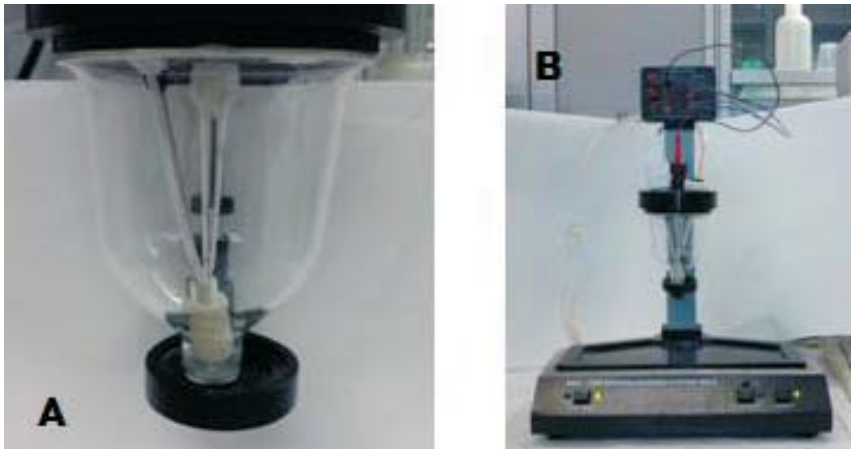

Fig. 1. Miniaturised detection cell for voltammetric determination of various analytes at soil extracts: (a) voltammetric cell with electrodes in vial, (b) complette assembly of measuring cell with Eco-Tribo polarograph.

Acknowledgments

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Comparison of HPLC and capillary liquid chromatography with tandem mass spectrometric detection for analysis of estrogen pollutants

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Keywords
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liquid chromatography
mass spectrometry

A relatively ubiquitous class of global environment pollutants are substances which affect the endocrine system of a wide spectrum of organisms [1, 2]. These substances, called endocrine disruptors, can mimic or antagonize functioning of steroid hormones, disrupt biosynthesis or metabolism of steroids or alter hormon receptor populations [1]. To the wide range of substances with endocrine-disrupting properties belong estrogens. It was reported that these compounds affect ecosystems, e.g., feminization of wild fishes living downstream from wastewater effluent [3, 4]. During the last decades, occurrence of testicular tumors and developmental defects of reproduction organs has increased in human population and the quality of human spermiogram has substantially deteriorated [5, 6]. The presence of estrogen pollutants, along with unhealthy lifestyle, may be one of the reasons. Typical representatives of endocrine disruptors are natural and synthetic hormones, such as 17β-estradiol, 17α-estradiol, estriol, estrone, and the synthetic contraceptives 17α-ethynylestradiol and mestranol. The estrogen concentrations in aqueous environmental samples are at nanogram per liter levels [7–11]. A sensitive, selective, and simple method to monitor these estrogens in water is therefore required.

The main aim of this study was to develop and optimize a method of high performance liquid chromatography and capillary liquid chromatography with tandem mass

spectrometric detection for the determination of selected estrogen pollutants, namely 17β-estradiol, 17α-estradiol, estriol, estrone and 17α-ethynylestradiol.

Standards of estrogens were purchased from Sigma-Aldrich. The HPLC experiments were perfomed using a Series 1200 Capillary Liquid Chromatograph and Series 1200 Liquid Chromatograph with a Triple Quad LC/MS 6460 tandem mass spectrometer (Agilent Technologies) with an electrospray ionization interface (ESI) and with an electrospray ionization interface using Agilent jet stream thermal gradient focusing technology (ESI-JT), which uses super-heated nitrogen to improve ion generation and desolvation, to get higher signal and reduced noise. The principle of ESI-JT is that super-heated nitrogen sheath gas surrounds the nebulizer spray increasing desolvation efficiency. More ions and fewer solvent droplets enter the sampling capillary resulting in higher signal/noise ratio. A Zorbax SB C18 capillary column (150 mm × 0.5 mm, particle size 5 μm, Agilent Technologies), Ascentis Express C8 fused core (4.6 mm × × 150 mm, particle size 2.7 μm, Supelco) and SunFire C18 (4.6 mm × 150 mm, particle size 5 μm, Waters) were tested.

For the MS/MS operation, ESI and ESI-JT were evaluated for determination of estrogens in both positive and negative ion modes. The ESI positive ion mode was more effective for the ionization of these estrogens. MS/MS detection was performed in the selected reaction monitoring. Two most intense characteristic molecular fragments were selected by tuning the values of the fragmentor voltage (from 10 to 350 V) in selected ion monitoring mode and the collision energy (from 10 to 250 V) in product ion mode for each analyte. The optimized conditions for the mass spectrometer were as follows: capillary voltage 5500 V, nebulizer pressure 12 psi, gas temperature 350 °C, gas flow 10 L/min for capillary liquid chromatopgraphy; capillary voltage 5500 V, nebulizer pressure 45 psi, gas temperature 300 °C, gas flow 10 L/min for LC using ESI; capillary voltage 5000 V, nozzle voltage 2000 V, nebulizer pressure 35 psi, gas temperature 350 °C, gas flow 10 L/min, sheath gas flow 12 L/min, sheath gas temperature 400 °C for LC using ESI-JT.

In order to develop a miniaturized capillary liquid chromatography and HPLC procedures for determination of estrogen pollutants three separation columns were selected (see above). A binary mixture of acetonitrile and water, containing 0.1% formic acid was used as the mobile phase. The separation system was optimized under the conditions of isocratic elution. The effect of the acetonitrile content on the retention and separation of estrogens was studied within a range from 25 to 45 vol.%. The degree of ionization of all the five analytes in the positive ionization mode also was examined. The optimum composition of the mobile phase, with respect to attain satisfactory retention of the most polar analyte, estriol, acceptable resolution of all the estrogens and sufficient detection sensitivity, was found to be 38/62 (*v/v*) acetonitrile/water mixture containing 0.1% formic acid for capillary liquid chromatography using Zorbax SB C18

Table 1
Limits of detection in ng/mL obtained for estriol, 17β-estradiol, 17α-estradiol, 17α-ethynylestradiol, and estrone, by capillary liquid chromatography using Zorbax SB C18 capillary column, HPLC using Ascentis Express C8 fused core with electrospray ionization interface (ESI) and electrospray ionization interface jet stream thermal gradient focusing technology (ESI-JT), and HPLC using SunFire C18 with ESI and ESI-JT.

Compound	Zorbax	Ascentis ESI	Ascentis ESI-JT	SunFire ESI	SunFire ESI-JT
estriol	0.68	0.59	1.72	1.21	1.95
17β-estradiol	0.64	0.20	0.18	0.69	0.59
17α-estradiol	0.52	0.20	0.10	0.67	0.32
17α-ethynylestradiol	0.51	0.46	0.59	1.53	1.66
estrone	0.67	0.69	0.85	2.97	2.89

capillary column. The acetonitrile/water ratio (with 0.1% formic acid) had to be shifted to 40/60 (*v/v*) if Ascentis Express C8 fused core column or SunFire C18 column were used in HPLC. The time of analysis did not exceed 13 minutes by capillary liquid chromatography (flow rate 18 μL/min), 10 minutes by HPLC using Ascentis Express C8 (flow rate 0.8 mL/min) and 25 minutes by HPLC using SunFire C18 (flow rate 0.8 mL/min). All the compounds were baseline resolved by HPLC using the both columns, the resolution of hardly separated 17α- and 17β-estradiol was poorer by capillary liquid chromatography.

Under the optimized MS/MS detection and separation conditions for each separation system, calibration curves were measured for all the five estrogens in a concentration range from 5 to 500 ng/mL, and the analytes were tested in a linearity range from limit of quantification of respective analyte to 500 ng/mL. Each measurement of the peak area was carried out in triplicate. The peak height versus concentration dependencies were treated by linear regression, to determine the limits of detection (Table 1) and limits of quantitation. The lowest limits of detection were achieved by HPLC using Ascentis Express C8 with ESI. The lowest baseline noise was achieved by ESI-JT but the signal of analytes was slightly suppressed in contrast to ESI.

The results clearly showed that the most suitable method for the analysis of estrogen pollutants was HPLC using Ascentis Express C8 column with ESI. The separation of the all studied estrogens is shown in Figure 1. The method provided good separation efficiency and very low limits of detection and the time of analysis did not exceed 10 minutes.

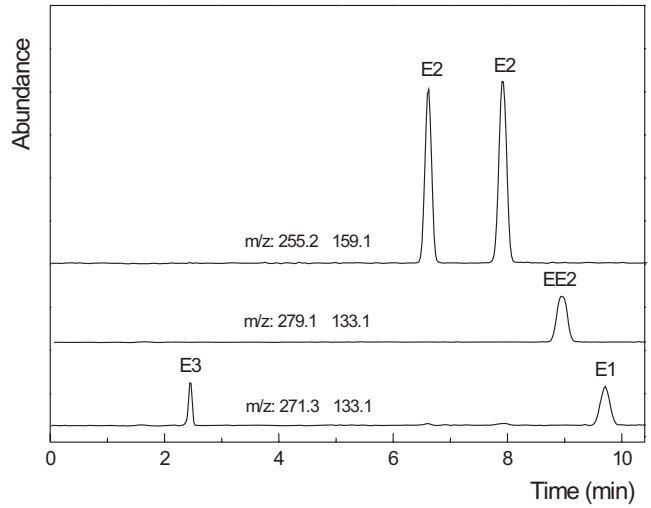

Fig. 1. Selected reaction monitoring chromatograms of 17β-estradiol (βE2), 17α-estradiol (αE2), estriol (E3), estrone (E1), obtained by HPLC using Ascentis Express C8 with electrospray ionization interface (50 ng/mL). Mobile phase: 40/60 (*v/v*) acetonitrile/water mixture containing 0.1% formic acid, flow rate 0.8 mL/min. MS conditions: capillary voltage 5500 V, nebulizer pressure 45 psi, gas temperature 300 °C, gas flow 10 L/min.

Acknowledgments

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GC-MS analysis of termite defensive compounds secreted from their frontal glands

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Keywords
frontal gland
GC-MS
termite defence

The ability of collective defence belongs to the most fascinating features of termite societies. The division of labour, characteristic to all social insects, is manifested in termites by the presence of specialized defenders – the soldiers (Fig. 1). In advanced termite families *Serritermitidae*, *Rhinotermitidae* and *Termitidae*, soldiers are equipped with a chemical weapon, the frontal gland. The chemistry of the frontal gland was studied in detail in soldiers of many termite species [1], revealing thus a fascinating of termite defensive chemicals. Nevertheless, this gland occurs also in termite imagoes

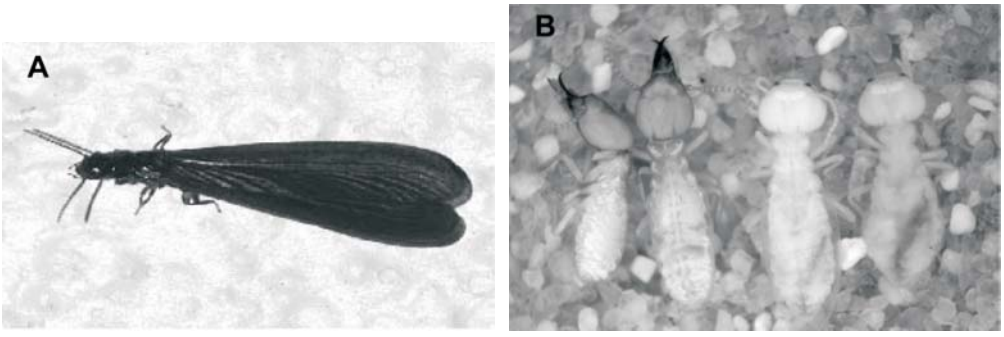

Fig. 1. Termite species *Coptotermes testaceus* (*Rhinotermitidae*): (A) swarming imago, (B) soldiers (left) and workers.

