Proceedings of the 9th International Students Conference "Modern Analytical Chemistry"

Prague, 23—24 September 2013

Edited by Karel Nesměrák

Charles University in Prague, Faculty of Science
Prague 2013

Proceedings of the
9th International Students Conference
"Modern Analytical Chemistry"

Proceedings of the 9th International Students Conference "Modern Analytical Chemistry"

Prague, 23—24 September 2013

Edited by Karel Nesměrák

Charles University in Prague, Faculty of Science
Prague 2013

CATALOGUING-IN-PUBLICATION – NATIONAL LIBRARY OF THE CZECH REPUBLIC
KATALOGIZACE V KNIZE – NÁRODNÍ KNIHOVNA ČR

Modern Analytical Chemistry (9. : 2013 : Praha, Česko)
Proceedings of the 9th International Students Conference
"Modern Analytical Chemistry" : Prague, 23–24 September 2013 /
edited by Karel Nesměrák. – 1st ed. – Prague : Charles
University in Prague, Faculty of Science, 2013. – 146 s.
ISBN 978-80-7444-023-6 (brož.)

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

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

The Proceedings publication was supported by the research project
MSM0021620857 of the Ministry of Education of the Czech Republic.

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

ISBN 978-80-7444-023-6

Preface

Dear friends and colleagues,

Welcome to the 9th International Students Conference "Modern Analytical Chemistry". This year, the conference is even more international than in previous years, as the attendees come from already five countries: Austria, Czech Republic, Germany, Poland, and Slovakia. Also, the number of participants – fifty one speakers – is the biggest in the history of the conference. We are very pleased that our forum has become so attractive for young analytical chemists.

We hope that the conference, as in previous eight years, will be interesting, challenging, and successful event. It is a platform for the presentation of new scientific results and shows the further directions of research in the field of analytical chemistry. We are convinced that the conference offers many possibilities for improvement of the presentation skills, provides the floor for discussion and exchange of experiences and opinions, and helps to master the English language to all the participants.

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

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

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

Sponzors

The organizers of 9th International Students Conference "Modern Analytical Chemistry" gratefully acknowledges the generous sponsorship of following companies:

http://www.apczech.cz/

http://www.thermofisher.cz/

http://www.ecomsro.cz/

http://www.lach-ner.com/

http://www.hpst.cz/

http://www.quinta.cz/

http://www.sigmaaldrich.com/

Programme

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

Monday, September 23, 2013

8:00–8:30	**Registration of participants**
8:30–8:45	**Opening ceremony, welcoming address**
	Session 1 ▪ chairperson: Zuzana Deáková
8:45–9:00	Kozlík P: *Characterization and comparison of cyclofructan and amide-based stationary phases in hydrophilic interaction liquid chromatography* (p. 11)
9:00–9.15	Szczygłowska M.: *Use of white cabbage for phytoremediation processes* (p. 13)
9:15–9:30	Slováková M.: *Determination of fluorotelomer alcohols in food contact materials and food* (p. 17)
9:30–9:45	Horáková E.: *Study of differential pulse voltammetric behavior of selected nitro-compounds at a hanging mercury drop electrode in the presence of double-stranded DNA* (p. 21)
9:45–10:00	Moravčík J.: *HPLC enantioseparation of underivatized amino acids using cyclofructan chiral stationary phases* (p. 24)
10:00–10:15	**Coffee Break**
	Session 2 ▪ chairperson: Justyna Małgorzata Płotka
10:15–10:30	Adamusová H.: *Determination of 17β-estradiol and 17α-ethynylestradiol in mouse fertilizing M2 medium* (p. 26)
10:30–10:45	Josypčuk O.: *Electrochemical flow biosensors based on silver solid amalgam electrodes* (p. 28)
10:45–11:00	Sadowski R.: *Anion-exchange chromatographic packings for analysis of heparin anticoagulants* (p. 30)
11:00–11:15	Fenzl Ch.: *Colloidal photonic crystal based sensor for ionic strength* (p. 32)
11:15–11:30	Pažitná A.: *The enantiomer distribution of major chiral volatile organic compounds in juniper flavoured distillates* (p. 34)
11:30–11:45	Bierhanzl V. M.: *Progress in analysis of phospholipid groups by instrumental methods* (p. 35)

11:45–12:30 **Lunch**

Session 3 ▪ chairperson: Pavlína Novotná

12:30–12:45 Płotka J. M.: *A novel methodology for the enantiomeric resolution of methamphetamine, its precursors and intermediates by GC-MS* (p. 37)

12:45–13:00 Habartová L.: *Diagnostic potential of chiroptical spectroscopy for type 1 diabetes mellitus* (p. 41)

13:00–13:15 Kotora P.: *Saliva analysis by GC-MS/MS techniques* (p. 44)

13:15–13:30 Klusáčková M.: *Phthalocyanine modified electrode utilized as electrochemical sensor for detection of unsaturated hydrocarbons* (p. 46)

13:30–13:45 Červinková B.: *Development of UHPLC/MS-MS method for determination of 8-hydroxy-2'-deoxyguanosine in human urine* (p. 48)

13:45–14:00 **Coffee Break**

Session 4 ▪ chairperson: Andrea Spevak

14:00–14:15 Kanakaki Ch.: *Method development for the analysis of gaseous emissions from lithium-ion cells used under extreme conditions* (p. 50)

14:15–14:30 Kupska M.: *Determination of terpene profile in Cape gooseberry (*Physalis peruviana *L.) with use of HS-SPME/GC×GC-TOFMS method* (p. 52)

14:30–14:45 Ashrafi A.: *Determination and mechanism study of antiviral drug Fosamprenavir using carbon paste electrode in the presence of Triton X-100* (p. 55)

14:45–15:00 Staňková M.: *Characterization of capillary monolithic columns for hydrophilic interaction chromatography* (p. 57)

15:00–15:15 Vozka J.: *Chiral stationary phases based on derivatized cyclofructan* (p. 60)

15:15–15:30 **Coffee Break**

Session 5 ▪ chairperson: Hana Adamusova

15:30–15:45 Słomińska M.: *The application of TD-GC-FID technique for production of matrix-free reference materials* (p. 64)

15:45–16:00 Mansfeldová V.: *Fast potentiometric detection system utilizing interface of two immiscible electrolytes for application in flow analysis* (p. 68)

16:00–16:15 Samková D.: *Gas chromatographic analysis of saccharides in urine* (p. 70)

16:15–16:30 Šlechtová T.: *The comparison of kinetic constants of different sequence motifs digested by trypsin* (p. 72)

16:30–17:00 **Sponzors' Presentations**

17:00 **Get-Together Party**

Tuesday, September 24, 2013

Session 6 ▪ chairperson: Juraj Adamuščin

8:30–8:45 Wasielewska M.: *Occurrence and determination of volatile fatty acids in landfill leachate and gas* (p. 74)

8:45–9:00 Deáková Z.: *Comparison of separation amino acid enantiomers on teicoplanin and teicoplanin aglycone* (p. 77)

9:00–9.15 Novotná P.: *Vibrational and electronic circular dichroism study of bilirubin interactions with model membranes and serum albumin* (p. 79)

9:15–9:30 Jędrkiewicz R.: *3-Monochloropropane-1,2-diol in infant foods and human breast milk: determination by GC-MS* (p. 82)

9:30–9:45 Ksandrová I.: *Voltammetric determination of caffeine at carbon paste-based electrodes. An initial study with unmodified carbon paste.* (p. 85)

9:45–10:00 Janás P.: *Hydrophilic interaction chromatography on the diol-based columns* (p. 87)

10:00–10:15 **Coffee Break**

Session 7 ▪ chairperson: Maciej Tankiewicz

10:15–10:30 Lankova D.: *Multi-analyte method for the determination of organo-halogen contaminants in dust* (p. 91)

10:30–10:45 van der Weerd B.: *A conductivity probe for determination of the carbon dioxide tension at the oxygenator exhaust outlet during extracorporeal membrane oxygenation* (p. 95)

10:45–11:00 Safaei Z.: *The influence of mobile phase pH on the analysis of oligonucleotides by RP-HPLC* (p. 98)

11:00–11:15 Rybínová M.: *Comparison of UV-photochemical and chemical volatile compounds generation for the determination of selenium by AAS* (p. 100)

11:15–11:30 Adamuščin J.: *Analysis of BTEX in water samples by large volume injection gas chromatography* (p. 103)

11:30–11:45 Müllerová M.: *Overall complexation. A usefull description of dual-cyclodextrin separation systems in capillary electrophoresis.* (p. 105)

11:45–12:30 **Lunch**

Session 8 ▪ chairperson: Magda Staňková

12:30–12:45 Pietrzak L.: *Chromatographic analysis of oligonucleotides* (p. 108)

12:45–13:00 Markechová D.: *Simultaneous determination of caffeine, caramel III, and riboflavin in energy drinks by synchronous fluorescence technique* (p. 110)

13:00–13:15 Geryk R.: *Comparison of enantioseparation potential of chiral stationary phases based on immobilized polysaccharides in reversed phase mode* (p. 113)
13:15–13:30 Zavazalova J.: *Voltammetric determination of selected aminonaphthalenes at different electrode surfaces* (p. 115)
13:30–13:45 Novotná H.: *Utilization of ambient mass spectrometry for saffron authentication* (p. 117)

13:45–14:00 **Coffee Break**

Session 9 ▪ chairperson: Peter Kotora
14:00–14:15 Franc M.: *Different types of frits in packed capillary columns* (p. 120)
14:15–14:30 Stocka J.: *Analysis of pesticide residues in fruits and vegetables using QuEChERS sample preparation method and gas chromatography with electron capture detection* (p. 123)
14:30–14:45 Zlámalová M.: *Development of poly(methylene blue) modified electrode sensor for hydrogen sulfide* (p. 126)
14:45–15:00 Spevak A.: *Use of molecularly imprinted polymers for determination of biologically active compounds in plant samples* (p. 128)
15:00–15:15 Stupak M.: *New fast strategy to determine ethanol in alcoholic beverages and overall quality check* (p. 130)

15:15–15:30 **Coffee Break**

Session 10 ▪ chairperson: Petr Kozlík
15:30–15:45 Tankiewicz M.: *'Green' multi-residue methods for the determination of high importance currently used pesticides in environmental samples* (p. 133)
15:45–16:00 Machyňák Ľ.: *Determination of arsenic in the waste waters by flow-through coulometry* (p. 136)
16:00–16:15 Vyviurska O.: *Characterization of volatile compounds in alcoholic beverage 'Oskurošovica'* (p. 137)
16:15–16:30 Ston M.: *Pressure modulator development and optimization for applications in comprehensive gas chromatography* (p. 140)

16:30 **Closing Ceremony**

Contributions

Characterization and Comparison of Cyclofructan and Amide-based Stationary Phases in Hydrophilic Interaction Liquid Chromatography

Petr Kozlík[a,*], Květa Kalíková[b], Eva Tesařová[b], Zuzana Bosáková[a]

[a] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague 2, Czech Republic* ✉ *kozlipet@centrum.cz*
[b] *Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague 2, Czech Republic*

Keywords
amide-based stationary phase
cyclofructan-based stationary phase
hydrophilic interaction liquid chromatography
linear free energy relationship
peptides

Hydrophilic interaction liquid chromatography (HILIC) attracts more attention since it offers an alternative approach for the separation of highly polar compounds. The primary retention mechanism in HILIC is believed to be analyte partitioning between the bulk eluent and the water-rich layer that is partially immobilized on the surface of the stationary phase [1]. Interactions between analytes and the functional groups of the stationary phase like dipole-dipole, hydrogen bonding and electrostatic interactions participate in HILIC systems [2]. Both the partition and adsorption mechanisms are thus believed to contribute to the overall retention of polar compounds in HILIC [2]. The predominant retention mechanism in HILIC separation is not unequivocal and it can differ when different analytes and/or different stationary and mobile phases are used [3, 4]. Any polar chromatographic surface can be used for HILIC separations. Various stationary phases are commercially available however, a development of new materials (sorbents) suitable for HILIC systems still continues.

In this work, two cyclofructan-based stationary phases (native cyclofructan 6 (CF6) (Frulic-N column) and isopropyl derivatized CF6 (Larihc CF6-P column)) and two amide-based stationary phases (XBridge Amide and TSK gel Amide-80 columns) were compared and characterized in HILIC mode. For this purpose,

peptides were chosen as model analytes. Mobile phases consisted of acetonitrile and ammonium acetate buffer (20 mM ammonium acetate), pH = 4.00, in various ratios. Model designed by Snyder-Soczewinski [5] was used for an indication whether partitioning or adsorption is the dominating retention mechanism. To investigate more subtle differences of the chosen stationary phases we utilized linear free energy relationship (LFER) method. Linear free energy relationship is a statistical model relating retention of a given analyte to its physicochemical and structural parameters. The main benefit of the LFER model lies in its ability to describe the contributions of individual types of molecular interactions to the retention process [6]. The detailed study of chromatographic behavior of peptides revealed that multimodal retention mechanism is present in systems with these stationary phases. The LFER method has revealed which type of molecular interactions play a role in retention process in studied separation systems. The LFER results indicated that significant interactions affecting retention and separation in all separation systems studied in this work are the same, namely hydrogen bond acidity (with positive regression coefficient values) and dispersion interactions (with negative values). Minor impact on retention process had dipolarity/polarizibility in the case of cyclofructan-based stationary phases and hydrogen bond basicity in the case of amide-based stationary phases.

Acknowledgments

The financial support of the Grant Agency of Charles University in Prague, SVV 267215, the Ministry of Education, Youth and Sports of the Czech Republic, project MSM0021620857 and KONTAKT AM 2010 project LH11018 is gratefully acknowledged.

References

[1] Alpert A. J.: *J. Chromatogr. A* **499** (1990), 177–196.
[2] Chirita R., West C., Zubrzycki S., Finaru A., Elfakir C.: *J. Chromatogr. A* **1218** (2011), 5939–5963.
[3] Hemstrom P., Irgum K.: *J. Sep. Sci.* **29** (2006), 1784–1821.
[4] Buszewski B., Noga S.: *Anal. Bioanal. Chem.* **402** (2012), 231–247.
[5] Snyder L. R., Poppe H.: *J. Chromatogr.* **184** (1980), 363–414.
[6] Janečkova L., Kalíkova K., Vozka J., Armstrong D. W., Bosáková Z., Tesařová E.: *J. Sep. Sci.* **34** (2011), 2639–2644.

Use of White Cabbage for Phytoremediation Processes

Marzena Szczygłowska*, Piotr Konieczka

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Gabriela Narutowicza 11/12 Street , 80-233 Gdansk, Poland ✉ marzena.sowinska@wp.pl

Keywords
AAS
cabbage
cadmium
phytoremediation
zinc

Forty or fifty years ago people did not think of the possible consequences of their decisions. All that mattered was that the standard of living went up. The upshot was the rapid expansion of transport, industry, and the economy in general. On the one hand, people achieved their aims in that their demand for all manner of consumer goods was sated, but on the other, their imprudent actions became the chief culprits responsible for the pollution of the air and surface/ground waters and for the decline in soil fertility [1]. Unlike substances polluting the air and water, contaminants can remain in the soil for a very long time, even for hundreds of years. This is all the more dangerous, since the soil takes up air pollutants arriving in rainwater, and also the pollutants in the runoff waters flowing over it.

Heavy metals are an important group of substances polluting waters and soils. The hazard they pose is due to their chemical nature: they are non-biodegradable. Metals are present in all soils, even those regarded as uncontaminated. But depending on the concentrations in which they occur, they may be harmful to the soil, and as a consequence, to vegetation, animals, water and human health.

Soil remediation has been a topic of research worldwide for many years. But traditional technologies have turned out to be extremely costly and time-consuming, and frequently require other, not always environmentally friendly, chemicals to be introduced into the soil. Hence, more effective and more economical technologies are constantly being sought. Among them is phytoremediation which are based on the activity of living organisms.

Nowadays, phytoremediation is variously defined in the literature, depending on the type of matrix intended for remediation (water, soil, air) and the type of

contaminant to be removed (organic/inorganic) [2]. The efficient clean-up of soil relies on plants known as hyperaccumulators, which are capable of taking up exceptionally large amounts of metals without visible toxicity symptoms [3]. The most effective plants used in the process of phytoremediation come from *Brassicaceae* family. Problems with use the plants recognized as hyperaccumulators is that, the plants needs to be harvested shortly after the plants becomes mature, which causes problems of disposal of obtained biomass. When these plants are dried, they easily crumble and flake off, greatly reducing the yield obtained, and the rest of the plant residues are a source of secondary emissions of toxic substances [4].

Quite a large biomass and the lack of difficulties after harvesting are advantages described for different types of cabbage. Our study reveals the usefulness of the white cabbage for the purification of the soil from zinc and cadmium by phytoextraction. Two cabbage (*Brasica oleracea* subsp. *capitata* f. *alba*) cultivars: early cv. Ditmarska Najwcześniejsza, late cv. Kamienna Głowa were used. In this research used crops grown under controlled conditions; where plants grown in the greenhouse of phytotron of University of Agriculture in Kraków in pots (10 L) filled with the local soil taken from the arable layer (0–20 cm). Cultivation was contaminated with a known amount of heavy metals in milligrams per kilogram dry weight of soil: Zn to 50 (Zn1) or 200 (Zn2) and in the case of Cd to 10 (Cd1) or 40 (Cd2). Control pots contained the same soil, but without addition of either cadmium or zinc. Each experimental group of plants consisted of four replicates.