(Fig. 1), future kings and queens. Suprisingly, the chemistry and anatomy of this gland in imagoes was ignored. Therefore, we compared the chemical composition of the frontal gland secretion in termite imagoes and soldiers by means of GC-MS and studied the connection between chemistry and anatomy of this gland in five genera from the families Rhinotermitidae and Serritermitidae.

Colonies of *Psammotermes hybostoma* (*Rhinotermitidae*: *Psammotermitinae*) were collected in Sahara, Egypt in 2010. Colonies of *Glossotermes oculatus* (*Serritermitidae*), *Dolichorhinotermes longilabius* (*Rhinotermitidae*: *Rhinotermitinae*), *Heterotermes tenuis* (*Rhinotermitidae*: *Heterotermitinae*) and *Coptotermes testaceus* (*Rhinotermitidae*: *Coptotermitinae*) were collected in Petit Saut, French Guyana in 2010. Soldiers and imagoes of both sexes were fixed for ultrastructural studies. In both studied castes, extracts of heads and/or abdomens cut into halves were prepared in hexane. We used tens of specimen per sample approximately. Extracts of workers were used as controls. Chemical analysis was performed using gas chromatography – mass spectrometry (GC/MS) technique on quadrupole instrument Thermo Scientific DSQ II with electron ionization (70 eV). For analyses a DB-5 fused silica capillary column has been used (15 m × 0.25 mm, film thickness 0.25 μm). Helium flow was 1 mL/min. Mass range was between 29 and 550. Initial temperature 50°C increased to 320°C, temperature gradient was adjusted for particular species.

A study of anatomy showed that the frontal gland of imagoes is a sac-like organ, smaller in size than that of soldiers, usually confined to the head, except for *Rhinotermitinae*, in which it can reach the abdominal cavity. The secretory epithelium usually consists of class 1 cells, with the exception of *Heterotermes* and *Coptotermes*, in which also the class 3 cells occur. The gland always opens through the frontal pore.

Imagoes of *Glossotermes oculatus* reveal a small frontal gland made by collumnar class 1 secretory cells surrounding a minute reservoir. The secretion of imagoes is not yellow in colour as in case of soldiers and the chemical analysis confirmed significant differences in its composition (Fig. 2). Both castes contain carboxylic acids and aldehydes.

Frontal gland of *Psammotermes hybostoma* imagoes lacks the reservoir, and collumnar class 1 secretory cells are attached directly to the cuticle of the anterior frons. This might explain the absence of any differences between extracts of *P. hybostoma* imagoes and workers (control sample). On the contrary, the frontal gland of soldiers contains a rich blend of compounds, especially sesquiterpenes.

Imagoes of *Heterotermes tenuis* reveal a large frontal gland filling the posterior part of the head capsule. Secretory epithelium consists of class 1 and class 3 secretory cells, and is much thicker in females compared to males. Despite the large size of the frontal gland in imagoes of *H. tenuis*, only few compounds different from workers were detected. The soldier secretion is richer than that of imagoes. In both castes, the gland contains hydrocarbons.

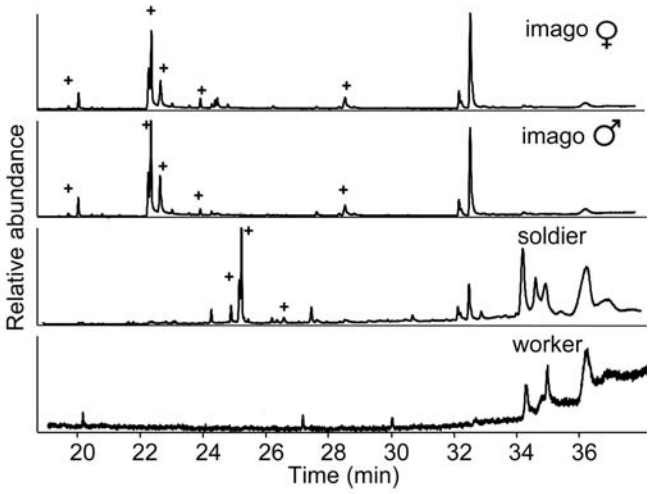

Fig. 2. Comparison of GC profiles of the frontal gland secretion in imagoes and soldiers of termite species *Glossotermes oculatus*; workers – control sample.

Huge frontal gland occurs in *Dolichorhinotermes longilabius*, where it is stretched from above the brain to the third abdominal segment. The thick epithelium consists of class 1 cells only. The frontal gland secretion in imagoes is richer in chemicals than that of soldiers; carbonyl compounds, saturated and unsaturated hydrocarbons and acetates were detected. Quantitive differences exist in some substances common to both castes.

In *Coptotermes testaceus*, the frontal gland is small, squeezed inbetween the brain and mandibular muscles. The secretory epithelium consists of class 1 and class 3 secretory cells. Imagoes and soldiers of *C. testaceus* share numerous compounds. In addition, each caste posseses a few specific compounds lacking in the other, such as carboxylic acids in soldiers and hydrocarbons in imagoes.

We compared the anatomy and chemistry of the frontal gland in imagoes and soldiers of four rhinotermitid and one serritermitid genera. In imagoes, we have detected volatiles in all studied genera, except for *Psammotermes*, in which the gland is very small and lacks reservoir. In some genera, the chemical composition does not differ from that observed in soldiers. Nevertheless, in a few cases, the imagoes posses specific blends of chemicals, including compounds lacking in the soldier caste – such a situation has been previously described also in the rhinotermitid *Prorhinotermes* [2, 3]. These results suggest that the gland in imagoes is not just an unperfect expression of the soldier frontal gland, but rather an organ with special adaptive value shaped by the life history of termite imagoes.

Acknowledgments

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Electrophoretic approaches to analysis of saccharides for enzyme kinetics studies of hexosaminidases

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Keywords
capillary electrophoresis
enzyme kinetics
hexosaminidase
N-acetylamino saccharides

Hexosaminidases are a group of glycosidases that is involved in hydrolysis of *N*-acetylhexosamines in poly- and oligosaccharides. Among other biological roles, hexosaminidases take part in chitinolitic systems, which are used by filamentous fungi for their adhesion to surfaces and also in competition with other fungus species. Study of activity and kinetics of these enzymes is a matter of great scientific interest. However, such studies cannot be performed without qualitative and quantitative analysis of saccharides. Saccharides are rather challenging analytes because their structures often differ only in configuration of their chiral centers. Due to this, chemical properties of different saccharides are very similar. Separation methods, however, are able to distinguish among these compounds on the basis of minute differences in physico-chemical properties. To study kinetic properties of hexosaminidases, determination of oligosaccharides as substrates and intermediates and monosaccharides as products of reaction is necessary. As the monomer and oligomers vary in their size, capillary electrophoresis can be considered a suitable method because electrophoretic mobility is a function of hydrated radius of an ion. However, electrophoretic separation of saccharides is not a trivial task. For capillary zone electrophoresis, ionic analytes are required. With neutral saccharides, this can be achieved by different ways. One way is application of a background electrolyte of a high pH value. In such conditions, hydroxyl groups of saccharides dissociate leaving thus peptides negatively charged. Rovio *et al.* [1] utilized this approach. They also took advantage of formation of sodium enediolate,

which takes place in highly basic solutions. Enediolate exhibits UV absorption at 270 nm which enables UV detection of normally non-absorbing saccharides. To speed up the analysis, small amount of a cationic surfactant can be used which reverses electroosmotic flow so that ions migrate in accordance with electroosmotic flow [2]. Borate-based background electrolytes are another option for electrophoretic separation of saccharides. Borate anion forms negatively charged esters with saccharides. Being negatively charged, esters can be separated using capillary electrophoresis employing simple experimental conditions [3, 4]. Another possibility is derivatization of analytes using charged aromatic derivatization agents [5, 6]. These agents enhance the UV absorbance of analytes and introduce a charged group into analyte molecule.

In this work, applicability of the approaches mentioned above to enzyme kinetics studies is discussed. Four different methods were tested. Namely, background electrolyte of high pH with and without cationic surfactant, borate-based background electrolyte and a method using sample derivatization. Enzymatic cleavage of *N,N′*-diacetylchitobiose and *N,N′,N″*-triacetylchitotriose with β-*N*-acetylhexosaminidase from *Aspergillus oryzae* was studied. In this case, a common problem with saccharides low UV absorbance is partly overcome by the presence of *N*-acetylamino groups which exhibit a moderate UV absorption. Results show that, however high pH background electrolyte really induces absorbance of saccharides at 270 nm, the absorption is not intensive enough at concentration levels required for the enzyme kinetics study. Furthermore, high pH background electrolytes result in extremely high current values which limit possibility of method optimization and adversely affect repeatability. Another disadvantage of this approach, which is connected to the high conductivity of background electrolyte, is limitation of separation voltage to relatively low values. This resulted in 30-minute long analyses. Borate-based buffer and high pH buffer with cationic surfactant provided baseline separations of all three analytes in

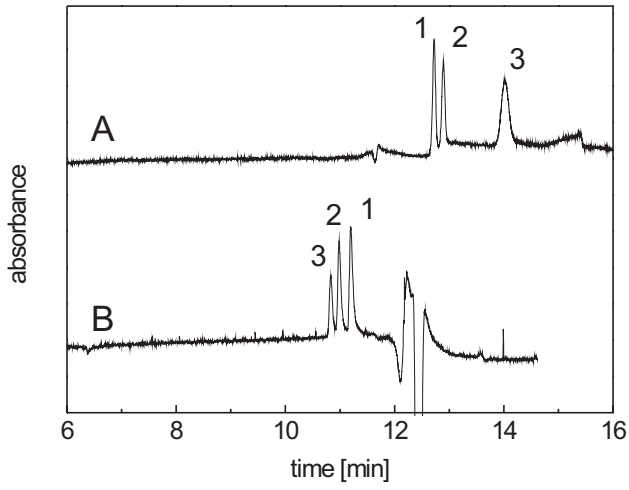

Fig. 1. Separation of chitotriose (1); chitobiose (2); and *N*-acetylglucosamine (3) using two different background electrolytes: (A) 20 mM tetraborate buffer, pH = 10.0, (B) 200 μM CTAB in 4.5 mM phosphate buffer, pH = 11.7.

similar analysis times, as can be seen in Figure 1. However, borate-based background electrolyte offered more reliable results which correlates with its simplicity. Based on the results, conclusion can be drawn that borate-based background electrolyte is the approach of choice where concentrations of analytes are not extremely low. This method was successfully employed to study pH dependence of enzyme activity and to determine preference of different substrates. On the other hand, detection limits of this method, though relatively low, were insufficient for determination of kinetic constants of the enzyme. In the moment, derivatization of saccharides is being optimized to enhance detection sensitivity. Preliminary results will be included in the presentation.

Acknowledgments

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Analysis of amino acids in degradation products of humic substances by RP-HPLC using pre-column derivatization with diethyl ethoxymethylenemalonate

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Keywords
amino acids
diethyl ethoxymethylenemalonate
humic acids
RP-HPLC

Humic substances are complex, amorphous, hydrophilic macromolecules, largely widespread in the environment, such as in soil, natural waters and sediments [1, 2]. Soil humic acids arise mainly due to (bio)chemical degradation of plant and animal residues and also from microbial synthetic activity. Humic substances constitute a significant fraction of the soil organic matter (about 20% of the total soil organic matter) [1].

Approximately 20–50% of total nitrogen occurring in soil organic matter was found in a form of amino acids, which are one of the most important natural compounds. The amino acids participate in the formation of peptide bonds, which can be release by acid hydrolysis [3, 4]. Majority of amino acids occurs in soils in bound form, e.g., in huminopeptide structures. They are usually bound to the central core (nucleus) of humic acids or fluvic acids, thus protected from fast degradation by microorganisms [5, 6].

Liquid chromatography belongs to the most employed analytical methods for determination of amino acids. However, the limits for direct detection are drawback of this method because usually amino acids do not exhibit fluorescence and only a few of them contain structural moieties with chromophores. For this reason, the most of the chromatographics methods designed for this purpose implement pre-column or post-column

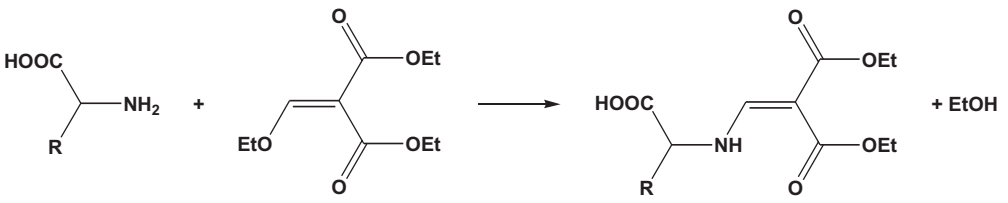

Fig. 1. Reaction scheme of amino acids with diethyl ethoxymethylmalonate [9].

derivatization. The necessary chromophores or fluorophores enabling spectrophotometric detection in UV region of the spectrum or fluorimetric detection are incorporated by the use of suitable derivatization steps [4, 7].

Several methods for amino acid pre-column derivatization were developed. Usually, this derivatization step results in creating the molecules with well-defined spectral properties, such as dansyl chloride, *o*-phtalaldehyde, phenyl isothiocyanate [8], diethyl ethoxymethylmalonate [9] under well defined conditions.

Based on the available information, it seems that diethyl ethoxymethylmalonate is the most suitable derivatization agent for amino acids and compounds with a primary or secondary amino groups (Fig. 1) [9]. Products of this derivatization reaction are highly stable and offer characteristic spectra in ultraviolet region with maximum at 280 nm [9].

Analyzed humic acids samples were decomposed by hydrolysis with 6 mol L^{-1} hydrochloric acid at 110 °C. Degradation products of humic acids were derivatized by diethyl ethoxymethylmalonate. Chromolith performance chromatography column RP-18e 100-4.6 was used for gradient separation of samples and standards of amino

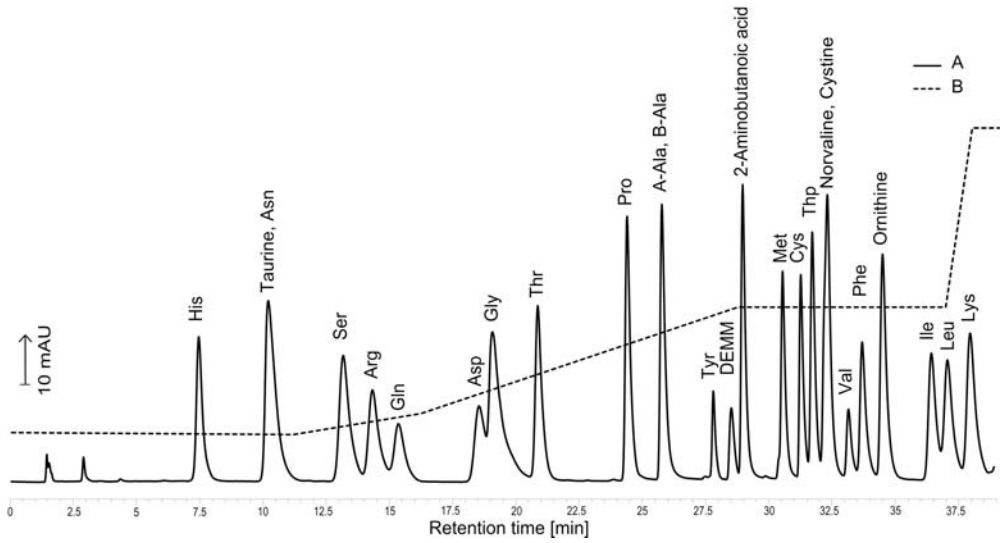

Fig. 2. Chromatographic profiles (UV 280 nm) of mixture of diethyl ethoxymethylmalonate derivatized 25 amino acids standards obtained by gradient elution (A), and shape of gradient curve (B,) see dashed line on the chromatogram.

acids hydrolysates. Following chromatographic conditions were employed: gradient elution by mixing 5 mmol L^{-1} sodium dihydrogen phosphate solution (pH = 3.0) (A) and methanol (B); a 20 μL injected volume; thermostated column at 35 °C with 1 mL min^{-1} flow rate. Under these chromatographic conditions, separation of twenty-five derivatized amino acids was achieved in 43 min (Fig. 2).

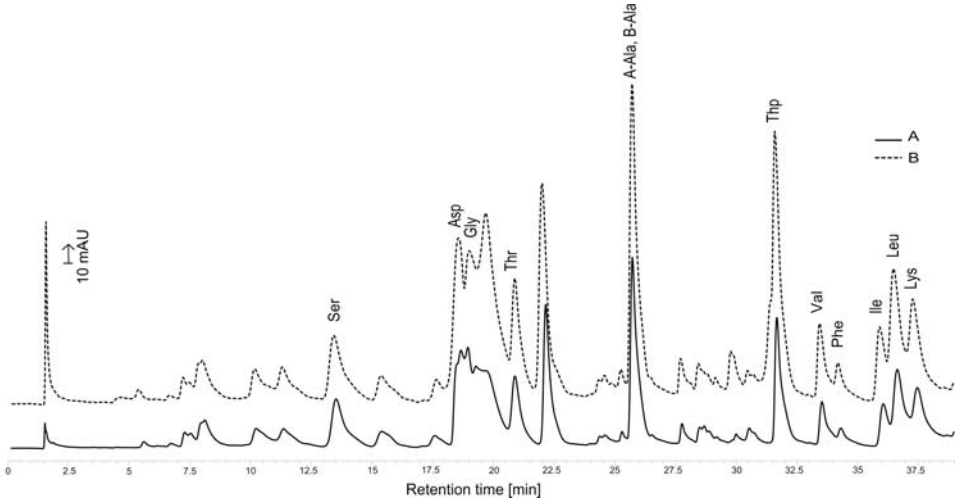

Fig. 3. Chromatographic profiles (UV 280 nm) of diethyl ethoxymethylmalonate derivatized hydrolysates of peat humic acids: (A) from locality Cerova, and (B) from locality Suchá hora.

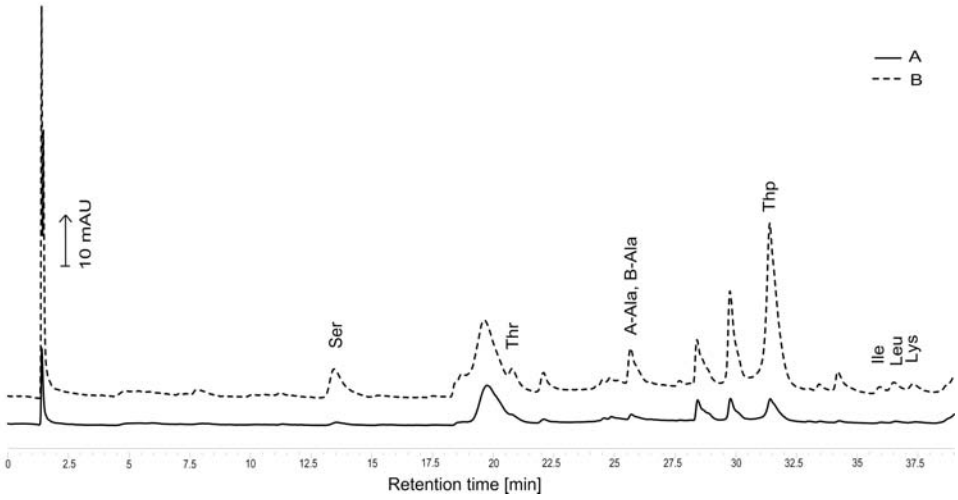

Fig. 4. Chromatographic profiles (UV 280 nm) of diethyl ethoxymethylmalonate derivatized hydrolysates of humic substances: (A) commercially available standard of humic acids from Sigma-Aldrich, and (B) humic acids preparative with high content of humic acids from Ekohum (Senica).

A majority of the analyzed samples consisted of proteogenic amino acids. Serine, glycine, alanine and tryptophan were the most prevalent amino acids in all examined samples of humic acids degradation products (Ekohum, Aldrich, Sucha hora, Cerova) (Fig. 3 and 4).

Acknowledgments

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Methodology of lipid analysis of vernix caseosa using MALDI-TOF MS

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Keywords
lipids
MALDI-TOF MS
TLC separation
vernix caseosa

Vernix caseosa is a white or yellowish creamy substance which covers the skin of the fetus and the newborn as shown in the Fig. 1. It is produced by the fetus itself during the third trimester [1]. In utero, vernix protects the fetus against maceration and helps the skin and other organs to mature properly [2]. During the birth it works as a lubricant and

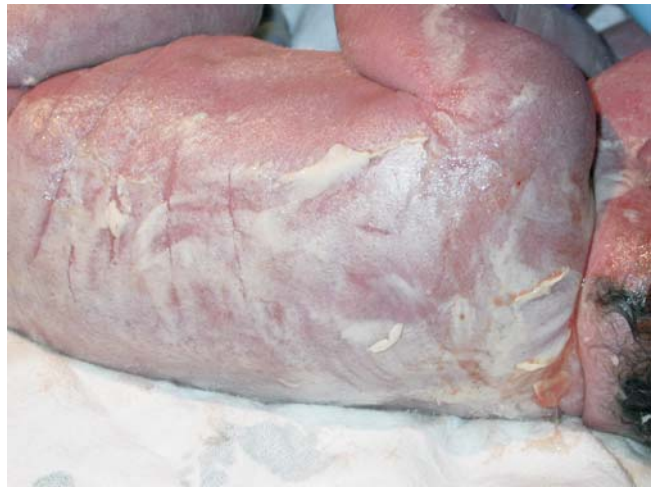

Fig. 1. Vernix caseosa covers the body of a newborn immediately after the delivery. (Source: http://dermatlas.med.jhmi.edu/derm/indexDisplay.cfm?ImageID=1080184503)

after birth, it provides the newborn the first immunity and protects the skin against desiccation [3].