For metal determinations, the air-dried soil samples and lyophilized cabbage samples were mineralized in a microwave-assisted Anton Paar mineralizator. Mineralization was performed at 240 °C and 60 bar for 0.5 g portions of cabbage or soil mixed with 5 mL 65% HNO_3 + 2 mL H_2O_2 or 7 mL HNO_3 as a mineralizing solution, respectively. After digestion, the acidic solutions were transferred into 25 mL PMP volumetric flask and brought to volume with deionized water. Metal were determined using AAS techniques such as:

- flame AAS (Zn, Cd),
- electrothermal AAS (lower content Cd).

The result for the experiments conducted are shown in Fig. 1. Based on the results can be concluded that:

- There is relationship between the content of zinc and cadmium of samples of soils, leaves and stems.
- The contents of cadmium and zinc, determined in sample leaves and stems of both cultivars exposed to different concentrations of the studied metals. In the case cultivars Kamienna Głowa, zinc and cadmium are mainly cumulated in the leaves.
- In the sample of leaves both varieties of cabbage, grown in soil spiked with Cd1, determined high concentrations this metal. This may prove that, cabbage can be use to phytoremediation of soil from cadmium.

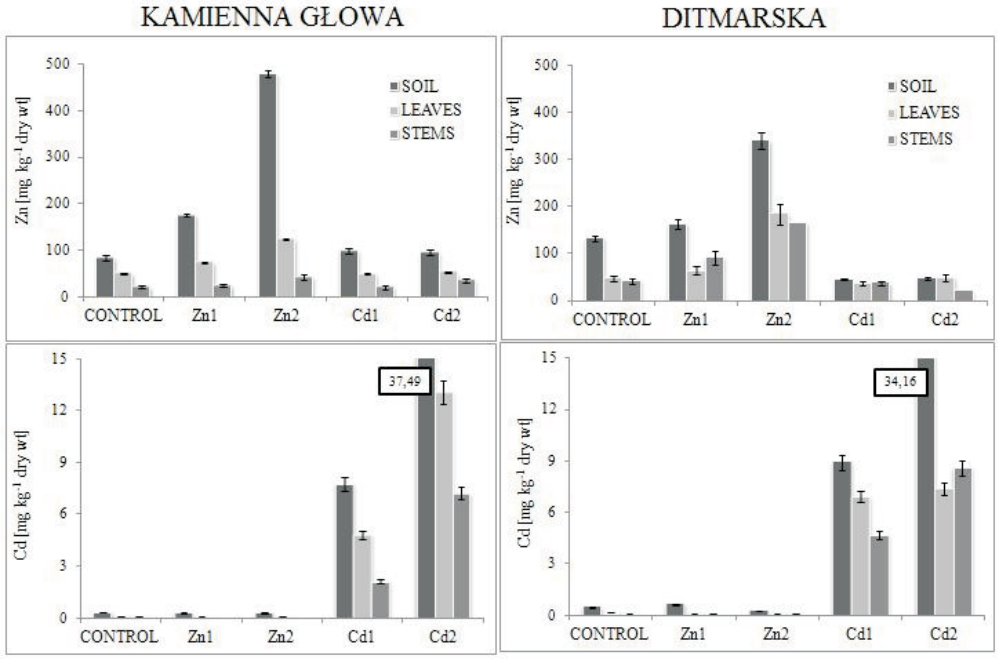


Fig. 1. Contents of cadmium and zinc (mg kg^{-1} dry wt) in samples of two cabbage cultivars, early Ditmarska Najwczesniejsza and medium-late Kamienna Głowa and in corresponding soil sample collected after harvest of plants.

The level of zinc content in the cabbages cultivated on the soil with average zinc content 200 mg kg^{-1} ranged 20–100 mg kg^{-1} dw, whereas for soil with concentration 500 mg kg^{-1} ranged 40–190 mg kg^{-1} dw. In the case cadmium, the obtained values were: 7 mg kg^{-1} in soil, 2–7 mg kg^{-1} dw in cabbage and 40 mg kg^{-1} in soil, 7–13 dw in cabbage. The metals content determined in different tissues (leaves and stems) of both cabbage and the yields of cabbage crops declared by registered producers of seedlings to range from 150 to 300 tons per hectare were the basis for calculating the output of pollutants from the environment. In the case of zinc, with a single crop of white cabbage (assuming that the cabbage is 20% dry weight and 80% water) was estimated to amount to 2–4 kg ha^{-1} for plants grown in normal soils (C). The plants grown in heavily zinc contaminated soils (Zn2 samples) might be capable of removing 5–10 kg ha^{-1} in the case of Kamienna Głowa and 10–20 kg ha^{-1}, in the case of Ditmarska. When it comes to cadmium, according to the results from the phytotron experiment, the estimated output of this pollutant from noncontaminated (C samples) and strongly Cd-contaminated (Cd2 samples) soils may vary from 0.006 to 0.012 kg ha^{-1} to 0.6 to 1.2 kg ha^{-1}, respectively assuming the low to high cabbage crop yield.

The results lead to the conclusion that white cabbage may represent a very promising crop for phytoremediation of farmlands polluted with either biogenic or toxic heavy metals.

Acknowledgments

The project 'The exploitation of white cabbage for phytoremediation and biofumigation of soils (AGROBIOKAP)' was cofinanced by the European Union from the European Regional Development Fund in a framework of the Innovative Economy Operational Programme 2007–2013. This research work was supported by the European Social Fund, the State Budget and the Pomorskie Voivodeship Budget according to the Operational Programme Human Capital, Priority VIII, Action 8.2, Under-action 8.2.2: 'Regional Innovation Strategy' within the system project of the Pomorskie Voivodeship 'InnoDoktorant – Scholarships for PhD students, Vth edition'. Thanks are due to Renata Bączek-Kwinta of the of University of Agriculture in Krakow, Poland, for her interest in the study.

References

[1] Weber J., Karczewska. A.: *Geoderma* **122** (2004), 105–107.
[2] Garbisu C., Alkorta I.: *Bioresour. Technol.* **77** (2001), 229–236.
[3] Kusznierewicz B., Bączek-Kwinta R., Bartoszek A., Piekarska A., Huk A., Manikowska A., Antonkiewicz J., Namieśnik J., Konieczka P.: *Environ. Toxicol. Chem.* **12** (2012), 1–8.
[4] Szczygłowska M., Piekarska A., Konieczka P., Namieśnik J.: *Int. J. Mol. Sci.* **12** (2011), 17760–7771.

Determination of Fluorotelomer Alcohols in Food Contact Materials and Food

Michala Slováková*, Jana Pulkrabová, Darina Lanková, Kamila Kalachová, Jana Hajšlová

Department of Food Analysis and Nutrition, Institute of Chemical Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic ✉ *Michala.Kockovska@vscht.cz*

Keywords
fluorotelomer alcohols
GC-TOF-MS
headspace solid phase microextraction

Perfluoroalkyl and polyfluoroalkyl substances represent a large and heterogeneous group of anthropogenic chemicals. They have unique properties of being hydrophobic and oleophobic at the same time and resistant towards degradation when exposed to high temperatures, chemicals or abrasion. Since 1950, perfluoroalkyl and polyfluoroalkyl substances, together with surfactants and polymers made with the aid of perfluoroalkyl and polyfluoroalkyl substances have been widely used in numerous industrial and commercial applications (e.g., lubricants and greases, cleaning agents, fire-fighting foams, polymers and surfactants, pigments, pesticides, pharmaceuticals) [1]. Due to their properties they are used for surface treatment of textiles, plastics and paper and board food contact materials to prevent them from being soiled or from soaking up fat or water. Their stability makes them convenient for high temperature applications, for instance when the foods in respective packaging are intended to be microwave heated [2].

The coating perfluoroalkyl and polyfluoroalkyl substance based additives for the surface treatment of paper and board materials contain as side groups fluorotelomer alcohols, which can potentially split off. The probability of such reactions increases with growing temperatures. On this account baking paper, muffin cups, popcorn bags etc. may be significant source of fluorotelomer alcohols that can migrate from the treated material to the food item. Moreover, depending on the type of food and heating conditions, fluorotelomer alcohols can be also released from polyfluorinated alkyl phosphate esters that are main components of the paper and board coating mixtures [3]. The predominant molecules in the

paper coating additives contain two C8 or C10 perfluoro groups. Several studies [4–7] have shown that fluorotelomer alcohols can undergo the degradationin the atmosphere and some organisms to perfluorocarboxylic acids that have been correlated to health effects such as endocrine disruption and metabolic changes [8].

Although the occurrence of polyfluorinated surfactants in food contact materials represent health concern issue, there are only few studies dealing with analysis of such chemicals in paper and board and the penetration of fluorotelomer alcohols and polyfluorinated alkyl phosphate esters to food items [9–11]. In the first phase of analysis, extraction of fluorotelomer alcohols with organic solvents (e.g. methanol, ethyl acetate) is commonly used. Unfortunately, a range of co-extracts is present in the extracts and, due to the high volatility of fluorotelomer alcohols, the extract must be handled with special care to avoid analytes losses. Fiedler et al. [12] developed a headspace gas chromatography-mass spectrometry (analyzer operated in electron ionization mode) method for screening of fluorotelomer alcohols (2-perfluorohexylethanol, 2-perfluorooktylethanol, and 2-perfluorodecylethanol) in food contact materials.

The aim of this study was to develop a simple and effective method for determination of fluorotelomer alcohols not only in baking paper and other food contact materials but also in food represented by bakery products. For this purpose, muffins were baked in perfluoroalkyl and polyfluoroalkyl substance containing paper cups. Simple, solvent free headspace solid phase microextraction (HS-SPME) technique was selected for analysis of volatile fluorotelomer alcohols. To obtain high sensitivity and good repeatability of determination of fluorotelomer alcohols in a paper matrix, careful optimization of HS-SPME was required. Therefore, the main factors influencing the analytical process (fiber type, headspace volume, extraction time, temperature, sample amount, sample ionic strength and agitation speed) were investigated. The identification of analytes was performed with gas chromatography coupled to a mass spectrometric detector with a high resolution time-of-flight mass analyzer using an Agilent 7200 GC/Q-TOF system in positive chemical ionization mode. Mass spectra were recorded in a range of m/z = 30–1000. The example of analysis is shown in Fig. 1. Contrary to electron ionization, high m/z value ions were obtained thus fairly better selectivity of detection could be achieved. The amount of four fluorotelomer alcohols (2-perfluorobutylethanol, 2-perfluorohexylethanol, 2-perfluorooktylethanol, 2-perfluorodecylethanol) was determined in the sample of paper experimentally impregnated with the fluorinated surfactants technical mixture. This sample was used for the optimization experiments. The quantification was performed using the standard addition method. Unfortunately, analytical standards are available only for four above-mentioned fluorotelomer alcohols, so only the content of these four compounds could be quantified in the food contact material samples. Using StableFlex DVB/CAR/PDMS 50/30 μm SPME fiber the achieved limits of quantification were 2, 2, 2 and 5 ng/g of paper for these compounds, respectively. Other substances such as 2-perfluorododecylethanol, 2-perfluorotetradecylethanol, and 2-perfluorohexadecylethanol were

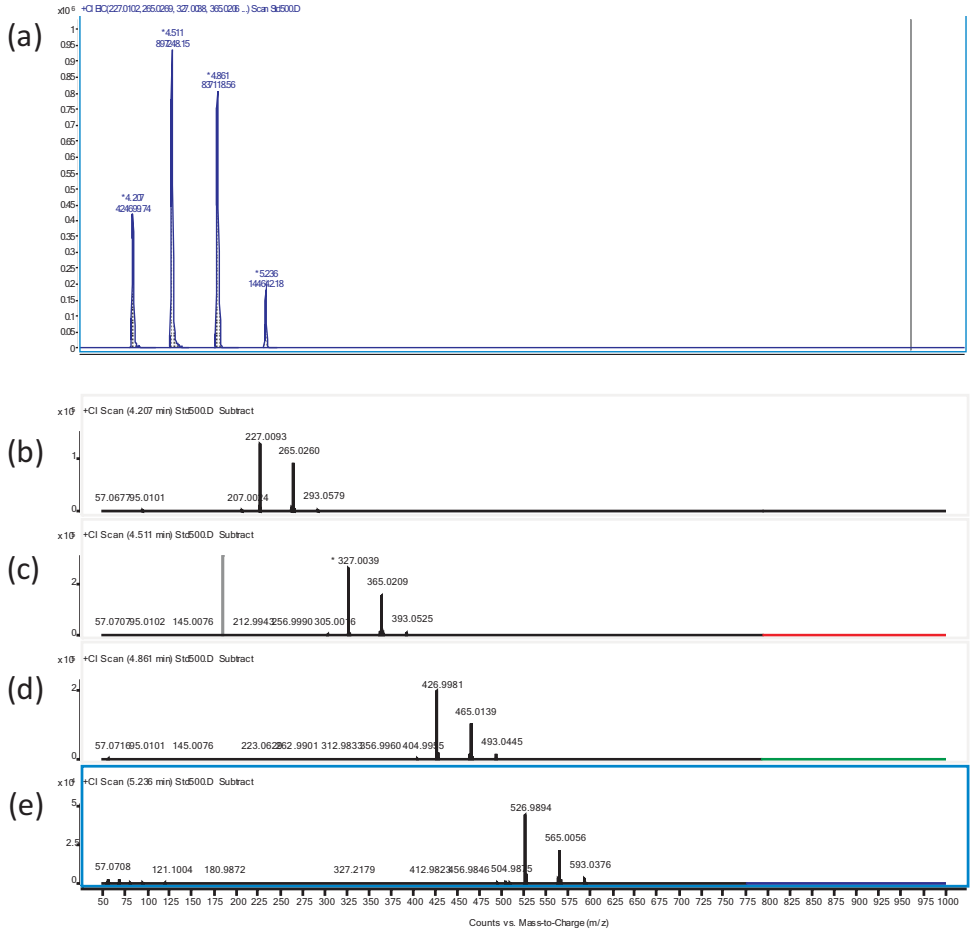


Fig. 1. (a) The chromatographic record of monitored analytes obtained by HS-SPME-GC-TOFMS analysis of the standard solution of concentration 500 ng mL^{-1} in methanol. Spectra of (b) 2-perfluorobutylethanol, (c) 2-perfluorohexylethanol, (d) 2-perfluorooktylethanol, and (e) 2-perfluorodecylethanol. Mass spectra recorded in a range of m/z = 30–1000.

present in some highly impregnated samples as well; they were identified based on exact masses of their molecular ions.

The method was subsequently modified to obtain optimal performance parameters for determination of fluorotelomer alcohols in different matrix bakery products (muffins, etc).

In summary, using the newly developed HS-SPME-GC-HRTOFMS method, fluorotelomer alcohols can be rapidly measured in baking papers, food packaging materials and also in some types of food, e.g. muffins. It will be used in following monitoring study of food and paper and board food contact materials available at the Czech market. Also the migration of fluorinated compounds from paper to food will be studied in a greater detail.

References

[1] Kissa E.: *Surfactant Science Series: Fluorinated Surfactants and Repellents.* 2nd ed. New York, Marcel Dekker 2001.
[2] *Trier X.: Polyfluorinated Surfactants in Food Packaging of Paper and Board.* Copenhagen, Dissertation Thesis on University of Copenhagen 2011.
[3] Fengler R., Schlummer M., Gruber L., Fiedler D., Weise N.: *Organohalogen Compd.* **73** (2011), 939–942.
[4] Dinglasan-Panlilio M.J.A., Mabury S.A.: *Environ. Sci. Technol.* **40**(2006), 1447–1453.
[5] Ellis D., Martin J.W., De Silva A.O., Mabury S.A., Hurley M.D., Andersen M.P.S., Wallington T.J.: *Environ. Sci. Technol.* **38**(2004), 3316–3321.
[6] Hagen D.F., Belisle J., Johnson J.D., Venkateswarlu P.: *Anal. Biochem.* **118** (1981), 336–343.
[7] Martin J.W., Mabury S.A., O'Brien P.J.: *Chem. Biol. Interact.* **155** (2005), 165–180.
[8] Nelson J.W., Hatch E.E., Webster T.F.: *Environ. Health Perspect.* **118** (2010), 197–202.
[9] Sinclair E., Kim S.K., Akinleye H.B., Kannan K.: *Environ. Sci. Technol.* **41** (2007), 1180–1185.
[10] Trier X., Granby K., Christensen J. H.: *Environ. Sci. Pol. Res.* **18** (2011), 1108–1120.
[11] Herzke D., Olsson E., Posner S.: *Chemosphere* **88** (2012) 980–987.
[12] Fiedler D., Schlummer M., Gruber L., Wolz G.: *2nd International Workshop Fluorinated Surfactants: New Developments, 2010*. Abstract Book, C 08.