Vernix caseosa consists of 80% water [4], which is enclosed in shed cells from the stratum corneum, 10% proteins [5], which affords the first immunity system for the infant, and 10% lipids. The lipid part is a very rich mixture of various lipid classes of a wide range of polarity: from the non-polar squalene to very polar phospholipids [6].

There were several attempts to contrive a proper substitution of vernix in order to protect the newborns who lack it [7]. However, it is not very easy to retain the functions of vernix. It is highly important to study vernix thoroughly. It is also useful to analyse each sample separately to find possible differences between the sexes, among races or gestational ages.

Both, chemical and biological properties have been studied so far using various methods of separation and detection. For lipids, mostly, TLC separation was utilized followed by transesterification and GC-MS/FID or HPLC-MS detection [8]. MALDI--TOF MS has never been used for vernix caseosa lipid research. This method enable to detect the intact molecules without previous transesterification or other modifications.

This project is aimed to lipid analysis of intact molecules and optimization of the MALDI-TOF MS method for these lipids [9]. First, lipid isolation has been optimized. TLC preparative separation has been processed on unconventional glass plates of size 9 × 12 cm, covered with a silicagel in a thick layer. For visualization a solution of rhodamine in ethanol has been used followed by a UV detection.

Table 1

The matrices that are suitable for each lipid component of the vernix caseosa. Spectra quality: +++ very good, ++ good, + visible (positive ion mode); – – good (negative ion mode). Matrices used for further measurements of particular lipid classes are marked with rounded squares.

	2,5-dihydroxybenzoic acid	sodium 2,5-dihydroxybenzoate	lithium 2,5-dihydroxybenzoate	2-mercapto benzothiazole	tetracyanoquinodimethane	4-nitroaniline	picolinic acid	proton sponge (1,8-bis(dimethylamino)naphthalene)	2,4,6-trihydroxyacetophenone
squalene	++		(+++)		+++		++		
wax esters		++	(+++)			+			
cholesteryl esters		++	(+++)			+			
diol diesters		+++	(+++)						
cholesterol	(– –)								
triacylglycerols	+++	(+++)		+++	++	+	+++		++
diacylglycerols	+++	(+++)		+++	+	++	++		++
monoacylglycerols	(+++)	+							
free fatty acids		++			++		+++	(– –)	
phospholipids	++	++		(+++)	++				+++

As for the MALDI-TOF measurement, many different matrices have been tested on standards in order to find a proper one for each lipid fraction from the TLC separation [10]. The results of the matrix optimization are displayed in the Table 1. Secondly, various ways of sample deposition have been examined and an optimal one has been found for each of the lipid classes. And finally, suitable laser intensities have been determinated.

Acknowledgments

We greatfully acknowledge the financial support from the Czech Science Foundation (project No. GA203/09/0139), the Grant Agency of the Charles University in Prague (project No. SVV 261204) and the Ministry of Education, Youth and Sports, Czech Republic (projects No. MSM0021620857 and No. RP 14/63). We also thank to the staff of the Department of Obstetrics and Gynecology for the vernix samples.

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Lipidomic profiling of patients with cardiovascular diseases using GC/FID and HPLC/MS

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Keywords
cardiovascular diseases
fatty acids
gas chromatography
lipids
UHPLC/MS

Lipids are important compounds in the organism due to their wide range of biological functions. Disruption of lipids metabolism can lead to serious diseases, i.e., diabetes mellitus, cardiovascular diseases, cancer, Alzheimer's disease, obesity, etc. Monitoring of the lipid composition can lead to distinguish patients with cardiovascular disease and healthy individuals. The goal of this work is the lipidomic profiling of erythrocytes, plasma and lipoproteins samples (very-low-density, low-density and high-density lipoproteins). Differences in lipid composition are determined based on changes in fatty acids and lipid classes profiles. The analysis of lipid classes is performed with ultra high-performance liquid chromatography – mass spectrometry (UHPLC/MS) using hydrophilic interaction liquid chromatography (HILIC) based on their polarity. The gas chromatography with flame – ionization detection (GC/FID) is used for the analysis of fatty acids profiles as fatty acid methyl esters. Fatty acid methyl esters are separated according to carbon number, number and location of double bonds – retention time increase with increasing acyl chain length and a number of double bonds. Fatty acids composition and composition of lipid classes is different among individual samples. In total, 35 fatty acids and 5 lipid classes were detected in analyzed samples. Developed method will be applied for the clinical study of patients with cardiovascular diseases.

Acknowledgments

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Application of novel capillary probes for the investigation of electrochemically assisted injection (EAI) using a scanning electrochemical microscope for EAI cell development

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Keywords
approach curve
capillary probe
electrochemical cell
electrochemically assisted injection
scanning electrochemical microscope

Electrochemically assisted injection (EAI) is a recently introduced hydrodynamic injection concept for capillary electrophoresis (CE). It enables the electrophoretic separation of neutral analyte species by electrochemical formation of charged species during the injection process [1]. A substrate electrode facing the capillary inlet is used for electrochemical analyte conversion. New amperometric capillary probes with an integrated platinum microwire electrode have been developed for studies of the mass transport behaviour in close proximity to the EAI substrate electrode after the injection of electrochemically formed ions. Initial experiments focused on the electrochemical properties of capillary probes with an internal platinum detection electrode. Amperometric repeatability tests using ferrocene methanol in aqueous medium and *n*-butylferrocene in acetonitrile have been carried out to characterize the capillary probes. Hydrodynamic cyclic voltammograms have been recorded to define the oxidative and reductive limiting current regions. Both experiments turned out, that a highly-resistive electrolyte path inside the capillary probes required an appropriate background electrolyte concentration to reduce the resulting IR-drop. In combination with a scanning electrochemical microscope (SECM) the capillary probes were used to

investigate the dependency of capillary-to-substrate electrode distance on the efficiency of EAI. The SECM micro-positioning unit enabled a precise and fast vertical capillary positioning with a resolution in the μm-range. The SECM bipotentiostat managed both the application of a fixed oxidation potential on a Pt-disk substrate electrode in the sample cell and the reductive amperometric *I-t*-detection inside the capillary. The detection signal made a quantitative interpretation of the injection efficiency possible. It turned out, that the amperometric signal response remained stable for capillary-to-substrate electrode distances smaller than 60 μm. Increasing the distance up to 150 μm led to a linear signal decrease [2]. Considering this result, a new semi-automated EAI cell was designed that ensured the required precision of capillary positioning using a small microprocessor-controlled piezo motor. The cell was built as a moveable Teflon® slide containing a CE buffer reservoir with an integrated platinum high-voltage electrode on one side and a substrate electrode holder on the other side. This allowed a manual change between CE-separation mode and EAI-injection mode. The substrate electrode holder was designed to incorporate a screen-printed electrode system. We have shown that EAI routine protocols could be realized faster and with better reproducibility using the semiautomated EAI cell developed. Integrating the EAI cell into a consisting CE time-of-flight mass spectrometry (TOF-MS) system has shown first promising results combining electrochemistry and CE-TOF-MS and opens new insights in mechanistic details of substrate electrode reactions.

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Anion-exchange chromatography in combination with stepwise gradient for characterization of humic substances in an alkaline medium

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Keywords
alkaline mobile phase
anion-exchange chromatography
fluorimetric detection
humic substance
stepwise gradient elution

Humic substances are ubiquitous natural materials occurring in huge amounts in soils, sediments and waters as a product of the chemical and biological transformation of animal and plant residua. Despite the great effort resulting in an enormous number of published papers, the structure and function of humic substances are not yet fully understood [1].

Humic substances considered as consisting of substituted aromatic rings linked together by aliphatic chains (polyols, peptides, sugars, etc.) [2]. The dominant functional groups which contribute to their surface charge and reactivity are phenolic, hydroxylic and carboxylic groups. Humic substances may chelate multivalent cations such as Mg^{2+}, Ca^{2+} and Fe^{2+}. By chelating the ions, they increase the availability of these cations to organisms, including plants [3].

The importance of separation methods in the chemistry of humic substances, including LC methods is currently stressed by a review article of Janoš [1]. From the literature results that the most frequently used separation methods for analysis and characterization of humic substances are column liquid chromatographic methods (mainly RP-HPLC, SEC, and their combination HPLC-SEC) [e.g., 4–6] and electroseparation methods (especially CZE, PAGE) [7–10]. Hutta *et al*. [4] showed the usefulness of stepwise gradient elution in RP-HPLC for characterization of humic

substances. In spite of direct evidence for potential of ion-exchange mechanisms, there is lack of articles on this topic. Therefore we decided to evaluate analytical potential of the application of stepwise gradient in combination with anion-exchange chromatography for characterization of humic acids (of various origin) in alkaline medium.

Experimental

Commercial humic acid were purchased from Sigma-Aldrich with relative mole-cular mass 500–1000, according to product catalogue. Humic substance Ecohum (isolated from peat) was a commercially available fertilizer. Humic substance Cerová was isolated from peat using fractionation procedure recommended by IHSS [4]. Solutions of humic substances were prepared daily fresh by dissolution of weighed humic substances at 3 mg/mL concentration level in 0.01 M NaOH. EDTA (Sigma Aldrich, Steinheim, Germany) of analytical grade was used for preparation on buffered mobile phases. Water for gradient HPLC was prepared by Labconco Pro-PS unit (Labconco, Kansas City, KS, USA). Sodium hydroxide (Merck, Darmstadt, Germany) of analytical grade was used for preparation of solutions of humic substances.

Study of retention behavior and evaluation of spectral characteristics of the selected groups of humic substances was carried out by the HPLC system LaChrom (Merck--Hitachi, Darmstadt, Germany) consisting of pump L-7100, autosampler L-7200, column oven L-7300, fluorescence detector L-7480, interface D-7000, PC data station with software HSM ver. 3.1 and on-line four channel solvent degasser L-7612. Digital pH meter WTW inoLab pH 730 (Weilheim, Germany) provided by combined glass/AgCl electrode was used for mobile phase pH measurement.

Separation was carried out using a 30 × 3 mm glass column filled with Separon HEMA-BIO 1000 DEAE sorbent (Tessek, Prague, Czech Republic) with 60 μm particles. Flow-rate was maintained at 0.50, 1.0, 1.5 and 2.0 mL/min, respectively. Mobile phase A composition was: aqueous solution of sodium salt of EDTA (pH = 12.0, 5 mM). Mobile phase B composition was: aqueous solution of sodium salt of EDTA (pH = 12.0, 500 mM). Gradient program was set from 0.0 to 1.9 min isocratic 0% B in A than from 2.0 to 3.9 min isocratic 1% B in A and from 4.0 min every 2 min there was isocratic step added by two-fold increasing of the content of B in A up to the last step increased by 36% ending in 100% B in A, maintained till 30.0 min isocratic 100% B in A, from 30.1 to 33.0 min linear decrease from 100% B in A to 0% B in A and between runs 5 min re-equilibration was maintained. The same gradient program was evaluated for all flow-rates, respectively. Column oven temperature was maintained at 40.0 ±0.1°C. Injection volume was 25 μL. Fluorescence detection parameters were set to excitation wavelength 480 nm and emission wavelength 530 nm according to published data [11].

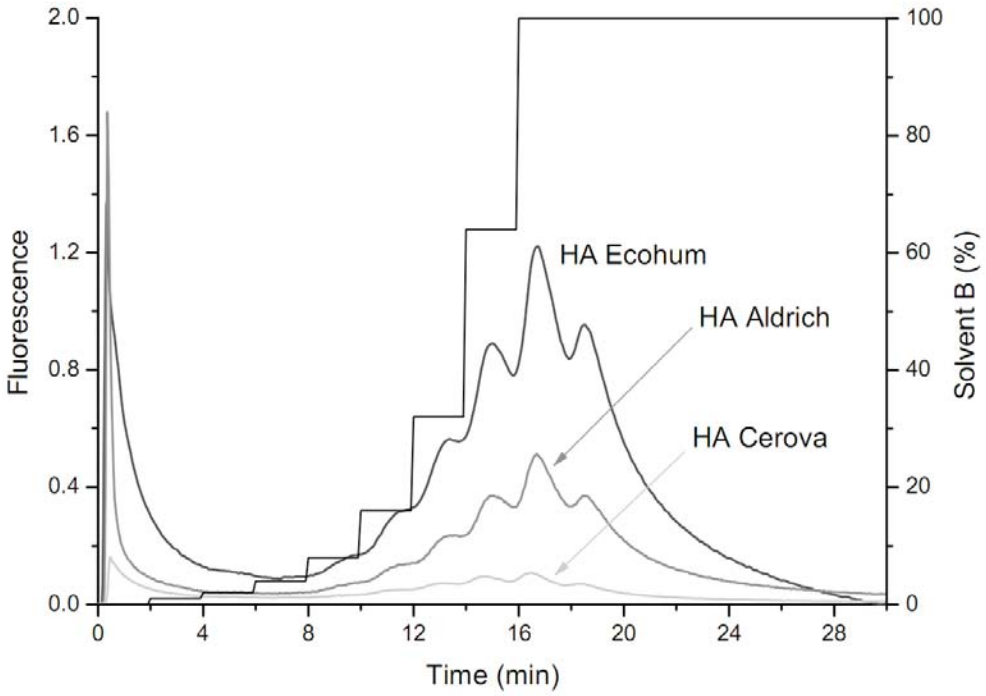

Fig. 1. Chromatographic profiles of selected humic substances (HA Cerova, HA Aldrich and HA Ecohum). Conditions: flow rate 1.0 mL/min, temperature: 40 °C, injection volume 25 μL. For the other conditions, see Experimental.

Results and Discussion

The devised stepwise gradient chromatography method with fluorimetric detection was used for characterization of humic substances by their chromatographic profiles. Fig. 1 shows typical examples of the profiles from analysis of humic substances of various origins. The peak profiles are enforced by the step gradient shape. From the chromatographic profiles is evident that we can see two parts: (i) unretained substances (eluated with weak elution component) and (ii) retained substances (eluated with strong elution component). From the drawing is evident also that profiles of selected humic substances are different, because of various origin these humic substances. Next we compared of profiles these selected humic substances at various flow-rate. These profiles we can see on Fig. 2. From this figure follows that the increased flow-rate is manifested in the reduction of the fluorescence signals, probably because of dilution of samples with higher amount of eluent at higher flow-rates. At higher flow-rate (1.0 and 2.0 ml/min) we observed an increased resolution of the chromatographic peaks separation.

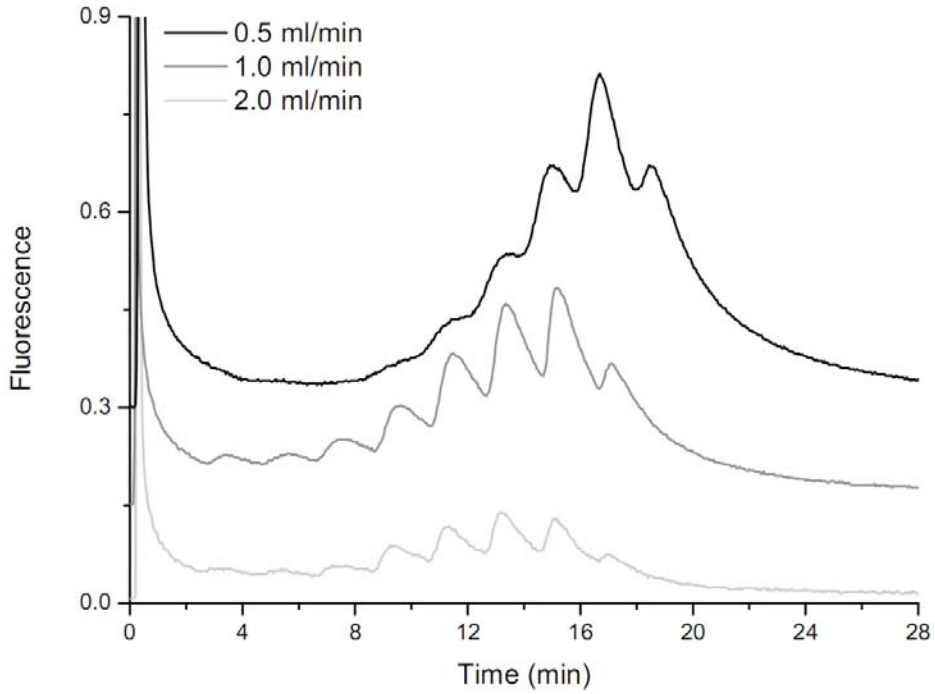

Fig. 2. Chromatographic profiles of selected humic substance (HA Aldrich) at various flow-rates (0.5, 1.0 and 2.0 ml/min). Conditions: temperature 40 °C, injection volume 25 μL. For the other conditions, see Experimental.

Conclusion

For the achieved results we can conclude that devised method of anion exchange chromatography based on short columns filled by medium basic annex containing diethylaminoethyl functional groups, combined with step gradient elution by EDTA solutions with pH = 12, is a promising method which enables characterization of various humic substances and soil types with regard to natural humic and fulvic acids ratio.

Acknowledgments

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Pulsed laser technique in conjunction with size exclusion chromatography as tool for determination propagation rate coefficient of free-radical polymerization of zwitterionic monomers

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Keywords
propagation rate coefficient
size exclusion chromatography
zwitterion

Pulsed laser polymerization in conjunction with size exclusion chromatography (PLP-SEC) has strongly improved the quality of determination of propagation rate coefficients in free-radical polymerization [1].

In this contribution we applied PLP-SEC technique to assess and evaluate propagation rate coefficients of free radical polymerization of methacrylates based on sulfobetaines and phosphobetaine (Fig. 1). Propagation rate coefficients show similar behaviour to ionized methacrylic acid polymerized at higher degrees of monomer ionization [2]. Moreover, optimalization of the condition of size exclusion chromatography will be discussed.

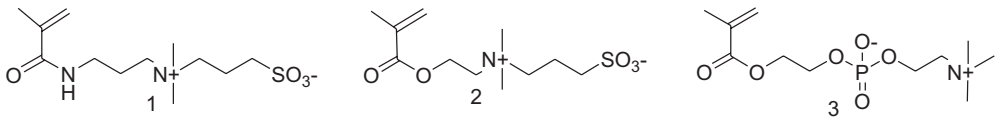

Fig. 1. Chemical structures of methacrylates based on sulfobetaines (1 and 2) and phosphobetaine (3).

Polymers form these monomers belong to zwitterionic polymers and exhibit unique solution as well as biocompatible properties [3]. However the kinetics and mechanism of free-radical polymerization of this type of zwitterionic monomers is insufficiently known and require deeply understanding and correlation.

Acknowledgments

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SPME-LC/MS^n for the analysis of selected biologically active compounds

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Keywords
antibiotics
drug monitoring
HPLC
mass spectrometry
solid phase microextraction

Determination of chemical compounds from various matrices, including environmental and biological (blood, plasma, urine) is a serious problem in a modern analytical chemistry. Chemical compounds analyzed at the ppm and ppb levels require the application of special detectors. The most important stage in trace analyses is undoubtedly a method of samples' preparation. Such methods of sampling as the liquid-liquid extraction, solid phase extraction or solid phase microextraction (SPME) have been known for many years. Methods of samples' collection, due to their meaning, are frequently called a bottle neck of the entire analytical procedure (70% errors). A quick development of the method and drawing up methods allowing an easy control of the properties of adsorptive layers used in SPME initiated a new stage of testing with the application of microextraction to the solid phase. From the end of the 1990s research was started on the possibility of using SPME in the medical analysis, mainly of very expensive pharmaceutical agents. Along with the development of chromatographic techniques and linking them with sensitive methods of detection, in recent years the application of metabolomics has largely increased. Metabolomics plays a major role in medicine, pharmacy, and agriculture. Thanks to it, the identification of compounds in biological samples for the purpose of a clinical diagnosing of diseases has become possible. In a pharmaceutical analysis it is an important trend to determine metabolic profiles after the administration of a drug in order to trace its lot in the organism. It is an example of a technological progress in the analytical chemistry.

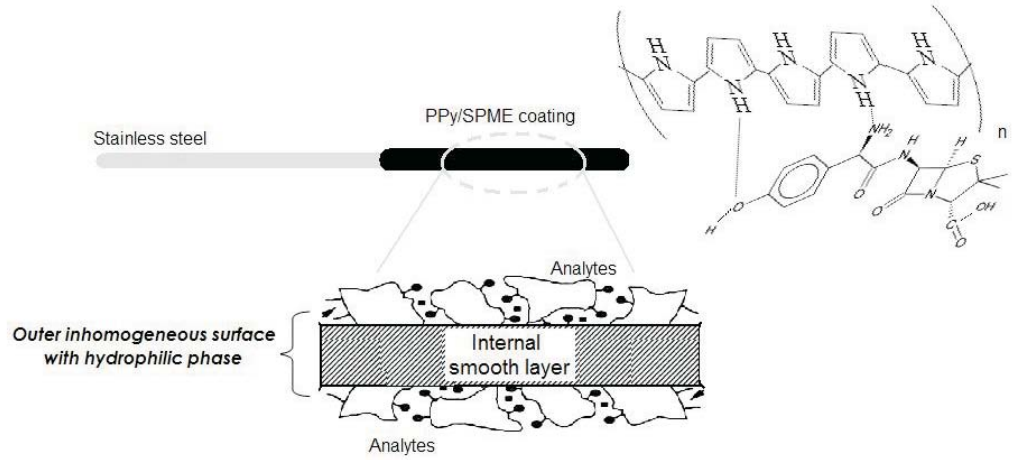

Fig. 1. Scheme illustrating the sorption mechanism of amoxicillin on the polypyrrole surface (PPy/SPME fibers).