Study of Differential Pulse Voltammetric Behavior of Selected Nitro-Compounds at a Hanging Mercury Drop Electrode in the Presence of Double-Stranded DNA

Eva Horáková*, Vlastimil Vyskočil, Jiří Barek

University Research Centre UNCE 'Supramolecular Chemistry' & UNESCO Laboratory of Environmental Electrochemistry, Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *eva.horakova@natur.cuni.cz*

Keywords
double-stranded DNA
hanging mercury drop electrode
aromatic nitro compounds
voltammetry

Aromatic nitro compounds constitute a group of chemicals with a wide spectrum of mutagenic and/or carcinogenic properties [1–3]. They are known as environmental pollutants that are formed in diesel and petrol engines during the combustion of fuels and in the atmosphere by reactions of their parent aromatic hydrocarbons with nitrogen oxides [1]. Because of their omnipresence in the environment, there is an enhanced interest in the monitoring of their environmental levels and in studying their biological effects [1, 2, 4–7]. For instance, mutagenicity screening studies are of great importance because about half of all carcinogenic chemicals tested in animals are mutagens that damage DNA in microbial short-term tests [8].

There are many electrochemical studies on interactions of DNA with some environmental pollutants or drugs [9, 10]. Three types of interactions of organic compounds with DNA are usually distinguished: (i) an intercalation of planar aromatic molecules between DNA base pairs, (ii) a binding into major and minor grooves of double-stranded DNA (*ds*DNA), and (iii) an electrostatic interaction of positively charged molecules with negatively charged DNA phosphate groups [10]. Different interactions are connected with different shifts in the peak

potential of the analyte in the absence or presence of *ds*DNA in the measured solution when voltammetric techniques (e.g., cyclic voltammetry or differential pulse voltammetry) are used. While a peak potential is shifted in the positive direction when the analyte binds to DNA by intercalation between the stacked base pairs of *ds*DNA, the peak potential is shifted in the negative direction when the interaction with DNA occurs by electrostatic attraction (interaction with the negatively charged nucleic sugar-phosphate structure) [10]. Therefore, it is possible to predict the type of the interaction of a test substance with *ds*DNA. Moreover, the fact that those interactions can cause the opposite potential shift can be used to separate voltammetric signals of studied compounds.

In this work, the effect of the presence of *ds*DNA in the solution (c_{DNA} = = 2–2500 μg mL^{-1}) on the electrochemical behavior of selected aromatic nitro compounds was investigated using differential pulse voltammetry at a hanging mercury drop electrode. Selected substances were as follows: 2-nitrobiphenyl, 3-nitrobiphenyl (both are not listed by IARC as proven or potential carcinogens), 4-nitrobiphenyl (it is listed in IARC Group 3, not classifiable as to its carcinogenicity to humans [11], however, it induces carcinomas on animals [12], and it is metabolically converted to 4-aminobiphenyl, which is carcinogenic to humans, IARC Group 1 [13]), 4-nitrophenol (it is not listed by the IARC, however, nitrobenzene is its metabolite [14]), nitrobenzene (it is listed in IARC Group 2B possibly carcinogenic to humans [15], and it is used in the production of aniline, precursor of dyes, pharmaceuticals, etc.).

All stock solutions of the test compounds ($c = 1\times10^{-3}$ mol L^{-1}) were prepared by dissolving pure substances in ethanol. Double-stranded DNA from salmon sperm was stored at 4 °C and its stock solutions (c_{DNA} = 10 mg mL^{-1}) were prepared by dissolving *ds*DNA in an aqueous mixture of EDTA ($c_{EDTA} = 1\times10^{-3}$ mol L^{-1}) and tris(hydroxymethyl)aminomethane hydrochloride ($c_{Tris.HCl}$ = 0.01 mol L^{-1}). All samples were prepared by adding an appropriate volume of a stock solution into a 10-mL volumetric flask, ethanol was added to a total volume of 1 or 5 mL, and the solution was filled up to the mark with a 0.25 mol L^{-1} phosphate buffer of pH = 7.0.

Initially, the influence of the composition of a supporting electrolyte was studied, since some of the studied analytes require a higher content of alcohol to be present in the solution phase because of their limited solubility in water. The effect was investigated in supporting electrolytes methanol–phosphate buffer and ethanol–phosphate buffer (in volume ratios of 1:9 and 1:1), and the influence of used organic solvent was tested. The testing criteria were as follows: a maximum width of the potential window, a minimum content of trace impurities, and a minimum background current. Thereafter, the influence of the matrix used in the *ds*DNA stock solution (i.e., the EDTA–Tris-HCl solution) was studied in the solution of 4-nitrobiphenyl ($c = 1\times10^{-5}$ mol L^{-1}) in ethanol–phosphate buffer (1:9 and 1:1); the content of the EDTA–Tris-HCl solution was subsequently increased, and the changes in voltammetric behavior of 4-nitrobiphenyl were observed.

Interactions of the studied compounds with *ds*DNA were investigated in the ethanol-phosphate buffer (1:9 and 1:1) medium. On the basis of the peak potential shifts (see the introduction of this text) [10], it was possible to estimate what type of interaction occurs. Moreover, based on the obtained results, it is suggested that the content of ethanol affects the rate of the reduction of the analyte, it causes differences in the peak heights (1:9 versus 1:1), and it also affects the ionic strength of the supporting electrolyte.

After the study of the influence of the presence of *ds*DNA on the voltammetric behavior of the studied compounds, a possibility to use the different interactions of the studied compounds with *ds*DNA for a separation of their voltammetric signals was investigated, too.

Acknowledgments

This research was carried out within the framework of the Specific University Research (SVV 267215). Financial support from the Grant Agency of the Czech Republic (Project P206/12/G151) is gratefully acknowledged.

References

[1] Moreira J.C., Barek J.: *Quím. Nova* **18** (1995), 362–367.
[2] Moller L.: *Environ. Health Perspect.* **102** (1994), 139–146.
[3] Jacob J., Karcher W., Belliardo J.J., Dumler R., Boenke A.: *Fresenius J. Anal. Chem.* **340** (1991), 755–767.
[4] Vyskočil V., Barek J.: *Curr. Org. Chem.* **15** (2011), 3059–3076
[5] Vyskočil V., Barek J.: *Crit. Rev. Anal. Chem.* **39** (2009), 173–188.
[6] Prchal V., Krejčová J., Vyskočil V., Pecková K., Fischer J., Zima J., Barek J.: *Int. J. Electrochem. Sci.* **8** (2013), 2524–2535.
[7] Tokiwa H., Nakagawa R., Horikawa K., Ohkubo A.: *Environ. Health Perspect.* **73** (1987), 191–199.
[8] Ames B.N.: *Environ. Mol. Mutagen.* **14** (1989), 66–77.
[9] Yola M.L., Özaltın N.: *J. Electroanal. Chem.* **653** (2011), 56–60.
[10] Vyskočil V., Blašková M., Hájková A., Horáková E., Krejčová Z., Stávková K., Wang J.: Electrochemical DNA biosensors – Useful diagnostic tools for the detection of damage to DNA caused by organic xenobiotics (A review). In: *Sensing in Electroanalysis*. *Vol. 7.* Kalcher K., Metelka R., Švancara I., Vytřas K. (edits.). Pardubice, University Press Centre 2012, p. 141–162.
[11] *Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents* (= IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 4). Lyon, IARC 1974.
[12] Ning S., Xiaobai X.: *Carcinogenesis* **18** (1997), 1233–1240.
[13] *Some Aromatic Amines, Organic Dyes, and Related Exposures* (= IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 99). Lyon, IARC 2010.
[14] http://whqlibdoc.who.int/ehc/WHO_EHC_230.pdf (accessed 4.4.2012).
[15] *Diesel and Gasoline Engine Exhausts and Some Nitroarenes* (= IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 46.). Lyon, IARC 1989.

HPLC Enantioseparation of Underivatized Amino Acids Using Cyclofructan Chiral Stationary Phases

Jakub Moravčík*, Katarína Hroboňová

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia ✉ *moravcikjakub@gmail.com*

Keywords
amino acids
chiral separations
cyclofructan-based chiral selectors
high performance liquid chromatography
thermodynamic study

Amino acids are biologically active compounds that have many functions in living organisms. They are the basic structural units of proteins. One of the most typical features of the proteinogenic α-amino acids is the optical activity related to the presence of a chiral carbon atom [1]. Enantiomers of amino acids and other organic compounds displaying the chiral character show diverse physiological activity in biological systems. As a consequence, the chiral separations of amino acids and other optically active compounds have been of great interest. Enantioseparations are largely realized by the methods of chromatography that may also be used in the process of asymmetric synthesis to determine the enantiomeric excess [2].

This study is concerned with the separation of underivatized amino acid enantiomers by high performance liquid chromatography. Aromatic-derivatized and aliphatic-derivatized chiral stationary phases of cyclofructan 6 and cyclofructan 7 were chosen for the HPLC separation of the target group consisting of α-amino acid racemic mixtures. The ability of this type of chiral selectors to recognize optical isomers was tested in the polar–organic separation mode with the selected mobile phase consisting of methanol/acetonitrile/acetic acid/triethylamine. Based on the results, the isopropylcarbamate cyclofructan 6 chiral stationary phase was found to be the most effective chiral selector for the enantioseparation of underivatized amino acids. Enantioseparation of selected analytes was achieved using this type of cyclofructan based chiral selector in polar organic separation mode. High performance liquid chromatographic enantioseparation of selected substances was significantly influenced by the mobile

phase composition. With the increasing value of methanol volume fraction in mobile phase, the values of the retention factors of the racemic mixtures of α-amino acids were decreasing. Although the highest values of the resolution factor were achieved in the mobile phase consisting of methanol/acetonitrile/acetic acid/triethylamine (50/50/0.3/0.2, v/v/v/v), this mobile phase composition resulted in the enantioseparation of a smaller group of the studied analytes. The mobile phase consisting of methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2, v/v/v/v) was used for experimental measurements in the following parts of the study, since this volume ratio of components in mobile phase resulted in enantioseparation of all studied analytes. In the next part of this study, the detection limit values were compared to the detection limits for various ways of the detection of the separated enantiomers. Apart from the spectrophotometric determination (λ_{max} = 210 nm), both polarimetric detector and circular dichroism detector were used. Using these chiral detectors, the limit of detection and limit of quantification values were ten times lower, as compared to the UV detector. Analytic column temperature has a strong effect on the elution characteristics. Chiral separations of underivatized α-amino acids were realized in the temperature range from 0 °C to 35 °C. Increasing the column temperature resulted in decreasing values of the resolution factor. The resolution factor of racemic mixtures of the selected group of analytes reached its maximum value at 0 °C. A calculation using the van't Hoff equation was applied in the thermodynamic study of HPLC enantioseparations of the aforementioned substances. Based on the values of enthalpic and entropic contribution to the change of Gibbs energy, enantioseparation of the studied substances was found to be an enthalpically controlled process. Consequently, the second eluting enantiomer and the chiral selector form a diastereomeric complex with a higher stability constant value than enantiomer with a shorter elution time. The acquired chromatographic conditions were applied in HPLC qualitative and quantitative analysis of methionine in a nutritional supplement sample.

Acknowledgments

This work was financially supported by grant of Scientific Grant Agency of the Ministry of Education of Slovak Republic No. 1/0164/11, and the Slovak Academy of Sciences.

References

[1] Ilisz, I., Aranyi, A., Pataj, Z., Péter, A.: *J. Pharm. Biomed. Anal.* **69** (2012), 28–41
[2] Subramanian, G.: *Chiral Separation Techniques : A Practical Approach.* 2nd Ed. Weinheim, Wiley–VCH 2000

Determination of 17β-Estradiol and 17α-Ethynylestradiol in Mouse Fertilizing M2 Medium

Hana Adamusová[a,*], Zuzana Bosáková[a], Petr Kozlík[a], Kateřina Hortová[b]

[a] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague Hlavova 8, 128 43 Prague 2, Czech Republic* ✉ *hana.adamusova@gmail.com*
[b] *Department of Zoology, Faculty of Science, Charles University in Prague Vinicna 7, 128 43 Prague 2, Czech Republic*

Keywords
estrogens
fertilizing medium
HPLC
MS/MS

Environmental estrogens (natural and synthetic) belong to a group of contaminants called endocrine disrupting chemicals [1]. These compounds have adverse effect on the endocrine system of human and animals. They can bind to natural estrogen receptors or block synthesis of endogenous hormones [2–4]. Estrogens are not accumulated in the environment and present at very low concentrations (ng/L), their impact on aqueous organisms and other wildlife and finally on humans could be significant [5]. 17β-Estradiol is a natural endocrine disruptor primarily of female origin. The synthetic 17α-ethynylestradiol is used as a main part of birth control pills. It was shown that estradiol and several estrogenic xenobiotics act towards an increase of germ cell apoptosis and a decrease of sperm count [6].

Capacitation is the key event in the study of sperm behavior prior to fertilization. Only capacitated sperm are sufficiently active and able to fertilize. In vitro experiments, simulating precisely an in vivo environment, are crucial for closer understanding of the process, when sperm gain the ability to fertilize an ovum. It is difficult to study these events in vitro. In order to monitor the effects of exogenous estrogen hormones such as 17β-estradiol and 17α-ethynylestradiol on sperm during in vitro capacitation, it is important to develop analytical method for determination of 17β-estradiol or the 17α-ethynylestradiol in M2 laboratory mouse in vitro fertilizing medium.

Our previous study [7] was focused on determination of free estriol in M2 medium during capacitation of mouse sperm in vitro by HPLC with UV detection. Due to the progressive UV absorption of BSA present in the fertilizing medium [8], the detection of estriol was carried out at 200 nm. Even though this method provided relatively high values of limit of detection and limit of quantification, it enabled only the starting concentration of 200 μg/L to be tested.

Based on the results reported for the separation of estriol by HPLC with UV detection [7], the reversed-phase separation system using SunFire C18 column as the stationary phase was selected for study of both the tested analytes. This column provided a high separation efficiency and symmetrical peak. For determination of 17β-estradiol and 17α-ethynylestradiol in M2 fertilizing medium, the UV detection was applied as the first one. Mixtures of acetonitrile and water in different volume ratios were tested as mobile phases. The optimization procedure was carried out with respect to obtain a sufficient sensitivity. However, neither 17β-estradiol nor 17α-ethynylestradiol was detected in any of tested mobile phases. The M2 medium is a complex mixture containing inorganic and organic components from which especially BSA (4.0 g/L) can cause difficulties during the separation process. At 200 nm, the signal of M2 medium was two orders of magnitude higher than the signal of analyte. Therefore, it was impossible to use the UV detection for this purpose and a tandem mass spectrometric detection, which can eliminate the matrix impact much better, was chosen.

Acknowledgments

Financial support from the MSMT CZ projects: MSM0021620857 and SVV2013-267215 is gratefully acknowledged.

References

[1] Lopez de Alda M. J., Barcelo D.: *J. Chromatogr. A* **892** (2000), 391–406.
[2] Della Seta D., Farabollini F., Dessi-Fulgheri F., Fusani L.: *Endocrinology* **149** (2008), 5592–5598.
[3] Safe S.H., Pallaroni L., Yoon K., Gaido K., Ross S., Saville B., McDonnellc D.: *Reproduction Fertility and Development* **13** (2001), 307–315.
[4] Golden R.J., Noller K.L., Titus-Ernstoff L., Kaufman R.H., Mittendorf R., Stillman R., Reese E.A.: *Crit. Rev. Toxicol.* **28** (1998), 109–227.
[5] Reinen J., Vriese E., Glatt H., Vermuelen N.P.E.: *Anal. Biochem.* **357** (2006), 85–92.
[6] Adeoya-Osiguwa S:A., Markoulaki S., Pocock V., Miligan S.R., Fraser L.R.: *Hum. Reprod.* **18** (2003), 100–107.
[7] Dvorakova-Hortova K., Honetschlaegerova M., Tesarova E., Vasinova J., Frolikova M., Bosakova Z.: *Folia Zoologica* **58** (2009), 75–81.
[8] Zhu L., Lee C.S., DeVoe D.L.: *Lab Chip* **6** (2006), 115–120.

Electrochemical Flow Biosensors Based on Silver Solid Amalgam Electrodes

Oksana Josypčuk[a, b, *], Jiří Barek[a], Bohdan Josypčuk[b]

[a] *Department of Analytical Chemistry, University research center UNCE 'Supramolecular chemistry', Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic* ✉ *oksana.yosypchuk@natur.cuni.cz*

[b] *Department of Biophysical Chemistry, J. Heyrovský Institute of Physical Chemistry of ASCR, v.v.i., Dolejškova 3, 182 23 Prague 8, Czech Republic*

Keywords
amperometry
electrochemical biosensor
flow injection analysis
silver solid amalgam electrode

In this paper, flow amperometric enzymatic biosensors for determination of glucose in honey and sarcosine in model sample were investigated. Though the most used electrochemical materials for preparation of biosensors are gold and various forms of carbon [1, 2], we would like to present the silver solid amalgam as a good alternative to them.

The first type of silver solid amalgam-biosensor is for determination of glucose (Fig. 1.A). It consists of a flow reactor based on porous silver solid amalgam and a flow tubular detector based on silver solid amalgam. The construction of it and determination of glucose occurred in three steps. First, a self-assembled monolayer of 11-mercaptoundecanoic acid was formed at the reactor. Second, an enzyme glucose oxidase was covalently immobilized in 11-mercaptoundecanoic acid layer using *N*-ethyl-*N*'-(3-dimethylaminopropyl) carboimide and *N*-hydroxysuccinimide chemistry. Finally, a decrease of oxygen concentration (directly proportional to the concentration of glucose) during enzymatic reaction is measured amperometrically in the detector under flow injection analysis.