Polypyrrole, polythiophene and poly(3-alikilothiopenes) SPME coatings with a molecular imprint were used and evaluated their ability to extract selected drugs from different classes with different physicochemical properties and of widely varying polarities. SPME fibres are manufactured electrochemically by coupling the homemade electropolymerization system to a new generation potentiostat/galvanostat connected to a three-electrode array consisting of an electrochemically roughened stainless steel (Ni–Cr) working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. Their physico-chemical properties, mechanical and chemical stabilities are characterized by scanning electron microscopy, optical stereo-microscope equipped with a CCD camera and Fourier transform infrared spectrometer. The SPME coatings were evaluated by analyzing clinically relevant antibiotic drugs: linezolid, daptomycin, amoxicillin, and moxifloxacin. Important factors in the optimization of SPME efficiency such as extraction time, temperature, pH of the matrix, influence of anticoagulants on sorption mechanisms, and desorption conditions are discussed. The polypyrrole film displayed high extraction efficiency (selectivity and sensibility) towards the target analytes among studied SPME coatings (Fig. 1). The results demonstrate the potential of in vivo SPME as a useful sample preparation tool for chromatographic based metabolomics drug monitoring in the biomedical application from patients receiving therapeutic dosages [1, 2].

Acknowledgments

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The use of miniaturized capillary electrophoresis in monitoring of some neurological diseases

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Keywords
cerebrospinal fluid
electrophoresis on a chip
nitrate / nitrite
solid phase extraction

This work deals with separation and determination of nitrite and nitrate in cerebrospinal fluid by on-line combination of isotachophoresis capillary zone electrophoresis (ITP-CZE) on a poly(methylmethacrylate) chip with coupled separation channels and conductivity detection (Fig. 1). These ions, taken orally or produced endogenously, are oxidation products of nitric oxide present in various body fluids. Nitrite and nitrate, as

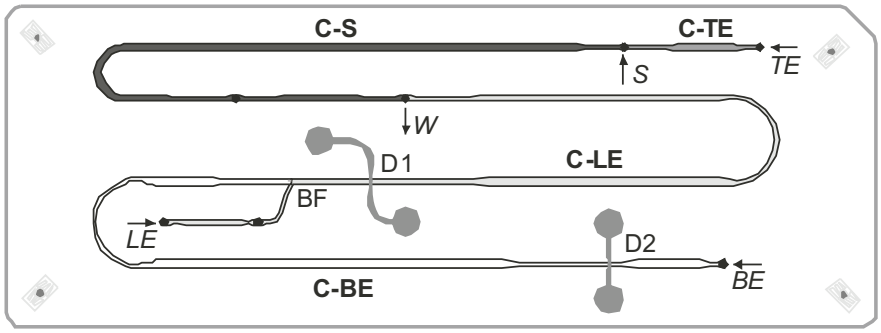

Fig. 1. A scheme of a poly(methylmetacrylate) chip with coupled channels and conductivity detectors. C-LE: the first (ITP) separation channel (a 4.5 μL volume) filled with leading electrolyte. C-BE: the second (CZE) separation channel (a 4.3 μL volume) filled with carrier electrolyte. C-TE: the third channel (a 0.8 μL volume) filled with terminating electrolyte. C-S: sample injection channel (a 9.9 μL volume). D1, D2: platinum conductivity sensors. BF: bifurcation section. BE, LE, TE, S: inlets for the carrier, leading, terminating and sample solutions to the chip channels, respectively. W: an outlet channel to waste container.

well as nitric oxide, are indicators of various neurological diseases. For example, increased concentrations of nitrite and nitrate in cerebrospinal fluid are symptoms of a multiple sclerosis [1] and bacterial meningitis [2]. On the other hand, decreased concentrations of these compounds in cerebrospinal fluid are indicators of Parkinson's disease [3].

Isotachophoresis separation conditions (pH of leading electrolyte was 3.6 and formate was used as a terminating anion) enabled a selective transfer of the analytes to the CZE separation stage on the coupled separation channels chip. In CZE stage, a zwitterionic detergent (3-(*N,N*-dimethyldodecylammonio)-propanesulphonate) present in the carrier electrolyte at a concentration (200 mmol/L) higher than its critical micellar concentration allowed quick resolution of the analytes. Coupled separation channels chip with enhanced sample loadability (a 9.9 μL volume of the sample loaded onto the chip was almost the same as a volume of both separation channels) was very effective in reaching favorable concentration limits of detection for nitrate and nitrite (ca. 0.2 μg/L corresponding to 3.1–3.5 nmol/L). Preferred working conditions (suppressed hydrodynamic and electroosmotic flow in the separation system on the coupled separation channels chip) have contributed to reproducible migration velocities (RSD values of migration times were 1.9–3.6%) and determinations of trace concentrations of nitrite and nitrate in model samples (RSD values of peak areas were 1.5–9.9%). Due to high concentration levels of chlorides present in cerebrospinal fluid (ca. 4.5 g/L), they were removed from real samples by solid phase extraction (SPE) performed on Ag^+ sorbent before ITP-CZE analysis (Fig. 2).

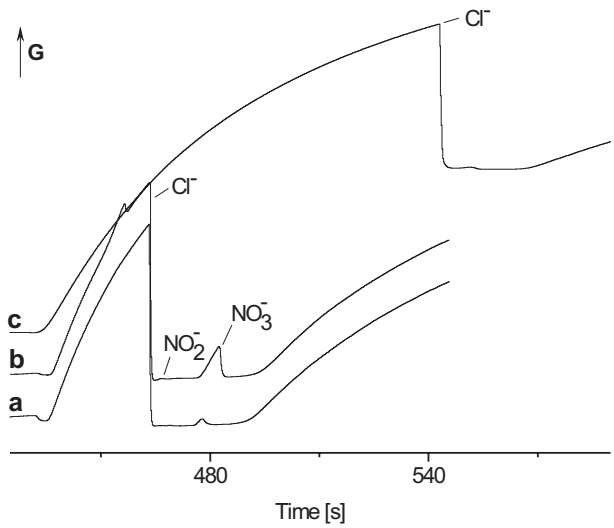

Fig. 2. Electropherograms from ITP-CZE analysis of real cerebrospinal fluid sample after its SPE pre-treatment on Ag^+ sorbent: (a) terminating electrolyte (a blank run), (b) 50-fold diluted cerebrospinal fluid sample after its SPE pre-treatment on Ag^+ sorbent, and (c) the same as in (b) without SPE pre-treatment step. The driving current was stabilized at a 20 μA in both separation channels. G – conductivity.

Elaborated SPE-ITP-CZE method on the coupled separation channels chip enabled quick (ca. 20 minutes total analysis time including also sample pre-treatment step) and reliable determination of trace concentrations of nitrate and nitrite in 10–50 times diluted cerebrospinal fluid samples.

Acknowledgments

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UHPLC-DAD-ToF-MS: A useful tool for chromatographic fingerprinting of fungal extracelullar metabolites

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fungal extracellular secondary metabolites
time of flight mass spectrometry
ultra high-performance liquid chromatography

In this paper, a new ultra high-performance liquid chromatography combine with diode array detection and time of flight mass spectrometry (UHPLC-DAD-ToF-MS) is proposed for chromatographic fingerprinting analysis of extracellular metabolites presented in fermentation broth of *Geosmithia* spp.

Chromatographic fingerprinting is method based on comparison of fingerprints (chromatograms) of highly complex samples that contain many unknown components [1, 2]. Chromatographic fingerprinting can be used as an effective tool for comparison, classification or identification of samples and have found widespread use in e.g. chemotaxonomic characterization of microorganisms [3].

Filamentous fungi of the genus *Geosmithia* (*Ascomycota*: *Hypocreales*) represent symbiotic fungi that enter complex chemical-based interactions with their hosts. Thus, they possess a diversity of secondary metabolites with various biological activities [4]. It is supposed that the production of secondary metabolites will reflect both the phylogenetical and ecological relatedness of their producers. For up-dating of taxonomic overview of the *Geosmithia* spp. strains maintained at the Institute of Microbiology, Academy of Sciences of the Czech Republic and in Culture Collection of Fungi in Prague, the method of chromatographic fingerprinting can be employed.

Since the fungal fermentation broth represents complex matrix, the combination of UHPLC with both DAD and ToF-MS detection was found to be an appropriate instrumentation for the purpose of chromatographic fingerprinting. UHPLC allows faster separations with higher separation efficiencies [5] and DAD detection provides additional spectral information of the sample components by generating the 3D chromatograms [6]. Moreover, the ToF-MS analyser is able to analyse theoretically unlimited number of compounds in the sample with high mass accuracy and high mass resolution [7].

For extraction of extracellular metabolites, SPE method employing Oasis MCX strong cation-exchange mixed-mode polymeric sorbent (Waters, Czech Republic) was chosen. The UHPLC-DAD-ToF-MS analyses were carried out on Waters Acquity UPLC System (Waters, Czech Republic) consisting of Acquity UPLC Solvent Manager, Acquity UPLC Sample Manager, Acquity UPLC Column Heater/cooler, and Acquity UPLC Diode Array Detector (set at 200-600 nm). The Waters LCT Premier XE orthogonal accelerated time of flight mass spectrometer (Waters MS, Manchester, UK) with an electrospray interface was operating in both positive and negative ion mode, using the following parameters: cone voltage 50 V; capillary voltage +2500 V (positive), –2500 V (negative). Full scan spectra from *m/z* 100 to 1600 were acquired with a scan time of 0.1 s and 0.1 s interscan delay. Mass accuracy was maintained by using lock spray and Mass Lynx V4.1 software was used for data processing. Analyses were performed on Acquity UPLC BEH C18 column (100 × 2.1 mm i.d.; particle size, 1.7 μm; Waters) with the mobile phase flow rate of 0.4 mL min^{-1} and column temperature of 25 °C. The mobile phase consisted of formic acid–water (0.1:99.9, *v/v*), and acetonitrile using gradient elution program.

The applicability of the method was proved by analysis of 38 strains produced by different species and isolated from different sources (hosts). Hierarchical cluster analysis and principal component analysis were performed to differentiate and classify the samples based on the generated fingerprints. The results of the study revealed an interesting relatedness of secondary metabolites production and taxonomical identity, which proved the applicability of the developed UHPLC-DAD-ToF-MS method for chromatographic fingerprinting analyses.