The second type of silver solid amalgam-biosensor is for determination of sarcosine in model sample (Fig. 1.B). In 2009, sarcosine, an *N*-methyl derivative of the amino acid glycine, was identified as a differential metabolite that was highly increased during prostate cancer progression to metastasis [3]. For this type of biosensor the polished silver solid amalgam electrode was used. First, the 0.5 mm thick layer of polysaccharide chitosan was formed at the surface of the electrode (silver solid amalgam/chitosan). Then, the chitosan layer was modified by –CHO groups by reaction with glutaraldehyde. Finely, the enzyme sarcosine oxidase was

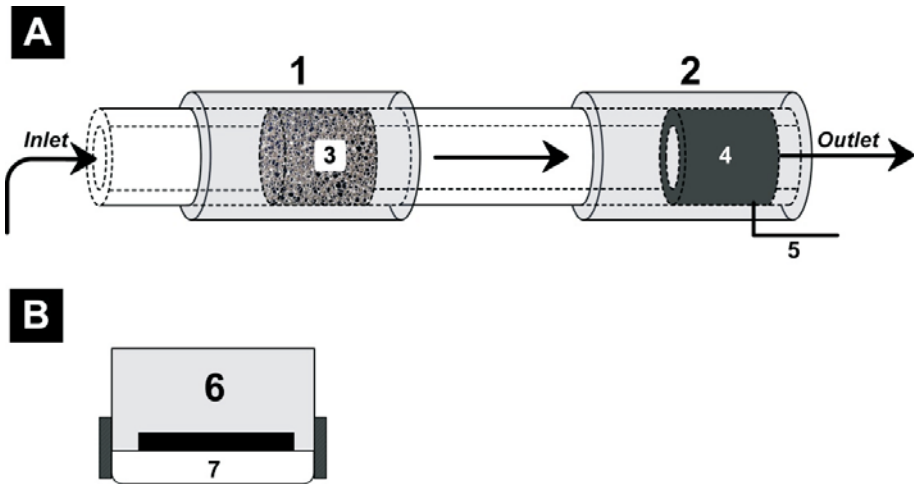


Fig. 1. (A) Construction of the flow enzymatic electrochemical biosensor contains (1) enzymatic reactor based on (3) porous silver solid amalgam**,** and (2) detector based on (4) compact silver solid amalgam, (5) platinum contact. (B) Construction of the flow enzymatic electrochemical biosensor using (6) polished silver solid amalgam electrode with (7) layer of chitosansarcosine oxidase.

covalently immobilized on the silver solid amalgam/chitosan/glutaraldehyde. As in previous case, the determination of sarcosine is based on decrease of oxygen concentration during enzymatic reaction. Sarcosine was measured amperometrically in the wall-jet arrangement under flow injection analysis.

The methods of determination of glucose and sarcosine included the optimization of following parameters with respect to amperometric response: the supporting electrolyte, its concentration, pH, the potential of detection and the flow rate.

The obtained results showed that the flow biosensors based on silver solid amalgam electrodes are relatively stable (it depends on stability of the enzyme), sufficiently sensitive and rapid-prepared devices which allows the measurement at highly negative potentials.

Acknowledgments

This work was financially supported by Grant Agency of the Czech Republic (Project P206/12/G151), Grant Agency of Charles University in Prague (Project 282111/2001/BCh/PrF), and Charles University in Prague (Project SVV).

References

[1] Ulman A.: *Chem. Rev.* **96** (1996), 1533–1554.
[2] Alaejos M.S., Montelongo F.J.G.: *Chem. Rev.* **104** (2004), 3239–3265.
[3] Sreekumar A., Poisson L.M., Rajendiran T.M., Khan A.P., Cao Q., Yu J., Laxman B., Mehra R., Lonigro R.J., Li Y., Nyati M.K., Ahsan A., Kalyana-Sundaram S., Han B., Cao X., Byun J., Omenn G.S., Ghosh D., Pennathur S., Alexander D.C., Berger A., Shuster J.R., Wei J.T., Varambally S., Beecher C., Chinnaiyan A.M.: *Nature* **457** (2009), 910–914.

Anion-Exchange Chromatographic Packings for Analysis of Heparin Anticoagulants

Radosław Sadowski*, Renata Gadzała-Kopciuch

Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarin St., 87-100 Toruń, Poland ✉ radoslawsad@gmail.com

Keywords
heparin
HPLC
ion exchange chromatography

The most commonly used ion exchange packings in liquid chromatography are modified silica gels. Chemical stability of materials and wide variety of modified derivatives determine the choice of this type of columns. However, silica gel columns are stable in the narrow range of pH from 2.0 to the maximum of 9.5. The alternative is polymer-based packings which can operate in the full range of pH. Polymeric materials used for the synthesis of anion-exchange stationary phase are poly(styrene-divinylbenzene) copolymers, polymethacrylates or vinyl polymers.

Ion exchange chromatography is becoming more popular in the distribution of biologically active compounds with ionic character. Examples of such compounds may be low molecular heparins. They are a group of pharmaceuticals used in the treatment of thromboembolic diseases. They are obtained by chemical or enzymatic depolymerization of natural heparin. Thus, small molecules are heparin fragments and their weight range is from 2 to 9 kDa. This range improves the bioavailability of low molecular heparins, making them subcutaneously active, improving their pharmacology, and altering their activity profile by decreasing their thrombin inhibitory activity [1–3].

In these studies, selected low molecular heparins were depolymerized at pH = 7.0 by a mixture of heparinases for 48 h at 25 °C. Then, tested samples were reduced by sodium borohydride to allow quantitative determination level of special forms before this process. High-performance anion-exchange liquid chromatography with diode-array detector was used for qualitative and quantitative analysis of those drugs. Commercially available packings based on silica gel and

synthesized polymer based on a styrene-divinylbenzene copolymer with ammonium salts, including hydrochloride, 2-chloro-*N,N*-diethylaminoethyl or hydrochloride of 2-chloro-*N,N*-dimethylaminoethyl were used as a stationary phase.

References

[1] Linhardt R.J., Liu J.: *Curr. Opin. Pharmacol.* **12** (2012), 217–219.
[2] Li L., Zhang F., Zaia J., Linhardt R.J.: *Anal. Chem.* **84** (2012), 8822–8829.
[3] Hoppensteadt D., Walenga J.M., Fareed J., Bick R.L.: *Hematology/Oncology Clinics of North America* **17** (2003), 313–341.

Colloidal Photonic Crystal Based Sensor for Ionic Strength

Christoph Fenzl*, Thomas Hirsch, Otto S. Wolfbeis

Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitaetsstr. 31, 93053 Regensburg, Germany ✉ *christoph.fenzl@ur.de*

Keywords
hydrogel
ionic strength
nanoparticles
photonic crystal
RGB readout
sensor

Reliable determination of ionic strength is very important in biology and biochemistry. For example, enzymes can be significantly inhibited if an applied buffer solution does not have the ideal ionic strength. There are very few optical systems which can reliably measure ionic strength in aqueous solutions [1, 2]. Therefore, photonic crystals have great potential in this sector.

This work used monodisperse, crosslinked polystyrene nanoparticles with highly negative surface charge, which were embedded in a polyacrylamide hydrogel film. Important advantages are: very quick response time, total reversibility of the signal change, which is insensitive to temperature changes, and complete lack of photobleaching, since the analytical signal is obtained by light reflection and not by light absorption. Sensitivity towards ionic strength with a linear range between 5×10^{-5} and 10^{-2} mol L^{-1} was achieved. The results were verified with three different salts: sodium chloride, di-sodium sulfate and tri-sodium citrate (Fig. 1). In addition, a veritable readout protocol for the film analysis with a digital single lens reflex camera was developed.

References

[1] Wolfbeis O.S., Offenbacher H.: *Sens. Actuators* **9** (1986), 85–91.
[2] Lee K., Asher A.S.: *J. Am. Chem. Soc.* **122** (2000), 9534–9537.

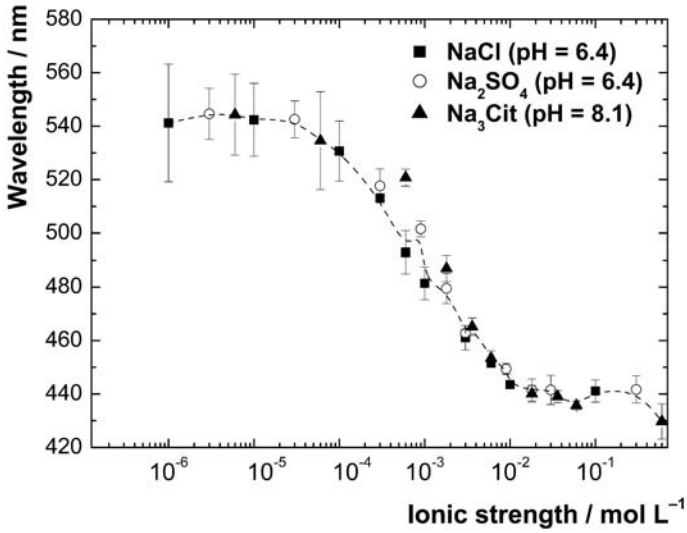


Fig. 1. Effect of the ionic strength on the wavelength of reflected light of colloidal photonic crystals in a hydrolyzed polyacrylamide hydrogel immersed in salt solutions of NaCl, Na_2SO_4, Na_3Cit at varying concentrations. Each data point presents the average value of five measurements; the error bars indicate the standard deviation of these measurements.

The Enantiomer Distribution of Major Chiral Volatile Organic Compounds in Juniper Flavoured Distillates

Alexandra Pažitná*, Ivan Špánik

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinskeho 9, SK-812 37 Bratislava, Slovak Republic ✉ *alexandra.pazitna@stuba.sk*

Keywords
cyclodextrin derivatives
enantiomer separation
juniper flavoured distillates
multidimensional gas chromatography

The enantiomer ratios of chiral volatile organic compounds in juniper flavoured spirits produced by various processing technologies in different EU countries were determined by multi-dimensional gas chromatography using solid phase microextraction and liquid-liquid extraction as a sample pre-treatment procedure. Totally, more than 260 compounds were detected that belong to various chemical classes, e.g. terpens, methyl and ethylesters of higher fatty acids, aldehydes and ketones. Linalool, α-terpineol, 4-terpineol, linalool oxides, α-pinene, and verbenone were found in all studied samples and those chiral volatile organic compounds were selected for further determination of enantiomer ratios since those were present at the highest concentrations level and/or did not coelute with other compounds. It was shown, that enantiomer ratio is independent on used sample treatment procedure. On the other hand, enantiomer distribution of some chiral organic compounds in juniper flavoured spirits depends on used technological procedure and consequently on country of its origin. The significant differences in enantiomer ratio of linalool and cis-linalool oxide allow distinguishing samples produced in Slovakia, Great Britain from those produced in Germany, Czech Republic and Belgium. The pure enantiomer of trans-linalool oxide was found only in samples from Germany.

Acknowledgments

This work was supported by kind assistance of EHP financial mechanisms under contract number SAV-EHP-2008-02-05 and Slovak Research and Development Agency under contract number APVV-0319-07.

Progress in Analysis of Phospholipid Groups by Instrumental Methods

VÁCLAV M. BIERHANZL*, RADOMÍR ČABALA

Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Albertov 6, 128 43 Prague 2, Czech Republic ✉ *ing.bierhanzl@gmail.com*

Keywords
capillary electrophoresis
HPLC
NMR spectroscopy
phosphates
phospholipids

Phospholipids are esters of phosphoric acid, glycerol and fatty acids. Phospholipids are divided into five main groups according to the phosphorylated group which could be phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and phosphatidic acid. They occur commonly in cells and are an important part of cell membranes. Currently, thin layer chromatography is used for the purposes of microbiological studies [1], which only allows for a limited quantitation and separation of certain phospholipids groups. Many instrumental methods have been tested for phospholipids determination, but publications about real-world applications or more detailed parameters of these methods are relatively rare. However, there was not such a work that had been focused to analysis of phosphorylated polar parts but only to the intact lipid structures [2].

The aim of the project was the development of fast, selective and robust instrumental methods (NMR, HPLC, CE) for determination of phospholipids and phosphorylated parts of phospholipids or their derivatives for group classification of phospholipids.

Capillary electrophoresis was performed in both aqueous and non-aqueous mode. Aqueous mode was successfully used for detection of phosphates with contactless conductivity detector. All investigated analytes were detected but they have not been determined yet. Currently, the high conductivity of separation electrolyte is the reason for high determination limit. Non-aqueous mode with spectrophotometrical detection was tested for intact phospholipids. Main phospholipids were detected in this mode and detection limit is much more lower

than in the former case. Low solubility of phosphatidylserine in non-aqueous electrolyte represents another problem. Solving this trouble should allow the determination of all phospholipids.

^{31}P-NMR was used for determination of phospholipids and phosphates. All phospholipids standards were successfully detected in artificially prepared samples. Unfortunately, real samples of bacterial membrane phospholipids (*Bacillus subtilis*) yielded signals at slightly different chemical shifts compared to the artificial standard. Those results were compared with previously published papers [3], however, without exact conclusion. The further progress of NMR spectroscopic analysis is limited by high detection limit of this method.

HPLC was tested in reverse-phase and HILIC mode. HILIC column was tested for separation of phosphorylated derivatives. All phosphates of interest are strongly polar compounds which were not retained at the HILIC (cyclofructan) column. Gradient RP-HPLC mode was used for intact phospholipids samples and some of phospholipids were detected. This method is still in development.

Acknowledgments

Financial support by The Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 0021620857), and by the Charles University in Prague (Project SVV267215).

References

[1] Dyńska-Kukulska K., Ciesielski W.: *Rev. Anal. Chem.* **31** (2012), 43–56.
[2] Raith K., Wolf R., Wagner J., Neubert R.H.: *J. Chromatogr. A* **802** (1998), 185–188.
[3] Schiller J., Arnold K.: *Med. Sci. Monitor.* **8** (2002), MT205–222.

A Novel Methodology for the Enantiomeric Resolution of Methamphetamine, Its Precursors and Intermediates by GC-MS

Justyna Małgorzata Płotka[a,*], Calum Morrison[b], Marek Biziuk[a]

[a] *Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdansk, Poland* ✉ *plotkajustyna@gmail.com*
[b] *School of Science, Faculty of Science and Technology, University of the West of Scotland, Paisley PA1 2BE, Scotland* ✉ *Calum.Morrison@uws.ac.uk*

Keywords
chirality
derivatization
gas chromatography
methamphetamine
nuclear magnetic resonance

According to a new report published by European Monitoring Centre for Drugs and Drug Addiction, the number of people who abuse methamphetamine is increasing every year, thus it became a global threat. This is mainly because many laboratories are engaged in clandestine manufacture of methamphetamine. The chemistry is well understood, and widely published, notably on the web, though not easy to achieve.

There are various routes of clandestine synthesis of methamphetamine but the most commonly used are: Leuckart, Emde, Nagai and Birch method. Methamphetamine coming from clandestine laboratories has variable composition. This is because during the synthesis of methamphetamine using each of these methods may produce different by-products. But also the samples synthesized using the same method may show different impurity patterns according to the various conditions, and the impurities may disappear or co-precipitate with methamphetamine during the crystallization process [1]. Subsequent analysis of the impurities of methamphetamine may supply valuable information about the conditions and the chemicals used in the illicit methamphetamine manufacture what can provide information on the original source of sample, thus the analysis of the impurities requires techniques which offer a high degree of resolution, specificity and sensitivity [2].

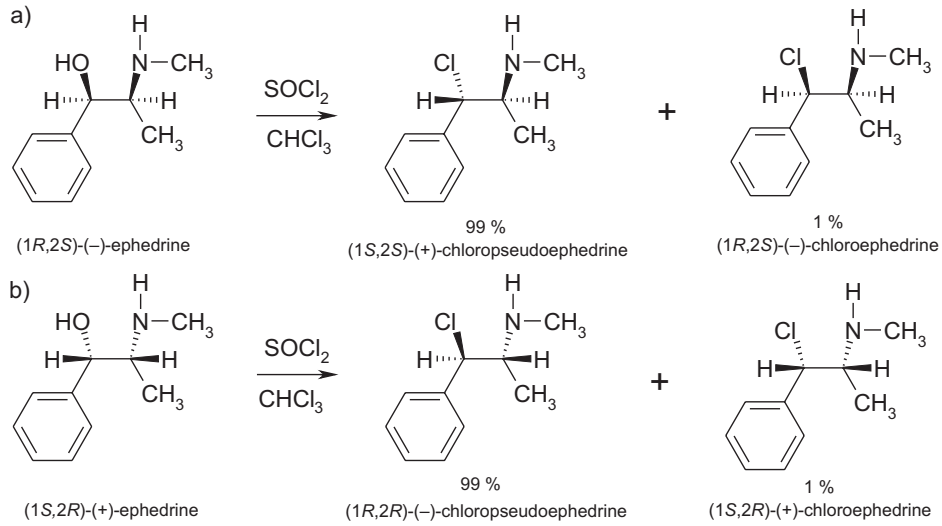


Fig. 1. Reaction of (a) (1*R*,2*S*)-(−)-ephedrine and (b) (1*S*,2*R*)-(+)-ephedrine with thionyl chloride.

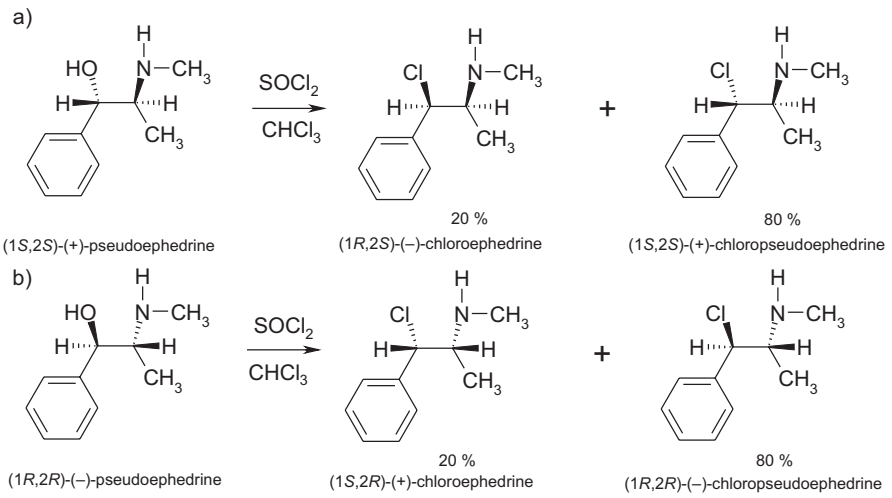


Fig. 2. Reaction of (a) (1*S*,2*S*)-(+)-pseudoephedrine and (b) (1*R*,2*R*)-(−)-pseudoephedrine with thionyl chloride.

Methamphetamine, because of its stereogenic center, has two optically active isomers of which (+)-enantiomer is more frequently abuse due to its stronger CNS stimulatory activity than (–)-methamphetamine, which does not possess any significant CNS activity or addictive properties [3]. For its high stimulant effect, (+)-methamphetamine is predominantly abused and illicitly smuggled in the black markets. On the other hand, (–)-methamphetamine can be used as a precursor for the manufacturing of l-deprenyl, an effective antiparkinsonian and antidepressant [4].

Because enantiomeric ratio of methamphetamine is closely related with the optical activity of precursors and reagents used for the synthesis, this knowledge can provide useful information concerning the origins and synthetic methods used for illicit manufacture [3]. This information is important, since it can be utilized for regulation of the precursors, investigation of the manufacturing sources, and resultant prevention of abuse. To obtain this information, analytical techniques which offers a high degree of enantioresolution are required.

Because of above, the knowledge about methamphetamine, analytical procedures to determine purity of drug as well as impurity profiling and enantiomeric composition, and also the detection and identification in different matrix types is significant for many reasons, including prediction and protection applied to reduction in drug addiction.

The aim of this work was to develop and optimize a chiral gas chromatographic method for the separation of compounds likely to be found in the EMDE synthesis of methamphetamine. However, this could not be achieved without pure enantiomers and diastereomers of chloroephedrine which are intermediates and possible contaminants formed when methamphetamine is manufactured using EMDE method. These chloroderivatives are not commercially available. Therefore, the synthesis of these compounds was required and thereafter the determination of conformations and configurations by NMR and GC-MS was carried out.

We have shown that chlorination of the ephedrine/pseudoephedrine compounds occurs via inversion (S_N2) and retention (S_Ni) of configuration around the α carbon and mixture of diastereoisomers (chloroephedrine and chloropseudoephedrine) were formed (Fig. 1, 2), with the ratio of the resulting compounds dependent on the precursors used [5].

Afterwards, the separation of the enantiomers of precursors, chlorinated intermediates and methamphetamine was carried out using fluorinated acid anhydrides as chemical derivatization reagents prior to gas chromatographic analysis on a 2,6-di-*O*-pentyl-3-propionyl silyl-cyclodextrin stationary phase (CHIRALDEX G-PN). A temperature-programmed method was developed and optimized. Trifluoroacetylated 3-(trifluoromethyl)phenethylamine hydrochloride was used as an internal standard, and mass fragmentation patterns are proposed for all derivatives analyzed. Qualitative validation of the optimized chromatographic conditions was completed in accordance with the guidelines

published by the United Nations Office on Drugs and Crime. The simultaneous enantioseparation of these compounds to produce a profile would provide valuable information to law enforcement agencies regarding the provenance of a methamphetamine seizure.

The combination of NMR and GC-MS should be considered as a suitable approach for chiral analysis of other compounds and useful for various fields which require accurate stereochemical data. The examples here are secondary amines required for forensic purposes, but equally can be applied to molecules of interest to pharmaceutical and general analytical science.

Acknowledgments

This research work was financial supported by the National Science Centre in Poland (Decision number: DEC-2012/05/N/ST4/ 02001) and "InnoDoktorant – Scholarships for PhD students, Vth edition". Project is co-financed by the European Union in the frame of the European Social Fund.

References

[1] Remberg B., Stead A. H.: Drug characterization-impurity profiling, with special focus on methamphetamine: recent work of the United Nations International Drug Control Programme. In: *Bulletin on Narcotics*, New York, UNDOC 2000, pp. 97–117
[2] Barker W. D., Antia W.: *Forensic Sci. Int.* **166** (2007), 102–109.
[3] Lee J. S., Yang W. K., Han E. Y., Lee S. Y., Park Y. H., Lima M. A., Chung H. S., Park J. H.: *Forensic Sci. Int.* **173** (2007), 68–72.
[4] Hasegawa M., Matsubara K., Fukushima S., Maseda C., Uezono T., Kimura K.: *Forensic Sci. Int.* **101** (1999), 95–106.
[5] Płotka J.M, Morrison C., Adam D., Biziuk M.: *Anal. Chem.* **84** (2012), 5625–5632.

Diagnostic Potential of Chiroptical Spectroscopy for Type 1 Diabetes Mellitus

Lucie Habartová[a,*], Michal Tatarkovič[a], Hana Logerová[b], Jan Vavřinec[c], Vladimír Setnička[a]

[a] *Department of Analytical Chemistry, Institute of Chemical Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic* ✉ *Lucie.Habartova@vscht.cz*
[b] *Department of Biochemistry, Cell and Molecular Biology, Third Faculty of Medicine, Charles University, Ruská 87, 100 00 Prague 10, Czech Republic*
[c] *Department of Children and Adolescents, Faculty Hospital Královské Vinohrady, Šrobárova 50, 100 34 Prague 10, Czech Republic*

Keywords
blood plasma
chiroptical spectroscopy
diabetes mellitus
electronic circular dichroism
Raman optical activity

Type 1 diabetes mellitus is a serious autoimmune metabolic disease characterized by impaired carbohydrate metabolism resulting in permanent hyperglycemia (elevated level of blood glucose), polyuria (frequent urination), polydipsia (intense thirst), and total exhaustion along with disruption of acid-base balance of the organism. The outbreak occurs at an early age, especially during puberty, and it can have a genetic basis [1, 2].

Current diagnostic tests of diabetes are carried out by measuring blood or plasma glucose concentration [2]. The detection of type 1 diabetes mellitus is usually impossible before its onset, and therefore it is very difficult to prevent acute complications of the disease. Positive diagnosis is usually made after the patient's sudden collapse or when specific symptoms of fully developed diabetes occur. Similarly to some other diseases, diabetes is also accompanied by changes in concentration and conformation of certain blood plasma proteins and other biomolecules in patients [3]. These structural changes take place probably long before the full manifestation of diabetes. These facts can be used for the development of new diagnostic methods for this disease that could help in the disease monitoring and even its early-stage diagnosis.

We investigated blood plasma samples of type 1 diabetes mellitus patients and healthy controls by means of chiroptical spectroscopy: electronic circular dichroism and Raman optical activity. The measurements were supplemented by common unpolarized methods of molecular spectroscopy: infrared and Raman spectroscopy. Unlike their unpolarized variants, chiroptical methods are among the few methods inherently sensitive to the 3D structure of chiral biomolecules [4, 5]. Therefore, chiroptical methods may be able to detect changes in the stereochemistry of several biomolecules occurring during many diseases. Real clinical samples have not been widely analyzed by chiroptical methods; however, studies focused on the possibility of using these methods for measuring blood plasma samples have been carried out [6, 7].

Blood from type 1 diabetes mellitus patients and healthy controls was collected by venipuncture and the isolated plasma was frozen immediately and stored at –70 °C. The electronic circular dichroism analyses of blood plasma samples were performed after thawing the samples at ambient temperature. All samples were diluted by a sterile phosphate buffer (pH = 7.4). In order to suppress very strong background fluorescence of the blood plasma samples in Raman optical activity and Raman spectroscopy, a fluorescence quenching method was applied [6, 7]. After obtaining the sample spectra, the data sets were evaluated by statistical methods (primarily using linear discriminant analysis) for each of the above mentioned spectroscopic method and also for their specific combinations. In the statistical evaluation, the attention was paid mainly to the spectral ranges corresponding to the protein secondary structures [5, 8, 9].

Spectral differences observed between the blood plasma samples of patients and healthy controls indicate a possible diagnostic potential of chiroptical methods for type 1 diabetes mellitus. It seems these methods might be useful for the prospective diagnostics and monitoring of diabetes mellitus.

Acknowledgments

The work was financially supported by Specific University Research MSMT No. 20/2013 – A2_FCHI_2013_003 and A1_FCHI_2013_003. We gratefully thank Miroslava Vojtová (Department of Biochemistry, Cell and Molecular Biology – Division of Biochemistry, Third Faculty of Medicine, Charles University, Prague, Czech Republic) for providing blood plasma samples and performing the electrophoresis of plasma proteins.

References

[1] Alberti K.G.M.M., Zimmet P.Z.: *Diabetic Med.* **15** (1998), 539–553.
[2] American Diabetes Association: *Diabetes Care* **35** (2012), S64–S71.
[3] Lasch P., Kneipp J.: *Biomedical Vibrational Spectroscopy.* New York, Wiley 2008.
[4] Berova N., Nakanishi K., Woody R.W. (edits.): *Circular Dichroism: Principles and Applications.* New York, Wiley 2000.
[5] Setnička V., Urbanová M.: Spektroskopie cirkulárního dichroismu. In: *Moderní přístupy k farmaceutické analýze.* Dohnal J., Jampílek J., Král V., Řezáčová A. (edits.). Brno, Farmaceutická fakulta Veterinární a farmaceutické univerzity Brno 2010, s. 12–73

[6] Synytsya A., Judexová M., Hrubý T., Tatarkovič M., Miškovičová M., Petruželka L., Setnička V.: *Anal. Bioanal. Chem.* **405** (2013), 5441–5453.
[7] Tatarkovič M., Fišar Z., Raboch J., Jirák R., Setnička V.: *Chirality* **24** (2012), 951–955.
[8] Tsuboi M., Suzuki M., Overman S.A., Thomas G.J.: *Biochemistry* **39** (2000), 2677.
[9] Zhu F., Isaacs N.W., Hecht L., Tranter G.E., Barron L.D.: *Chirality* **18** (2006), 103–115.

Saliva Analysis by GC-MS/MS Techniques

Peter Kotora*, Viktória Ferenczy, Peter Podolec,
Alexandra Hengerics Szabó, Róbert Kubinec

Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovakia ✉ *kotora@fns.uniba.sk*

Keywords
GC-MS/MS
metabolites
saliva

The aim of this work was the development of a new method for the analysis of metabolites in saliva by gas chromatography and tandem mass spectrometry, (GC-MS/MS). Developed method includes a one-step sample pretreatment and extraction by isopropanol (due to its good extraction capacity and higher freezing point than methanol, which is most often used for this purpose), preconcentration of the analytes by lyophilization and derivatization using silylation agents hexamethyldisilazane (HMDS) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and subsequent chromatographic analysis. This method is suitable for the identification of various metabolites and other substances such as pharmaceuticals, nicotine and caffeine in saliva samples.

Saliva is a body fluid possessing several functions involved in oral health and homeostasis, with an active protective role in maintaining oral health. Saliva helps bolus formation by moistening food, protects the oral mucosa against mechanical damage and plays an important role in the preliminary digestion of food. It also has a role in maintaining teeth enamel mineralization. Healthy adult subjects normally produce 500–1500 mL of saliva per day, at a rate of approximately 0.5 mL/min [1], but several physiological and pathological conditions can modify saliva production quantitatively and qualitatively, e.g., smell and taste stimulation, chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene [2], and physical exercise [1, 3].

Each type of salivary gland secretes a characteristic type of saliva. Differences in the concentration of salts/ions [4] and total proteins [3] among glands can be observed. Salivary proteins are expressed differently among individual glands, like submandibular and sublingual glands. For example cystatin C is secreted by the submandibular gland and MUC5B mucin and calgranulin are secreted by the sublingual gland [5]. Moreover, salivary composition varies, depending upon whether salivary secretion is basal or stimulated [4].

Saliva tests are in a permanent development because of the easy and noninvasive sample collection. Saliva does not give a view of longtime exposure, but the compounds present in saliva are in high concentrations levels, because the salivary glands are linked to blood capillaries and there is a straight exchange between blood and saliva. Saliva tests are often used for forensic purposes, e.g. for the determination of narcotics with relatively good sensitivity. Besides drugs metabolites, endogenous metabolites can also be determined in saliva. High levels of glucose, or low levels of albumin and immunoglobuline in saliva can indicate metabolic diseases like diabetes [6]. The main advantages of saliva analysis are the easiness and nonivasiveness of sample collection, the similar chemical composition to blood, sample analysis performable without medical personnel, possibility of obtaining information about concentration levels of illegal substances, toxic pollutants or naturally presented metabolites [7].

This work was focused to the analysis of metabolites in saliva by GC-MS/MS techniques. The aim of this study was the development of a new extraction method, preconcentration of anaytes by lyophilisation, and derivatisation using silanization agents HMDS and BSTFA. We had confirmed the presence of the target compounds -paracetamol, ibuprofen and caffeine in real saliva samples by MS/MS detection. The amounts of these compounds were affected by absorption, saliva production, weight, and sex of the subjects. We have confirmed that the male subject (with higher weight) had also faster metabolism and by that also faster secretion of these substances into saliva.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0416-10, and by Comenius University in Bratislava (grant Nos. UK/227/2013 and UK/229/2013).

References

[1] Chicharro J.L., Lucia A., Perez M., Vaquero A.F., Urena R.: *Sports Med.* **26** (1998), 17–27.
[2] Aps J.K.M., Martens L.C.: *Forensic Sci. Int.* **150** (2005), 119–31.
[3] Walsh N.P., Laing S.J., Oliver S.J., Montague J.C., Walters R., Bilzon J.L.J.: *Med. Sci. Sports Exerc.* (2004), 1535–1542.
[4] Kalk W.W., Vissink A., Stegenga B., Bootsma H., Nieuw Amerongen A.V., Kallenberg C.G.: *Ann. Rheum. Dis.* **61** (2002), 137–144.
[5] Hu S., Denny P., Denny P., Xie Y., Loo J.A., Wolinsky L.E., Li Y., McBride J., Ogorzalek Loo R.R., Navazesh M., Wong D.T.: *Int. J. Oncol.* **25** (2004), 1423–1430.
[6] Aps J., Martens L.C.: *Forensic Sci. Int.* **150** (2005), 119–131.
[7] Chiappin S., Antonelli G., Gatti R., De Palo E.F.: *Clin. Chim. Acta* **383** (2007), 30–40.

Phthalocyanine Modified Electrode Utilized as Electrochemical Sensor for Detection of Unsaturated Hydrocarbons

Monika Klusáčková[a, b, *], Pavel Janda[a]

[a] *J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Dolejškova 3, 182 23 Prague 8, Czech Republic* ✉ *monika.klusackova@jh-inst.cas.cz*
[b] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic*

Keywords
phthalocyanine
sensor
unsaturated hydrocarbons

Generally, unsaturated hydrocarbons such as ethylene and propylene are not only gases of considerable industrial significance but also typical air pollutants that are formed and produced globally [1, 2]. These gases are mainly released from combustion processes such as automobile exhaust, industrial combustion processes and biomass burning [3]. In addition, ethylene is more often referred in a number of gas sensing applications in contrast to propylene. It is promising fast on-line tool for medical applications in non-invasive oxidative stress diagnostics [4] and important plant hormone that commercially applied to control the fruits ripening in storage facilities [5]. This explains particular significance of detection these gases and to determine their concentration respectively. Consequently, the development of sensitive, reliable and portable gas replacing expensive and bulky conventional analytical techniques [6] represents important task.

We have studied the electrochemical oxidation of ethylene and propylene on two different electrodes including annealed gold (Au111) and highly ordered pyrolytic graphite (HOPG), both modified by thin layer *N,N´,N´´,N´´´*-tetramethyltetra-3,4-pyridinoporphyrazinocobalt, (abbreviated: CoTmt-3,4-ppa) and utilized this compound in construction of electrochemical gas sensor. The choice of complex CoTmt-3,4-ppa, as an electron transfer mediator between the electrode surface and analyte in solution, was given by its redox properties and ability to form low soluble and electrically conducting film on electrode surface [7, 8]. In this work we modified annealed Au111 and HOPG electrode respectively by three

different deposition techniques: adsorption, electrochemical deposition and spin coating leading to deposition of an insoluble film on electrode surface.