Acknowledgments

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Magnetic and upconverting luminescent core/shell nanoparticles for sensor applications

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magnetic nanoparticles
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We describe the preparation of a new type of hybrid core/shell nanoparticles offering magnetic response and upconversion luminescence together. Monodisperse nanocrystals of super-paramagnetic magnetite (Fe_3O_4) with an average size of 10 nm and capped with oleic acid were used as seed crystals to further grow, on their surface, a layer of hexagonal $NaYF_4$ doped with Yb^{3+} and Er^{3+} ions [1, 2]. Hexagonal $NaYF_4$(Yb, Er) nanomaterials are known as efficient infrared-to-visible up-conversion phosphors, emitting visible light on excitation with near-infrared light. Compared to conventional fluorophores, the upconverting luminescent nanoparticles have distinct advantages. For example, its light can deeply penetrate tissue, and does not cause photodamage to living organisms, and autofluorescence of biomatter, thus allowing for a high

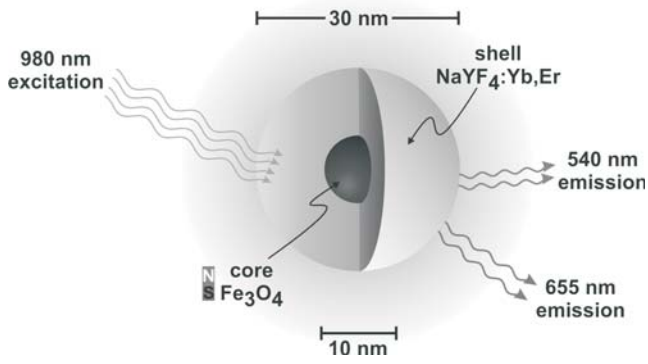

Fig. 1. Schematic representation of magnetic upconverting luminescent core/shell nanoparticles consisting of a magnetite core and a shell of hexagonal $NaYF_4$ doped with 20% of Yb^{3+} and 2% of Er^{3+} ions.

sensitivity in detection. Hybrid core/shell nanoparticles possessing both magnetic response and upconversion luminescence offer numerous possibilities. They may be functionalized with receptor molecules, manipulated with a magnetic field for separation or purification, applied to magnetic resonance imaging, or used as "nano-lamps" in optical biosensors [3] and for bioimaging purposes [4]. We are currently investigating on how magnetic upconverting luminescent nanoparticles can be modified at their surface, with the final goal to immobilize probes and biorecognition elements thereon. We expect the resulting magnetic upconverting luminescent nanoparticles to offer a potential that is comparable to that of magnetic quantum dots, for example for microRNA [5], and for stem cell labeling [6], or of magnetic nanoparticles covered with fluorescent conjugated polymers [7].

Acknowledgments

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Determination of platinum on different concentration levels by inductively coupled plasma mass spectrometry

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cytostatics
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Platinum with other platinum group elements are nowadays mainly emitted from automotive catalytic converters, but it is also used in medicine in cytostatic drugs. Its levels in the environment are increasing. The aim of presented work was development, validation and use of the method for determination of platinum by inductively coupled plasma mass spectrometry (ICP-MS) on different concentrations levels in various samples. The optimal parameters and suitable platinum isotopes were found, rhenium as an internal standard (1 or 10 μg Re/L) was added to all standards and samples. Spectral interferences from hafnium oxides ions were studied and corrected.

Platinum levels in the environment have being increased due to its role in catalytic converters [1–3]. Further platinum source is the waste from a production and use of cystostatic drugs (e. g., cisplatin, carboplatin, oxaliplatin) used in the therapy of cancer decease [4, 5]. Emitted platinum and its compounds can cause allergy, asthma [6–9] or other health risks as carcinogenicity, mutagenicity or teratogenicity [4, 10].

For the determination of total platinum, instrumental techniques such as electrothermal atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry or mass spectrometry, neutron activation analysis [4, 5] or cathodic stripping voltammetry are used [1, 11].

In this work the method of ICP-MS has been applied because of excellent detection limit, selectivity, a wide linear dynamic range and speed of measurement [12, 13]. The

Table 1
Instrumental operating conditions.

Plasma	
RF Power	1100 W
Ar flow rate	~ 0.83 L min^{-1}
T spray chamber	5 ºC
Lens voltage[a]	~ 8.5 V
Mass Spectrometer	
Sampling cone	nickel
Skimmer	nickel
Analyser vacuum	~ 7.0×10^{-6} Torr
Acquisition parameters	
Measurement mode	peak jumping
Platinum group elements' isotopes	$^{194}Pt^{+}$, $^{195}Pt^{+}$
Monitored Interferents	$^{178}Hf^{16}O^{+}$, $^{179}Hf^{16}O^{+}$
Dwell time	100 ms
Internal standard	$^{187}Re^{+}$

[a] Optimization of lens voltage was done daily by using of a solution of 1 µg Pt per litre.

serious limitation of this technique is spectral interferences, which have to be widely studied [1, 2, 14] and corrected by mathematical correction equations.

Platinum determinations were carried out on a Perkin Elmer quadrupole ICP-MS Elan DRC-e (Perkin ElmerSCIEX Instrument, Canada) equipped with nickel cones, concentric quartz SeaSpray nebulizer and cooled cyclonic spray chamber (Peltier, at 5 °C). To optimize the ICP-MS system parameters 1 µg L^{-1} solution of platinum was used. Results are summarised in Table 1.

Reagents of highest purity as 65% nitric acid (Suprapur, Merck) were used. Stock solution of Pt, interfering species Hf and internal standard Re were prepared from single element standard solutions of 1000 mg L^{-1} Merck ICP CertiPUR. Working standards of individual elements were prepared from the standard solution and stabilized by 1% (v/v) nitric acid. Demineralised water (18.2 M Ω cm^{-1}) from a Milli-Q water purification system (Millipore, France) was used. ^{187}Re as an internal standard was used due to its very low abundance in samples and especially because of similar chemical and physical behaviour, comparable atomic mass and ionisation potential to platinum. It helps to control instrumental drift or non-spectral interferences [15].

Because of a lack of suitable certified reference materials with declared platinum value, accuracy was confirmed by our successful participation in international round robin tests G-EQUAS (The German External Quality Assessment Scheme for Analyses in Biological Materials, Erlangen, Germany). For samples with higher platinum amount a method of spiked samples was used. Results are shown in Table 2.

Table 2
Certified and measured values of platinum in used reference materials.

reference material	certified value (acceptable range) [μg L^{-1}]	measured value [μg L^{-1}]	recovery [%]
EQUAS 2009 8A	0.0418 (0.0289–0.0547)	0.041	99.0
EQUAS 2009 8B	0.1022 (0.0755–0.1289)	0.103	100.5
spiked urine samples	spike of 25.0	24.4	97.7

Elaborated method was used for the platinum determination in various samples: water (waste, rinse), urine (oncological patient, nurse), extracts of smear (oncological departments with robotic and hand operated drug dilution), etc. Samples were diluted by 1% HNO_3 (v/v) before analyses (from 5 up to 40 000 times). Two calibration ranges 0.1–10 μg Pt L^{-1} and 5–100 μg Pt L^{-1} were used according to platinum content.

Platinum has six isotopes (190, 192, 194, 195 and 196), these with the highest natural abundance and no isobaric interferences ^{194}Pt (32.9%) and ^{195}Pt (33.8%) were chosen. Concentrations of platinum in samples of clean water and non-exposed urine were very low and therefore the sums of both isotopes' signals were used. The same mass to charge ratio have ions $^{178}Hf^{16}O^+$ and $^{179}Hf^{16}O^+$. As the content of hafnium in these samples could be similar to platinum concentration the possible affect was studied and the correction factors calculated from signal ratios were used. The final equation for determination of corrected platinum amount was designed:

$$(^{194}Pt^+ + {}^{195}Pt^+)_K = (^{194}Pt^+ - k_1\,{}^{178}Hf^+) + (^{195}Pt^+ - k_2\,{}^{179}Hf^+) \quad (1)$$

$$k_1 = \text{Mass } 194/{}^{178}Hf^+ \quad (2)$$

$$k_2 = \text{Mass } 195/{}^{179}Hf^+ \quad (3)$$

where k_1 and k_2 are correction factors calculated as a ratio of Mass 194 signal and intensity $^{178}Hf^+$ and Mass 195 and $^{179}Hf^+$ respectively in aqueous solution of 1 μg Hf L^{-1}. On the other hand this interference was negligible in case of rinse and waste water samples and urine of oncological patients. Intensities of hafnium were only monitored. The problem of spectral interferences from HfO^+ ions had been solved.

Validation of method was provided. Specificity and selectivity is done by the mass properties of individually isotopes, other validation parameters are given in Table 3.

The method was successfully used for platinum determination in various real samples. Platinum in nurses' urine was on ng Pt L^{-1} level (8 samples: median 26.0; min 6.7; max 2350), concentration in patients' urine lay on mg Pt L^{-1} level (12 samples: median 4.5; min 0.8; max 139.2). The extracts of smear from different places in oncological preparation room with robotic and hand operated drug dilution were measured. Platinum amount was on ng Pt per smear level, but higher concentrations

Table 3
Validation parameters of ICP-MS determination of platinum: limit of detection and quantification for different sample matrix and repeatability.

	LOD	LOQ
urine samples	4.0 ng L^{-1}	12.0 ng L^{-1}
water	7.0 ng L^{-1}	24.0 ng L^{-1}
waste water	0.1 µg L^{-1}	0.3 µg L^{-1}
waste organic	4.5 µg L^{-1}	15.0 µg L^{-1}
smear	0.07 ng	0.24 ng
repeatability	< 10 %	

were found in case of hand operated room (robotic; 80 samples: median 0.10; min 0.003; max 5.7; hand operated; 60 samples: median 5.3; min 0.06; max 1380). Platinum in waste and water from Pt drugs production reached up to g Pt L^{-1} level.

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Electrochemical biosensor for determination of nucleic acid bases

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Keywords
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cytosine
electrochemical biosensor
monolayer
thioguanine

In this work an electrochemical biosensor based on thioguanine (6-amino-8-purine-thiol) modified mercury film covered silver solid amalgam electrode (MF-AgSAE) for determination of cytosine is represented. In general, for preparation of electrochemical biosensors based on formation of *thio*monolayer, electrode material as gold [1, 2] is often used. Big disadvantage of this material is very long formation of the *thio*monolayer on the electrode surface (obviously several hours). Therefore, MF-AgSAE was proposed for better affinity of silver liquid amalgam film to sulphur.

The first step of preparation of the biosensor is covalently binding thioguanine on electrode surface via sulfur atom to form a S–Hg bond (Fig. 1). The monolayer of thioguanine can be carry out by either the self-assembled method [3] or by imposition an appropriate potential on the working electrode. Self-assembled monolayer is formed

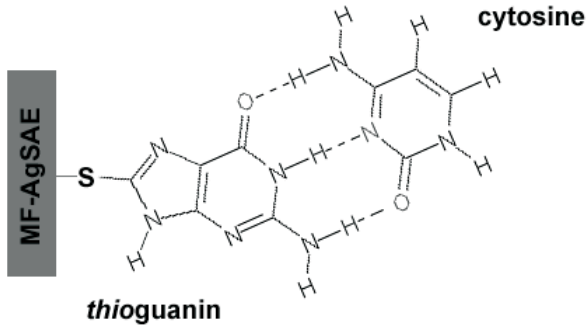

Fig. 1. Schematic drawing of the thioguanine monolayer on mercury film covered silver solid amalgam electrode and hydrogen bonding with cytosine in solution.

by the chemosorption of thioguanin from solution onto the surface of silver liquid amalgam film (adsorbate molecules organize spontaneously). In case when the monolayer is prepared under potential control, the monolayer is adsorbed onto the silver liquid amalgam surface during some seconds when the optimal potential is imposed. Just this method was used in this work, because it is better reproducible than self-assembled one and such prepared monolayer has the higher surface concentration. The formation of thioguanine monolayer was studied by cyclic voltammetry, by alternating current voltammetry and by electrochemical impedance spectroscopy. The next step is immersion of the electrode with thioguanine monolayer into the solution with cytosine. Detection of cytosine in solution is carried out with the selective hydrogen bond interaction between thioguanine and cytosine. Concentration of cytosine is determined by measuring of an impedance and/or capacity of the formed double layer thioguanin-cytosine.