Our main interest has been focused on optimizing conditions of electrocatalytic detection unsaturated hydrocarbon on each modified electrode. The electrochemical behaviour of hydrocarbons was studied in aqueous phosphate buffer by cyclic voltammetry at laboratory temperature. Furthermore, thin layer ionic liquid appeared to be more promising electrolyte due to its low toxicity and reportedly higher solubility of unsaturated hydrocarbons compared to water, which can increase the sensor response [5]. The electrochemical results were completed by in situ backscattering VIS spectroscopy and ex situ atomic force microscopy which allow to get better understanding of deposition process mechanism and film stability respectively. The applicability of individual deposition techniques for electrochemical detection was considered.

Acknowledgments

We are grateful to Yu-Hong Tse, York University, Toronto, for supply of CoTmtppa used in this work. This work was supported by the research project MSM0021620857 of the Ministry of Education, Youth, and Sports of the Czech Republic, and by grant project SVV267215 of Charles University in Prague.

References

[1] Wama, R., Plashnitsa, V. V., Elumalai, P., Utiyama, M., Miura, N.: *Solid State Ionics*. **181** (2010), 359–363.
[2] Graewe, B., Rang, A., Schalley, Ch. A., Haubrich, J., Bargon, J.: *Sensor Actuator.* **119** (2006), 302–307.
[3] Kesselmeier, J., Staudt, M.: *J. Atmos. Chem.* **33** (1999), 23–88.
[4] Berkelmans, H. W. A., Moeskops, B. W. M., Bominaar, J., Scheepers, P. T. J., Harren, F.J.M.: *Toxicol. Appl. Pharm.* **190** (2003), 206–213.
[5] Zevenbergen, M. A. G., Wouters, D., Dam, V-A. T., Brongersma, S. H., Crego-Calama, M.: *Anal. Chem.* **83** (2011), 6300–6307.
[6] Hodgson, A. W. E., Jacquinot, P., Jordan, L. R., Hauser, P.C.: *Anal. Chim. Acta.* **393** (1999), 43–48.
[7] Tse Y. H., Janda P., Lever A. B. P.: *Anal. Chem.* **66** (1994), 384–390.
[8] Chen J., Zhang J., Tse Y. H., Janda P., Christendat, D., Lever, A. B. P.: *J. Porphyrins Phthalocyanines* **10** (2006), 1238–1248.

Development of UHPLC/MS-MS Method for Determination of 8-Hydroxy--2'-deoxyguanosine in Human Urine

Barbora Červinková[a, b, *], Lenka Krčmová[a, b], David Čechlovský[a], Dagmar Solichová[a, b], Bohuslav Melichar[c], Luboš Sobotka[b], Petr Solich[a]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic* ✉ *barbora.cervinkova@gmail.com*
[b] *III. Internal Gerontometabolic Clinic, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic*
[c] *Department of Oncology, Palacký University Medical School & Teaching Hospital, Třída Svobody 8, 771 26 Olomouc, Czech Republic*

Keywords
clinical research
human urine
8-hydroxy-2'-deoxyguanosine
UHPLC/MS-MS

Nucleic acids, lipids and membranes are constantly attacked by reactive oxygen radicals which leads to damage of these biological structures. One of the attacked DNA nucleotide might be guanine, which is transformed to 8-hydroxy-2'-deoxyguanosine [1]. 8-Hydroxy-2'-deoxyguanosine is considered as a marker of DNA oxidative damage. It can be determined in the different kinds of human fluids such as urine, plasma, serum, saliva [2], in which 8-hydroxy-2'-deoxyguanosine is excreted after enzymatic DNA repair. High 8-hydroxy-2'-deoxyguanosine levels were observed in many serious diseases such as different types of cancer (lung, hepatocellular, breast cancer and colorectal carcinoma [3]), degenerative diseases (Parkinson), hematological disorders (acute leukemia, refractory anemia [4]), arsenic-related Bowen's disease [5] and chronic liver disease [6].

Creatinine is a major breakdown product of mammalian metabolism produced in muscle tissue and eliminated in urine. The urinary creatinine concentration is often used to correct urinary excretion of other chemical substances due to urine dilution effects, since the excretion of creatinine is reasonably constant throughout the day.

This work is focused on the development of new analytical method for the determination of 8-hydroxy-2'-deoxyguanosine and creatinine in human urine.

Ultra High Performance Liquid Chromatography in combination with mass spectrometry (UHPLC/MS/MS) was used. During method development chromatographic and mass spectrometry conditions and sample preparation procedure were optimized. Solid phase extraction and simple precipitation followed by centrifugation and filtration were tested in sample preparation procedure. Different kinds of solid phase extraction phases (Spe-ed™ Flow C18, Oasis MCX, HLB, LiChrolut® EN) were tested. Method of simple precipitation included sample dilution, precipitation, centrifugation and filtration using microtitration plate. Based on the results of the sample preparation, solid phase extraction method using Oasis HLB Vac Cartridge C18 200 mg/3 mL (Watters) was selected. Chromatographic analysis was performed on UHPLC system Nexera (Shimadzu) with stationary phase Aquity UPLC BEH Amide at dimension 1.7 μm 3.0×150 mm (Waters). As the mobile phase, combination of acetonitrile and 10 mM ammonium formate buffer with flow rate 0.4 mL/min was used. Creatinine was detected by UV detection at 235 nm. Detection conditions for 8-hydroxy-2'-deoxyguanosine were optimized using triple-quadrupole mass spectrometer LC-MS 8030 (Shimadzu) with electrospray ionization in positive mode. MS/MS detection was based on Multiple Reaction Monitoring transitions 284/168, 284/140, 284/112.

Development of new method for determination of 8-hydroxy-2'-deoxyguanosine could have a large benefit for clinical practice. The determination might contribute to easier prognostics and therapy monitoring of various types of serious diseases, which are linked to DNA damage.

Acknowledgments

This work was supported by project SVV 267 002, the European Social Fund and the state budget of the Czech Republic, TEAB, project no. CZ.1.07/2.3.00/20.0235. The authors also acknowledge financial support of the Charles University in Prague – project UNCE 204026/2012.

References

[1] Valavanidis A., Vlachogianni T., Fiotakis C.: *J. Environ. Sci. Health. C* **27** (2009), 120–139.
[2] Hosozumi C., Toriba A., Chuesaard T., Kameda T., Tang N., Hayakawa K.: *J. Chromatogr. B* **893–894** (2012), 173–176.
[3] Hsu W., Chen W., Lin W., Tsai F., Tsai Y., Lin C., Loa W., Jeng L., Lai C.: *Clin. Chim. Acta* **402** (2009), 31–37.
[4] Honda M., Yamada Y., Tomonaga M., Ichinose H., Kamihira S.: *Leukemia Res.* **24** (2000), 461–468.
[5] Matsui M., Nishigori C., Toyokuni S., Takada J., Akaboshi M., Ishikawa M., Imamura S., Miyachi Y.: *J. Invest. Dermatol.* **113** (1999), 26–31.
[6] Ichiba M., Maeta Y., Mukoyama T., Saeki T., Yasui S., Kanbe T., Okano J-I., Tanabe Y., Hirooka Y., Yamada S., Kurimasa A., Murawaki Y., Shiota G.: *Liver Int.* **23** (2003), 338–345.

Method Development for the Analysis of Gaseous Emissions From Lithium-Ion Cells Used Under Extreme Conditions

Chrysoula Kanakaki[a,*], Erwin Rosenberg[a], Atanaska Trifonova[b], Joong-Hee Han[b]

[a] *Vienna University of Technology, Institute of Chemical Technologies and Analytics, Getreidemarkt 9, 1060 Vienna, Austria* ✉ *chrysoula.kanakaki@tuwien.ac.at*
[b] *AIT Austrian Institute of Technology GmbH, Mobility Department, Giefinggasse 2, 1210 Vienna, Austria*

Keywords
lithium-ion cells
multiplex
vacuum outlet

In order to reduce carbon dioxide emissions and fossil fuel dependence the development and uptake of electric vehicles play a key role. Achieving performances acceptable for a wide range of users, and not only for the early adapters, requires the development of innovative energy storage with higher energy density, higher safety and lower environmental impact. To serve this purpose, larger lithium-ion batteries are developed, increasing the necessity of the risk assessment for unexpected chemical hazards, due to misuse or extreme operating conditions. The aim of this research work is the development of a standardized test method for the characterization of the gaseous emissions of lithium-ion cells, focusing on the improvement of safety and environmental issues.

The qualitative and quantitative analysis of gaseous products, generated during the cycling operation and misuse conditions, is typically performed by Gas Chromatography/Mass Spectrometry (GC/MS). In order to enhance the time resolution, two different approaches of fast GC techniques will be investigated.

The first technique chosen is the vacuum outlet GC/MS technique, with which we can develop a fast chromatographic method without the need of special injection or detection equipment. Conventional GC/MS instrumentation can be used in this case, which represents a significant advantage regarding the other fast chromatographic techniques. Under vacuum-outlet conditions, short, mega-bore columns are the most suitable to use, extending the vacuum across the whole column length, and providing a higher analysis speed than the same column

operated at atmospheric outlet pressure [1]. In such applications, a restriction coupled to the column inlet is necessary for restricting the carrier gas flow to an acceptable level for the detector and prevailing above-atmospheric pressures for the operation of the injection system and still low-pressure conditions throughout the entire column [2]. The lower elution temperatures required for vacuum outlet applications allow heavy components to elute in shorter time, improving analysis time and sensitivity and generate less stationary phase bleed, providing better mass spectra, with higher signal to noise ratios and reduced risk of pollution of the ion source [3].

The second applied technique is the Multiplex-Gas Chromatography. 'Multiplexing' techniques, for example, Fourier and Hadamard transformations, are commonly used in spectroscopy and mass spectrometry to increase the duty cycle and improve the signal-to-noise ratio [4]. To achieve high time resolution, short and precise injection intervals in the range of 500 to 2000 ms and short and highly reproducible injection pulses are applied, so that sample is injected continuously while a run is still in progress [5]. In this way, on-line measurements are also enabled and the relative concentrations of the analytes in each sample are obtained by the use of a deconvolution algorithm for the raw data, which has to be developed and applied to the data sets [6].

The significance of this work is basically lying in the ability to perform qualitative and quantitative analysis of gas emissions from lithium-ion cells used under extreme conditions and to estimate the potential hazards arising. Despite of their great importance, the aforementioned aspects have not been studied systematically yet. In addition, the application of the vacuum outlet GC/MS technique, for the fast and precise analysis of the emitted gases, and the Multiplex-GC/MS for on-line measurements under extreme heating or charging conditions of the cells, lead to the development of a successful tool for the characterization of rechargeable lithium-ion batteries. For these reasons, the results of this work can be used for future optimizations on the components of the batteries, contributing to the development of highly efficient, safe and environmentally friendly Li-ion batteries.

Acknowledgments

Financial support of this study, provided by the Austrian Research Promotion Agency (FFG, Project 'SiLithium', Project Number 835790), is gratefully acknowledged.

References

[1] Ravindra K., Dirtu A. C., Covaci A.: *Trends Anal. Chem.* **27** (2008), 291–303.
[2] van Deursen M., Janssen H. G., Beens J., Lipman P., Reinierkens R., Rutten G., Cramers C.: *J. Microcol. Sep.* **12** (2000), 613–622.
[3] Mastovska K., Lehotay S. J.: *J. Chromatogr. A* **1000** (2003), 153–180.
[4] Phillips J. B.: *Anal. Chem.* **52** (1980), 468–478.
[5] Trapp O.: *J. Chromatogr. A* **1217** (2010), 6640–6645.
[6] Valentin J. R., Hall K. W., Becker J. F.: *J. Chromatogr.* **518** (1990), 199–206.

Determination of Terpene Profile in Cape Gooseberry (*Physalis peruviana* L.) with Use of HS-SPME/GC×GC-TOFMS Method

Magdalena Kupska*, Tomasz Wasilewski, Renata Jędrkiewicz, Justyna Gromadzka, Jacek Namieśnik

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland ✉ *magdalena.kupska@gmail.com*

Keywords
cape gooseberry
food analysis
HS-SPME
two dimensional gas chromatography

In recent years, more and more attention is given to the search for natural diet ingredients, regular consumption of which may be important for the protection of human health against civilization diseases, mainly cardiovascular and cancer. Fresh fruits and products made of them, especially fruit juice, provide the basic energetic substances (simple and complex sugars) and are a source of health beneficial vitamins, macro and microelements, antioxidants and compounds which are classified together with essential oils (for example terpenes), which positively affect human's health. These last two groups of bioactive compounds are very important for the human organism because they associate free radicals and heavy metals, by acting in anti-inflammatory, anticancer, antibacterial, antifungal, antiviral and antiallergic way [1–3]. Moreover, the terpenes present in fruits are responsible for taste and fragrances values of these products. This knowledge has contributed to search for so called 'superfruits' [4, 5] and the development of new food products and pharmaceuticals based on such fruits.

One of the most promising tropical fruit is a Cape gooseberry (*Physalis peruviana* L.), also known as Golden berry, Physalis, Inca berry, Aztec berry, Giant ground cherry, Peruvian groundcherry, Peruvian cherry. These fruits have been cultivated for a long time in the Andes, as well as in California, South Africa, India, New Zealand, Australia and Egypt [6]. Goldenberries are gold juicy beads with size of about 2 cm. During growth the fruits are protected by the surrounding leaves

(fruits are mechanically protected by a surrounding dehydrated calyx), from which after ripening stage, is formed a paper husk – looking like Chinese lanterns.

In Poland these fruits are available in some specialized stores with national origin organic food or sporadically occur in supermarkets as fruits imported from South America. These fruits, like other exotic fruit can be eaten not only as fresh or dried fruit, but also as an interesting components of salads, desserts, jams and juices.

Previous work on the fruit *Physalis peruviana* L. discussed content of compounds represents the group of physiologically active steroid lactones, which are present in the leaves [7] and roots [8].

Profile of volatile compounds for the first time was studied in 1989 using liquid-liquid extraction technique in combination with gas chromatography with flame ionization detector, gas chromatography olfactometry, and gas chromatography mass spectrometry [9]. It has been shown that the cape gooseberry fruits is a research source of compounds from the group of aldehydes, ketones, acids, lactones, terpenes and esters.

There are various chromatographic techniques used for analysis of solvent extracts obtained from fruits. For many years these techniques were in the area of great interest because of the wide range of application. At present, conventional gas chromatography offers high peak capacity and resolution power. However, it does not enable the separation of all components of complex matrices introduced to the column. The reason for unreliability of this technique is insufficient selectivity of applied columns and extended chromatographic bands during migration of the sample components through the column. That is why the combination of two chromatographic columns with different selectivity started to be used for separation of the components of complex matrices.

The terpenes profile of cape gooseberry was determined by headspace solid--phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME/GC×GC-TOFMS). The procedure was based on extraction and preconcentration of analytes to the stationary phase of the fiber from headspace phase and further thermal desorption from the fiber in the GCxGC injector port. The GC×GC system (Agilent 7890A), equipped with liquid nitrogen-based quad-jet cryogenic modulator and time-of-flight mass spectrometer (LECO Pegasus 4D) was applied for the whole analysis. Helium was used as the carrier gas with a constant flow rate of 1mL/min. The obtained GC×GC total ion chromatograms were processed using automated spectral deconvolution software ChromaTOF (version 4.44, LECO). Mass spectral match factor was used to decide whether a peak was correctly identified or not.

The berries were found to contain around 110 terpenes identified by GC×GC--TOFMS. According to their chemical structure, the compounds were organized in different groups: monoterpene hydrocarbons and monoterpene oxygen-containing compounds (oxides, alcohols, aldehydes, and ketones). Positive identification of some of the terpenes was performed using 22 standard compounds, while

tentative identification of the rest of analytes was based on deconvoluted mass spectra and comparison of linear retention indices with literature values.

The results indicate that the used method HS-SPME/GC×GC-TOFMS is a good alternative for determination of volatile and semi-volatile compounds compared with other concentration techniques and separation methods of volatile analytes, mainly terpenes. Furthermore, this method enables the analysis of complex matrix composition with improved sensitivity and selectivity. Also it limits the use of chemical reagents in the step of sample preparation.

Tentative identification of terpenes was verified by comparing their linear temperature-programmed retention indices with literature values. Furthermore, the identification of some terpenes was proved by comparison of their linear retention indices and mass spectra with those of authentic standards. GC×GC analysis of C8–C20 *n*-alkanes series was performed for the calculation of linear retention indices.

Acknowledgments

The authors wish to thank LECO Poland for financial and technical support.

References

[1] Grajek W., Beer-Dubowska W.: *Przeciwutleniacze w żywności: aspekty zdrowotne, technologiczne, molekularne i analityczne.* Warszawa, Wydawnictwo Naukowo-Techniczne 2007. (In Polish.)
[2] Zwenger S., Basu C.: *Biotechnol. Mol. Biol. Rev.* **3** (2008), 1–7.
[3] Trytek M., Paduch R., Fiedurek J., Kandefer-Szerszeń M.: *Biotechnologia* **1** (2007), 135–155.
[4] Bogacz K.: *Przem. Ferm. Owoc.-Warz.* **9** (2009), 33–34. (In Polish.)
[5] Yildiz Ö., Eyduran S.P.: *Afr. J. Agric. Res.* **4** (2009), 422–426.
[6] Ramadan M. F., Morsel J. T.: *J. Sci. Food Agric.* **87** (2007), 452–460.
[7] Ray A. B., Ali A., Sahai M., Schiff P. L., Knapp J. E., Slatkin D. J.: *Chem. Ind. (London)* **2** (1981), 62–64.
[8] Neogi P., Sahai M., Ray A.B.: *Phytochemistry* **26** (1986), 243–247.
[9] Berger R.G., Drawert F., Kollmannsberger H.: *Z. Lebensm. Unters. Forsch.* **188** (1989), 122–126.

Determination and Mechanism Study of Antiviral Drug Fosamprenavir Using Carbon Paste Electrode in the Presence of Triton X-100

AMIRMANSOOR ASHRAFI[a,*], MEHMET GUMUSTASB[b], KAREL VYTŘAS[a], DAVOOD NEMATOLLAHI[c], BENGI USLUB[b], TOMAS MIKYSEK[a], ROBERT JIRASKO[a], SIBEL A. OZKAN[b]

[a] *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic* ✉ *amirmansoor.ashrafi@yahoo.com*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara 06100, Turkey*
[c] *Department of Chemistry, Faculty of Science, University of Bou-Ali-Sina, Hamedan, Iran*

Keywords
carbon paste electrode
Fosamprenavir
mechanism
Triton X-100
voltammetry

Fosamprenavir (Fig. 1) is an antiretroviral medication that belongs to a group of drugs known as protease inhibitors. Fosamprenavir is the phosphate ester prodrug of amprenavir. Fosamprenavir is absorbed into the blood stream through the wall of the small intestine where it is converted to amprenavir. Amprenavir interferes with the life cycle of HIV to stop it from producing more virus [1–2].

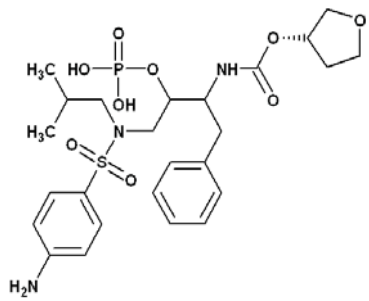


Fig. 1. Structure of Fosamprenavir

The new procedure has been developed for determination Fosamprenavir. Developed procedure is based on a carbon paste electrode as working, the Ag/AgCl/3 mol L^{-1} KCl reference and a platinum counter electrode in Britton Robinson buffer (pH ~ 2) using square wave voltammetry. Triton X-100 was found to have a positive effect on the analytical signal. In the presence of Triton X-100 the signal increased almost five times. The promising analytical

performance was obtained with developed method. Concentration calibration was linear in the range of 1×10^{-5} to 5×10^{-5} mol L^{-1} Fosamprenavir. In sense of low detection limit (4.8×10^{-7} mol L^{-1}) and good repeatibilty and reproducibity of the results (4.50%) and (4.07%) respectively, The method was used to determine Fosamprenavir in pharmaceutical dosage form successfully. Study the mechanism of electrochemical reaction also was carried out and aromatic amine part of the molecule found to be involved in electrochemical oxidation of Fosamprenavir. The oxidized form takes part in follow up reactions and the products also are electroactive and participate in redox reaction.

References

[1] http://depts.washington.edu/madclin/pharmacy/patientinfo/fosamprenavir.pdf (accesed 1st August 2013)
[2] Clercq E.D.: *Curr. Opin. Virol.* **2** (2012), 572–579.
[3] Durga B.L., Devi P.V.L., Babu K.M., Rifath K.S., Abbulu M., Devanaboyina N.: *International Journal of Research and Reviews in Pharmacy and Applied Science* **2** (2012), 429–441.

Characterization of Capillary Monolithic Columns for Hydrophilic Interaction Chromatography

Magda Staňková*, Pavel Jandera, Jiří Urban, Veronika Škeříková

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 532 10 Pardubice Czech Republic ✉ magda.stankova@student.upce.cz

Keywords
capillary columns
hydrophilic interaction chromatography
liquid chromatography
organic polymer monolith
phenolic compounds

Miniaturization is an actual trend in liquid chromatography. A micro-bore or capillary columns allow lower volumes of mobile phases and of samples to be used. Monolithic stationary phases formed by a single piece of highly porous material are very suitable for miniaturization.

Organic polymer-based monolithic columns are prepared by in-situ radical polymerization in fused silica capillary. The polymerization mixture contains a crosslinking monomer, a functional monomer, porogen solvents and an initiator of polymerization. Optimization of composition of the polymerization mixture allows control of hydrodynamic and separation properties of columns such as porosity, efficiency, and selectivity.

Various crosslinking monomers were used for the preparation of capillary monolithic columns suitable for hydrophilic interaction chromatography. We have applied crosslinking monomers (Fig. 1) with different lengths of alkyl chain between two methacrylate units (ethylene, tetramethylene, hexamethylene dimethacrylate), a crosslinking monomer with oxyethylene chain (dioxyethylene dimethacrylate), a crosslinking monomer with three acrylate units (pentaerythritole triacrylate), and crosslinking monomers with aromatic units in their structures (bisphenol A dimethacrylate and bisphenol A glycerolate dimethacrylate). *N, N*-dimethyl-*N*-metacryloxyethyl-*N*-(3-sulfopropyl) ammonium betaine has been used as a functional monomer for preparation of monolithic columns in hydrophilic interaction chromatography. We have used 1-propanol, 1,4-butanediol and water as a porogen solvents and azobisizobutyronitrile as initiator of the reaction.

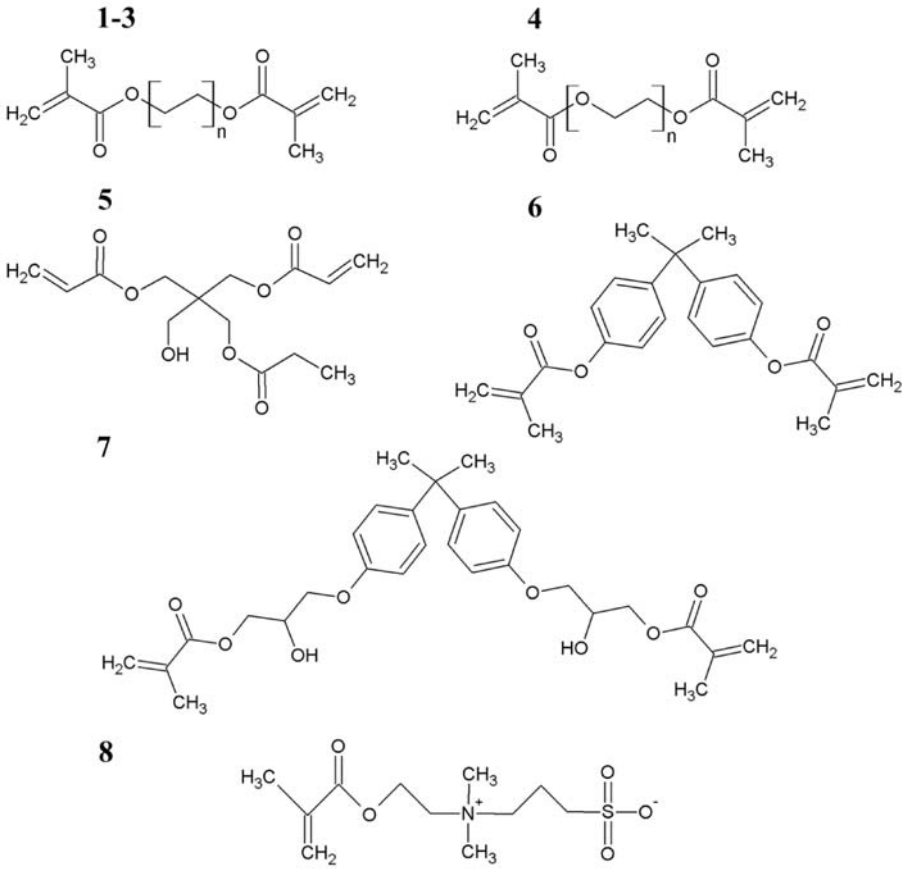


Fig. 1. Structures of methacrylate monomers. Crosslinking monomers: (1) ethylene dimethacrylate, *n* = 1 (EDMA), (2) tetramethylene dimethacrylate, *n* = 2 (BUDMA), (3) hexamethylene dimethacrylate, *n* = 3 (HEDMA), (4) dioxyethylene dimethacrylate, *n* = 2 (DiEDMA), (5) pentaeritritole triacrylate (PETRA), (6) bisphenol A dimethacrylate (BIDMA), (7) bisphenol A glycerolate dimethacrylate (BIGDMA). Functional zwitterionic monomer: (8) *N,N*-dimethyl-*N*-methacryloxyethyl-*N*--(3-sulfopropyl) ammonium betaine (MEDSA).

To investigate the effects of the crosslinkers on the pore size distribution, inverse size-exclusion chromatography has been used. We also determined the effects of the applied crosslinkers on the efficiency of prepared columns using van Deemter plots. Columns prepared with dioxyethylene dimethacrylate and bisphenol A glycerolate dimethacrylate showed the efficiency up to 70 000 theoretical plates/m. The columns provided a dual retention mechanism and were used for isocratic separation of model mixture of phenolic compounds both in acetonitrile-rich mobile phases in HILIC mode and in the mobile phases with higher concentrations of water in the reversed-phase mode.

These columns have been also applied in the separation of polystyrene standards and toluene in size-exclusion chromatography. These columns provide good run-to-run and batch-to-batch repeatability of the elution volumes with relative standard deviation of 1.3% or lower on both columns.

The result of this work confirmed significant effect of the length and chemistry of the chain in the crosslinking monomer on the efficiency of polymethacrylate capillary columns and importance of its optimization to obtain suitable pore morphology enabling isocratic separations of complex mixture of phenolic compounds. The optimized columns can be used not only in the one-dimensional but also in comprehensive two-dimensional liquid chromatography.

Acknowledgments

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

Chiral Stationary Phases Based on Derivatized Cyclofructan

Jiří Vozka*, Květa Kalíková, Eva Tesařová

Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 2030, 128 43 Prague, Czech Republic ✉ *vozka@natur.cuni.cz*

Keywords
chiral stationary phase
cyclofructans
HPLC
linear free energy relationship
supercritical fluid chromatography

In 2009 Armstrong et al. introduced chiral stationary phases based on native and derivatized cyclofructans [1]. Since that time cyclofructans as chiral selectors found applications in many separation techniques including HPLC, gas chromatography, and capillary electrophoresis [2–5].

The structure of cyclofructan molecule is composed of six, seven or eight D-fructofuranose units connected via β (2→1) linkage [6]. The connected saccharide units form a crown-ether ring, which significantly determinates chromatographic behavior of cyclofructans. Common abbreviations CF6, CF7 and CF8 indicates a number of saccharide units forming the ring. Native cyclofructans have their crown-ether skeleton sterically blocked by intramolecular hydrogen bonds, therefore their separation abilities are quite limited. As a result of introducing of derivatization groups into the cyclofructans molecule the molecule relaxes and crown-ether core becomes more accessible and the enantioselective potential significantly increases. Character of the derivatization moiety has substantial impact, since aliphatic derivatization moieties particularly improve enantioselectivity especially for amines, while aromatic derivatization groups improve enantioselectivity among various compounds.

The aim of our work was to study chiral stationary phases based on derivatized cyclofructans under the conditions of normal phase mode HPLC. Overall, three cyclofructans chiral stationary phases were studied. DMP-CF7 chiral stationary phase contained 3,5-dimethylphenyl carbamate bonded onto silica gel (Larihc CF7-DMP column), RN-CF6 chiral stationary phase contained *R*-naphthylethyl carbamate (Larihc CF6-RN column) and IP-CF6 isopropyl carbamate (Larihc CF6-P columns) both bonded onto silica.

To determine and quantify the interactions participating in the retention process a linear free energy relationship model (LFER) was employed. This model is based on the idea that retention (expressed as log *k*, where *k* is a retention factor) is composed of independent contribution according to the involved interaction type. The most common LFER equation works with five type of interactions (dispersion interactions, hydrogen bond acidity, hydrogen bond basicity, polarity/polarizibility, and ability to interact via *n*- and/or π-electron pairs).The LFER set contained 35 compounds, whose chemical features were described. The description is based on the physical measurements/calculations and expressed as a number (a descriptor). After analysis of LFER set, logarithms of obtained retention factors undergo multilinear regression analysis (log *k* against the descriptors). Results of multilinear analysis, regression coefficients, indicate whether the particular interaction type is dominant in the stationary phase (positive value of the regression coefficient) and thus increases the retention or in the mobile phase (negative value of the descriptor) and decreases retention or the same in both phases (statistically insignificant regression coefficient)

At first, individual cyclofructan chiral stationary phases were compared (Table 1). The same types of interactions in a different extent were shown to be

Table 1
Comparison of regression coefficients of the LFER equation and correlation coefficient *R* for systems with three cyclofructan chiral stationary phases under normal phase mode HPLC. CI represent ±95% confidence interval; × insignificant interaction; OM optimal model of the LFER equation; the *p*-values express probability of the error that the individual coefficient does not contribute to the model (i.e., *p*-values represent the significance of the individual coefficients).

Column	Mobile phase	Model	*e*	*s*	*a*	*b*	*v*	*c*	*R*
CF6-RN	hex/IPA/TFA	OM	×	1.128	×	1.596	–1.169	–0.944	0.953
	80/20/0	± 95% CI		0.298		0.302	0.382	0.307	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	
CF6-IP	hex/IPA/TFA	OM	×	0.518	×	1.573	–0.887	–0.401	0.963
	80/20/0	± 95% CI		0.228		0.211	0.282	0.232	
	v/v/v	*p*-value		0.000		0.000	0.000	0.001	
CF7-DMP	hex/IPA/TFA	OM	×	0.803	×	1.729	–0.703	–1.048	0.950
	80/20/0	± 95% CI		0.305		0.287	0.391	0.315	
	v/v/v	*p*-value		0.000		0.000	0.001	0.000	
CF6-RN	hex/IPA/TFA	OM	×	0.891	×	1.535	–0.918	–0.892	0.965
	80/20/0.5	± 95% CI		0.244		0.231	0.316	0.254	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	
CF6-IP	hex/IPA/TFA	OM	×	0.594	×	1.486	–0.821	–0.553	0.957
	80/20/0.5	± 95% CI		0.242		0.226	0.311	0.254	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	
CF7-DMP	hex/IPA/TFA	O.M	×	0.927	×	1.622	–0.765	–1.110	0.956
	80/20/0.5	± 95% CI		0.287		0.267	0.368	0.300	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	

preferred by all three chiral stationary phases, i.e. hydrogen bond acidity and dipolarity/polarizibility. Also the effect of hydrophobicity as the retention reducing factor plays a role with all tested cyclofructan based chiral stationary phases. Hydrogen bond basicity and interactions with *n*- and π-electron pairs seemed to be insignificant.

The logical continuance of our work was to study interaction of cyclofructan chiral stationary phases in supercritical fluid chromatography. This separation method uses carbon dioxide in a supercritical state as the component of the mobile phase. In the first article introducing cyclofructan chiral stationary phases was mentioned the possibility of use cyclofructan chiral stationary phases in supercritical fluid chromatography. However, the detailed study has never been done.

To this purpose DMP-CF7 chiral stationary phase was chosen. The same model as for HPLC was used for the characterization and further comparison of retention interaction under HPLC and supercritical fluid chromatography conditions. The LFER model confirmed that different interactions participated to different degrees in the retention process on DMP-CF7 chiral stationary phase in supercritical fluid chromatography and HPLC (Table 2). The results suggested that the adsorption of some components of the mobile phases is more important in supercritical fluid chromatography than in HPLC.

Table 2
Comparison of regression coefficients of the LFER equation and correlation coefficient *R* for DMP-CF7 chiral stationary phases in supercritical fluid chromatography (SFC) and HPLC. CI represent ±95% confidence interval; × insignificant interaction; OM optimal model of the LFER equation; the *p*-values express probability of the error that the individual coefficient does not contribute to the model (i.e., *p*-values represent the significance of the individual coefficients).