Acknowledgments

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Analysis of plant membrane lipids by RP-HPLC/HR-ESI-MS

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high-resolution mass spectrometry
monogalactosyldiacylglycerols
RP-HPLC

Mono- and digalactosyldiacylglycerols are glycolipids forming the major part of biological membranes in photosynthesizing organisms (Fig. 1). The individual species differ by fatty acids linked to *sn*-1 and *sn*-2 possition on glycerol backbone [1]. The distribution of saturated and unsaturated fatty acids and ratio of mono-/di- galactosyldiacylglycerols is influenced by environmental conditions [2]. The complexity of their mixtures is increased by enzymatic or chemical oxidation of unsaturated species [3, 4]. Based on the predominant fatty acids linked to *sn*-2 position in monogalactosyldiacylglycerols molecules, plants are divided into two groups, namely "18:3 plants" and "16:3 plants" [5]. Moreover, composition of fatty acyls in plant species provides chemotaxonomical informations [6]. Galactolipids are also of interests due to their anticancer, antiviral and anti-inflammatory activity [7–9]. Nowadays, there are used for curing joint pain [10].

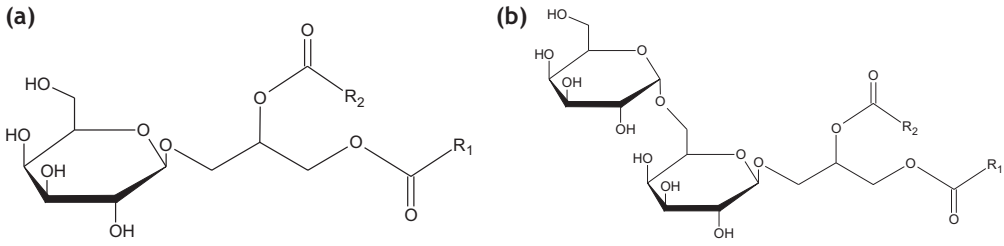

Fig. 1. Structures of (a) monogalactosyldiacylglycerols, and (b) digalactosyldiacylglycerols.

The aim of this work was to develop and optimize RP-HPLC/HR-ESI-MS^2 methods for analysis of oxidized and non-oxidized mono- and digalactosyldiacylglycerols in plant samples. Mono- and digalactosyldiacylglycerols were isolated from *Arabidopsis thaliana* and *Melissa officinalis.* The analysis was performed on a HPLC/MS system with an ion trap mass spectrometer LCQ FLEET (low resolution) and a hybrid mass spectrometer LTQ Orbitrap XL (high resolution) with an electrospray ion source (Thermo Fischer Scientific, USA). A stainless steel column Nucleosil C18 (250 × 2 mm, 5 μm, Macherey-Nagel, Germany) with a C18 precolumn (4 × 2 mm, Phenomenex, USA) was used. For each analysis 5 μL of sample was injected. The flow rate of the mobile phase was 200 μL/min and the column temperature was 30 ºC.

We investigated the retention behavior of mono- and digalactosyldiacylglycerols in the solvent systems with water, acetonitrile and methanol. Generally, methanol/water systems provided better chromatographic resolution for monogalactosyldiacylglycerols, whereas acetonitrile/water systems were preferable for separation of digalactosyldiacylglycerols. The high initial concentration of methanol (85% or more) caused rapid elution of monogalactosyldiacylglycerols and loss of the chromatographic resolution. Too low concentration of methanol at the beginning of the analysis (65% or less) resulted in a very high retention of some monogalactosyldiacylglycerols and long analyses. The optimum separation of monogalactosyldiacylglycerol molecular species was achieved using gradient elution starting from 75% of methanol and 25% of water. The concentration of methanol linearly increased to 100% in 80 minutes. The separation of digalactosyldiacylglycerols was initially tested in acetonitrile/water solvent systems. The best separation between the molecular species was found in a gradient system with linear increase of acetonitrile from 65% to 100% within 50 minutes. Even though this system provided good separation, some digalactosyldiacylglycerols were strongly retained on the column. Therefore, we optimized a ternary solvent system with water, acetonitrile and methanol. Interestingly, methanol itself caused very rapid elution of all digalactosyldiacylglycerol molecular species, but any methanol/water combinations resulted in strong retention of digalactosyldiacylglycerols in the column. The optimum gradient program for digalactosyldiacylglycerols started from 65% of acetonitrile and 35% of water. The acetonitrile portion linearly increased to 80% in 50 minutes and simultaneously methanol linearly increased to 20%. The final step was linear increase of methanol up to 100% in 10 minutes.

The analytes were detected either by low resolution ion trap or high resolution orbitrap mass spectrometer. The CID MS/MS spectra of sodium adducts ($[M+Na]^+$) contained fragments corresponding to elimination of fatty acids (both mono- and digalactosyldiacylglycerol) and dehydrated galactose (digalactosyldiacylglycerol; monogalactosyldiacylglycerol only rarely) as described previously [11]. The

non-oxidized and oxidized galactolipids provided similarly looking spectra dominated by galactosylmonoacyl fragments. Based on these fragments the low resolution ion trap mass spectrometer was unable to distinguish between oxidized and non-oxidized species. The *m/z* values of singly oxidized galactolipid and non-oxidized galactolipid having one extra carbon and lacking one double bond differ just by 36 mDa, which is beyond the resolving power of ion traps and other low resolution instruments. Consequently, the spectra of galactolipids can be interpreted incorrectly and the use of high resolution mass spectrometry is essential to avoid erroneous identifications of galactolipids.

Using optimized conditions 61 mono- and 20 digalactosyldiacylglycerol molecular species were identified in both plants in this work. *A. thaliana* sample contained more galactolipid species than *M. officinalis*, with substantional number of them being oxidized. We have detected 52 mono- (21 non-oxidized, 31 oxidized) and 17 digalactosyldiacylglycerols (15 non-oxidized, 2 oxidized) in *A. thaliana* and 25 mono- (15 non-oxidized, 10 oxidized) and 13 digalactosyldiacylglycerols (11 non-oxidized, 2 oxidized) in *M. officinalis.* The total peak area of the oxidized species was substantialy lower than that of non-oxidized; for instance, the oxidized species formed 17% of mono- and 5% of digalactosyldiacylglycerols in *A. thalliana.* The basepeak chromatogram of monogalactosyldiacylglycerols identified in *A. thaliana* is shown in Fig. 2.

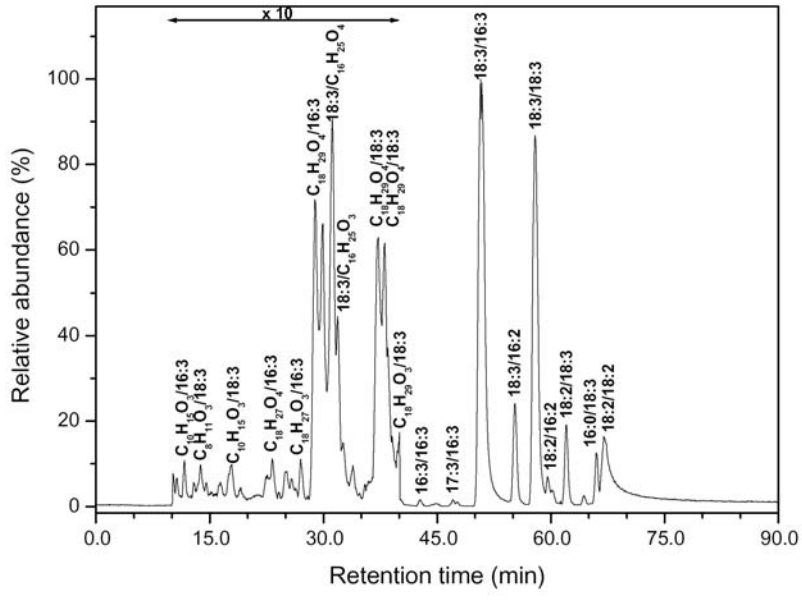

Fig. 2. The basepeak chromatogram of monogalactosyldiacylglycerols from *Arabidopsis thaliana*; the region 10–40 min is ten times enlarged. Sample was separated on Nucleosil C18 column (250 mm × 2 mm, 5 μm) with methanol/water gradient and HR-ESI/MS detection.

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LC/MS separation of natural antioxidants in herbs and honey extracts

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Keywords
antioxidants
herb
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polyphenolic compounds

A liquid chromatography mass spectrometry method has been developed for separation of polyphenolic compounds including phenolic acids and flavonoids in natural matrices. The composition of mobile phase, type of the column and gradient profile were optimized. Five different columns with C18, phenyl-hexyl and pentafluorophenyl chemically bonded stationary phases were compared using mobile phases with methanol and acetonitrile mixtures with addition of 0.3% (v/v) formic acid. The best separation of selected polyphenolic compounds was achieved using column with pentafluorophenyl chemically bonded stationary phase. The segmented linear gradient profile, i.e. initial concentration of acetonitrile and gradient steepness in each segment, was optimized by numerical iteration procedure using regression parameters of experimental dependencies of retention factors of antioxidants under isocratic conditions on concentration of acetonitrile in mobile phase. One of the most important steps for separation of phenolic compounds in natural matrices is a sample preparation. In this work, solid phase extraction employing columns packed with C18 chemically bonded stationary phase was used. The best results were achieved by elution with methanol, drying of eluent under nitrogen and its dilution in 30% methanol. The identification phenolic compounds was performed using mass spectrometry with electrospray ionization in negative ion mode. The full mass scan range was m/z = 100–800. Structures of phenolic compounds were confirmed by tandem mass spectrometry.

The water extracts of flower of wild chamomile, lemon balm, sage and water extract of honey have been analyzed under optimal conditions.

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Utilization of modern extraction methods for analysis of propellant components

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Analysis of explosives, mainly smokeless powders and explosives mixtures, is an integral part of the production of explosives, fulminating compounds or propellants. Nowadays, chromatographic methods are used for these analyses, for example high performance liquid chromatography with ultraviolet detection (HPLC/UV) [1]. Modern methods and classical methods of propellant analysis (titration, gravimetry) require sample preparation to analysis. It means that target analytes have to be isolated from sample for their analysis. Isolation step of propellant analysis includes extraction methods usually. The extraction method is always selected according to the matrix of a sample and kind of a sample. Conventional method for preparation of propellant samples and explosives samples is Soxhlet extraction with dichloromethane or diethyl ether as extraction agent [2].

Replace of Soxhlet extraction and development of new chromatographic method for analyzing of propellant components are major goals of our work. Pressurized fluid extraction or ultrasonic extraction with ultrasonic probe are modern and efficient extraction methods. The same efficiency or higher efficiency of extraction using pressurized fluid extraction and ultrasonic extraction is observed than using Soxhlet extraction. Time of extraction is shorter using modern extraction methods than using Soxhlet extraction (8 hours). Combination of gas chromatography and mass spectrometry (GC/MS) is faster, more sensitive and more accurate method for analyses of propellant components than conventional HPLC/UV. First of all, advantage of GC/MS

method is detection of newly tested propellant components, because some of them do not provide of response using ultraviolet detector (for example acetyl tributyl citrate – gelifying agent in propellants). Secondly, substances can be immediately identified by comparing measured mass spectra with library spectra. Therefore, the GC/MS method fulfills also the qualitative part of smokeless powder analysis (especially the unknown ones).

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