Method	Mobile phase	Model	*e*	*s*	*a*	*b*	*v*	*c*	*R*
HPLC	Hex/IPA/TFA	OM	×	0.789	×	1.597	–0.810	–0.881	0.95
	80/20/0.0	± 95% CI		0.277		0.310	0.389	0.330	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	
HPLC	Hex/IPA/TFA	OM	×	0.933	×	1.516	–0.917	–0.940	0.96
	80/20/0.5	± 95% CI		0.254		0.274	0.348	0.296	
	v/v/v	*p*-value		0.000		0.000	0.000	0.000	
SFC	CO_2/IPA/TFA	OM	0.459	0.514	×	0.949	–1.011	–0.410	0.94
	80/20/0.0	± 95% CI	0.250	0.263		0.251	0.494	0.359	
	v/v/v	*p*-value	0.001	0.000		0.000	0.000	0.026	
SFC	CO_2/IPA/TFA	OM	0.537	0.472	×	0.936	–1.204	–0.227	0.94
	80/20/0.5	± 95% CI	0.253	0.267		0.255	0.501	0.364	
	v/v/v	*p*-value	0.000	0.001		0.000	0.000	0.212	
SFC	CO_2/MeOH/TFA	OM	0.557	0.495	0.891	1.020	–0.806	–0.685	0.97
	95/5/0.0	± 95% CI	0.308	0.351	0.279	0.314	0.735	0.512	
	v/v/v	*p*-value	0.001	0.007	0.000	0.000	0.033	0.010	
SFC	CO_2/MeOH/TFA	OM	0.627	0.553	0.795	1.113	–1.165	–0.422	0.93
	95/5/0.1	± 95% CI	0.446	0.508	0.404	0.455	1.064	0.741	
	v/v/v	*p*-value	0.007	0.034	0.000	0.000	0.033	0.254	

Acknowledgments

The Grant Agency of the Charles University in Prague, project No. 356411, the Ministry of Education, Youth and Sports of the Czech Republic, project KONTAKT AM 2010 project LH11018, and the long-term project MSM0021620857 are gratefully acknowledged for the financial support. Furthermore, Prof. Ch. Roussel and Dr. N. Vanthuyne from the Chirosciences team of Aix-Marseille University are thanked for their kind help during the supercritical fluid chromatography experiments.

References

[1] Sun P., Wang Ch., Breitbach Z. S., Zhang Y., Armstrong D.W.: *Anal. Chem.* **81** (2009), 10215–10226.
[2] Sun P., Wang, Ch., Padivitage N. L. T., Nanayakkara Y. S., Perera S., Qiu H., Zhang Y., Armstrong D.W.: *Analyst* **136** (2011), 787–800.
[3] Zhang Y., Breitbach Z. S., Wang Ch., Armstrong D. W.: *Analyst* **135** (2010), 1076–1083.
[4] Jiang Ch., Tong M., Breitbach Z. S., Armstrong D.W.: *Electrophoresis* **30** (2009), 3897–3909.
[5] Gondová T., Petrovaj J., Kutschy P., Armstrong D.W.: *J. Chromatogr. A* **1272** (2013), 100–105.
[6] Immel S., Schmitt G. E., Lichtenthaler, F. W.: *Carbohydr. Res.* **313** (1998), 91–105.

The Application of TD-GC-FID Technique for Production of Matrix-Free Reference Materials

Marta Słomińska*, Piotr Konieczka, Jacek Namieśnik

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, G. Narutowicza 11/12 Str., 80-233 Gdansk, Poland ✉ korpala.m@poczta.onet.pl

Keywords
BTX compounds
reference materials
standard gas mixture
thermal decomposition

Atmospheric air is one part of the environment ensuring the survival of life on Earth. It contains not only the essential, natural components (oxygen, nitrogen, carbon dioxide, etc.) but also a whole range of undesirable, unnatural substances. The presence of pollutants in the air has a deleterious effect on the health of humans, animals and plants; it also leads to irreversible changes in water and soil (World Health Organization). The emission of BTX compounds (benzene, toluene, xylenes) into the air is a subject of particular interest, principally in view of the adverse effect on the environment and their possible transport into all of its compartments. Benzene, toluene, and/or xylenes compounds are among the most hazardous and ubiquitous air pollutants, emitted from natural and anthropogenic sources. Once released to the atmosphere, they can easily distribute in the environment and therefore affect humans' health. The dangers of the presence of benzene and its alkylated derivatives in the environment stem from their toxicity, mutagenicity and carcinogenicity towards living organisms. From environmental point they play significant role in the formation of secondary pollutants and they have influence on global environmental problems. For these reasons particularly much attention is being paid to the determination of trace and ultra-trace levels of these pollutants [1–4].

In the last few years particular pressure is exerted on finding new analytical methods which would provide real possibilities of monitoring and control of the state of the environment. The necessity to ensure proper quality of life for living organisms at all levels of their evolution causes that all actions leading to an ever-increasing quality of results of analytical measurements gain importance. To

ensure the proper quality of the results of analytical measurements, two types of tools are needed:

- appropriate analytical procedures;
- measuring equipment.

The basic types of tools at the disposal of analysts, are used to perform research on appropriate representative samples with the aim of obtaining measurement data which constitute the basis of reliable analytical information.

Intensive development of analytical techniques for measurement of gaseous media and the negative effects of BTX compounds make it necessary to produce new gaseous reference materials. Currently the most popular producers of gaseous reference materials are: The National Institute of Standards and Technology, National Research Council of Canada, Institute for Reference Materials and Measurements, International Atomic Energy Agency [5].

One novel approach to generating standard gaseous mixtures of toxic, reactive, volatile, labile, and malodorous compounds involves thermal decomposition of immobilized compounds. This approach is based on making use of surface compounds, chemically bonded to the surface of a carrier, which, under defined temperature conditions, undergo thermal decomposition or rearrangement, releasing specific amounts of volatile compounds. Generated standard gas mixture is analyzed on gas chromatograph coupled with flame-ionization detector (GC-FID) [5–7].

Suitable preparation of surface of the 'candidate' for reference material is the most difficult stage in the production of standard gas mixture. The main objective of the research is to prepare new matrix-free reference materials for volatile organic compounds such as BTX compounds using a glass fibers covered with thin layer of aluminum and pure aluminum fibers as a support. The starting point for the project is conducted from many years research on the production of new matrix-free reference materials of gaseous substances, with the use of thermal decomposition of surface compounds. The research is focussed on:

- chemical modification of solid materials which are characterized by properly developed the active surface in order to obtain the desired surface compounds;
- searching for optimal conditions for the thermal decomposition of surface compounds in order to obtain well-defined quantities of the desired analytes;
- searching for quick and convenient calibration techniques.

An essential role in the application of thermal decomposition process to obtain an appropriate standard gas mixture is played by the type of carrier for immobilized compounds: the amount of released analyte directly depending on its choice.

In the research glass fibers modified with aluminum are used. Fibers are placed in a glass tube and treated with high temperature for a definite period of time. Four-port valve is closed at the time of thermal decomposition. Then, after a given period of time position of valve is changed, carrier gas stream flushes the analytes from the chamber of thermal desorber, and the stream of gaseous mixtures is

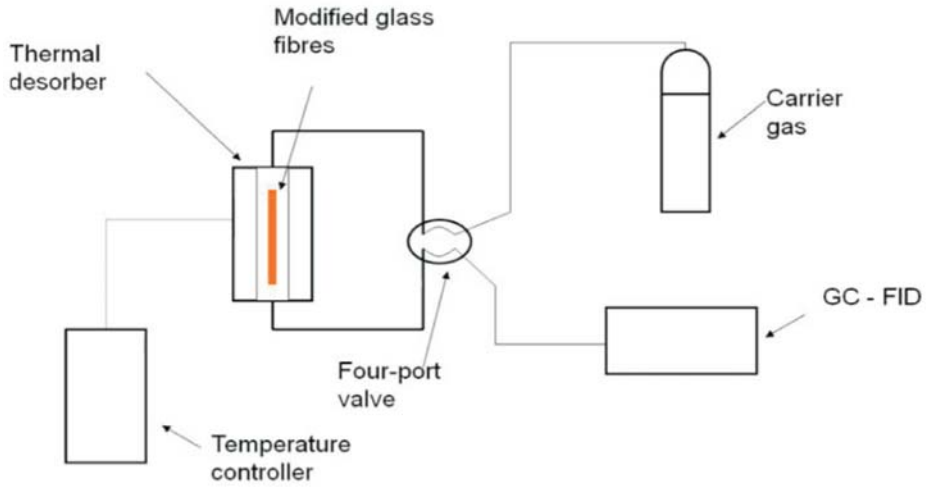


Fig. 1. The apparatus used in the investigation.

directed into the gas GC-FID system. Thermal decomposition-GC-FID apparatus is presented in the figure 1.

Homogeneity and stability are two basic parameters which play a decisive role in the preparation of reference materials. In order to determine the degree of homogeneity, a series of comparisons of results obtained for randomly chosen samples of the reference material is carried out. Such process is always carried through, after having previously divided the reference material among individual packages and then ascertained the homogeneity inside the packages and between them [8].

On the other hand, stability is determined on the basis of comparison of measured values for samples stored at a so-called reference temperature and those for samples stored at the temperature recommended for the given material. Two types of stability are distinguished: long- and short-term stability. Stability of gaseous reference materials is the main problem in their production[9].

The last important parameter related to reference materials are the given reference value with calculated an expanded uncertainty value. Reference value and expanded uncertainty value are calculated taking into account the results obtained in interlaboratory comparisons campaign. Expanded uncertainty of measurement is calculated according to ISO guidelines [10]. The necessity to meet these requirements causes that the production of reference materials, gaseous reference materials in particular, is time-consuming labor-intensive and consequently very costly.

On the basis of the results it will be possible to:

- shorten the time of analysis,
- eliminate sources of error,
- obtain higher level of precision and accuracy of the analysis.

Moreover, this form of reference materials will provide a useful tool for routine screening in daily laboratory practice.

Acknowledgments

The research is funded by the National Science Centre (UMO-2011/03/N/ST4/02467). Further Marta Słomińska expresses her gratitude for financial support from the funds of the project 'Development of interdisciplinary postgraduate studies in modern technologies at the Gdańsk University of Technology' (Project: POKL.04.01.01-00-368/09).

References

[1] Gelancsér A., Siszler K., Hlavay J.: *Environ. Sci. Technol.* **31** (1997), 2869–2872.
[2] Pyta H.: *Polish J. Environ. Stud.* **15** (2006), 785–791.
[3] Keymeulen R., Görgényi M., Héberger K., Priksane A., Langenhove H.: *Atm. Environ.* **35** (2001), 6327–6335.
[4] Gallego E., Roca F. X., Guardino X., Rosell M. G.: *J. Environ. Sci.* **20** (2008), 1063–1069.
[5] Konieczka P., Świtaj-Zawadka A., Namieśnik J.: *Trends Anal. Chem.* **23** (2004), 450–458.
[6] Przyk E., Świtaj-Zawadka A., Konieczka P., Szczygelska-Tao J., Biernat J.F., Namieśnik J.: *Anal. Chim. Acta.* **488** (2003), 89–96.
[7] Kupiec K., Konieczka P., Namieśnik J.: *Crit. Rev. Anal. Chem.* **39** (2009), 311–322.
[8] Van der Veen A. M. H., Lisinger T., Pauwels J.*: Accred. Qual. Assur.* **6** (2001), 26–30
[9] Słomińska M., Konieczka P., Namieśnik J.: *Anal. Bioanal. Chem.* **405** (2013), 1773–1778.
[10] Van der Veen A.M.H., Lisinger T., Schimmel H., Lamberty A., Pauwels J.: *Accred. Qual. Assur.* **6** (2001), 290–294.

Fast Potentiometric Detection System Utilizing Interface of Two Immiscible Electrolytes for Application in Flow Analysis

VĚRA MANSFELDOVÁ[a, b, *], PAVEL JANDA[b], HANA TARÁBKOVÁ[b]

[a] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic*
[b] *Department of Electrochemical Materials, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, v.v.i, Dolejškova 3, 182 23 Prague 8, Czech Republic* ✉ *vera.mansfeldova@jh-inst.cas.cz*

Keywords
dichlorobenzene
flow analysis
ITIES
phthalocyanine
potentiometry

In method, based on the electrochemistry at the interface between two immiscible electrolyte solutions (ITIES), transfer of ions across the ITIES provides the analytical signal besides the oxidation or reduction of the analyte as in case of other electrochemical methods. Koryta, Samec, Mareček, and coworkers were among those who developed this topic of electrochemistry [1, 2]. Since then, ITIES found wide range of applications from classical electrochemical methods including amperometry [3] to solar cells [4]. Moreover, ITIES can be used as a biomimetic (model) system for membrane charge transfer processes in biological systems [5]. Important application of ITIES can be found in drug delivery (e.g. heparin [6, 7], tetracyclines [8, 9] dopamine [10]) across the cell membrane. Substantial miniaturization of ITIES technique was introduced by Girault and Taylor [11].

Our aim was to construct potentiometric sensor utilizing ITIES that would be suitable for flow analysis. In our experiments we have used the water/1,2-dichlorobenzene interface. Aqueous phase served as a mobile phase while nonaqueous phase contained phthalocyanine mediator. Phthalocyanine compounds were chosen as mediators offering a wide variety of modifications affecting their physico-chemical and redox properties. The cell design was optimalized by

determination of model reductive analytes such as sulphides and cysteine, which can be detected in open-to-air conditions. Under these conditions, oxidation form of mediator is regenerated competitively by oxygen, establishing equilibrium interface/membrane potential. This type of cell is resistant towards gas bubbles in mobile phase and it appears to be the suitable choice for detection of sulfide-containing compounds.

Acknowledgments

We are grateful to N. Kobayashi, Tohoku University in Japan, and J. Kaleta, Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., in Prague, for supplying phthalocyanine used in the work. The work was supported by the research project MSM0021620857 of the Ministry of Education, Youth, and Sports of the Czech Republic, and grant project SVV of Charles University in Prague.

References

[1] Koryta, J.: *Electrochim. Acta* **31** (1986), 515–520.
[2] Koryta, J.: *Electrochim. Acta* **33** (1988), 189–197.
[3] Osborne, M.D., Girault, H.H.: *Electroanalysis* **4** (1995), 714–721.
[4] Reymond, F., Fermín, D., Lee, H.J., Girault, H.H.: *Electrochim. Acta* **45** (2000), 2647–2662.
[5] Lu, X., Hu, L., Wang, X.: *Electroanalysis* **17** (2005), 953–958.
[6] Ma, S.C., Yang, V.C., Meyerhoff, M.E.: *Anal. Chem.* **64** (1992), 694–697.
[7] Amemiya, S., Kim, Y., Ishimatsu, R., Kabagambe, B.: *Anal. Biol. Chem.* **399** (2011), 571–579.
[8] Kozlov, Y.N., Koryta, J.: *Anal. Lett.* **16** (1983), 255–263.
[9] Fernández, R.A., Dassie, S.A.: *J. Electroanal. Chem.* **585** (2005), 240–249.
[10] Berduque, A., Zazpe, R., Arrigan, D.W.M.: *Anal. Chim. Acta* **611** (2008), 156–162.
[11] Taylor, G., Girault, H.H.: *J. Electroanal. Chem.* **208** (1986), 179–183.

Gas Chromatographic Analysis of Saccharides in Urine

Dušana Samková[a,*], Peter Podolec[a], Alexandra Hengerics Szabó[a], Róbert Kubinec[a], Jozef Višňovský[b]

[a] *Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovakia* ✉ *samkova@fns.uniba.sk*
[b] *SynthCluster, s.r.o., Komenského 1439, 900 01 Modra, Slovakia*

Keywords
derivatization
gas chromatography
saccharides
urine

Inherited metabolic disorders belong to the major causes of morbidity and mortality of children and adults. Therefore, the need for a simple, rapid and reliable method for their diagnosis increases. Galactosemia belongs to the most serious metabolic disorders of carbohydrate metabolism affecting newborns [1].

Although carbohydrate analysis is not a new research area, methods for their simple and rapid analysis which can be performed routinely are still in development. One of the greatest difficulties of carbohydrate analysis is the number of possible isomeric forms of the saccharides, e.g., the simple monosaccharide glucose can exist in six different forms (simple open chain, its hydrated form, α- and β-glucopyranose, α- and β-glucofuranose). The second difficulty is the fact that simple monosaccharides differ only slightly; in their hydroxyl groups, what makes their identification in complex mixtures nearly impossible. Moreover, they are very polar and nonvolatile, lack easily ionizable groups, do not have fluorescence and most of them do not absorb light in the analytically usable area of the light spectrum, what prevents their sensitive detection [2, 3]. As they are very polar and nonvolatile compounds, even their GC analysis is a complex problem, as they have to be derivatized before gas chromatographic analysis. Their analysis without derivatization is nearly impossible as they give unreproducible tailing peaks and their polar groups interact with the column and other parts of the analytical separation system degrading and shortening its lifetime. Derivatization makes mono- and disaccharides more volatile and less polar so that they can be analyzed by gas chromatography.

A variety of derivatization procedures were developed in dependence on the task to be solved. Most frequently the saccharides are analyzed as acetyl, trifluoroacetyl or trimethylsilyl derivatives. The trimethylsilyl derivatives however are sensible to the presence of water, in which presence their decomposition may occur. Another problem related to their determination is their mutarotation. If the anomeric centres are not eliminated, derivatization fixes the carbohydrate in its actual form in the solution. Therefore, prior to the derivatization step, the elimination of the anomeric centers is often performed. Carbohydrates containing carboxyl groups require an additional derivatisation step, otherwise some of the carboxyl groups remain underivatized, resp. are transformed to lactones or esters [3, 4–7].

Undigested carbohydrates are in an unaltered or a reduced form excreted in urine, where they can be reliably determined. The need of immediate diagnosis is obvious, but the medical diagnosis is often lengthy and even painful, therefore new methods of rapid and simple diagnosis are needed. Saccharides present in urine either in unaltered form or as polyols can be easily determined by gas chromatography in a relatively short time without the need of blood tests.

The aim of this work was the development of a new method for the gas chromatographic determination of carbohydrates. The main object of study was the development of a simple method of sample pretreatment and derivatization by silylation. The published methods of carbohydrate derivatization by silylation are time-consuming and complicated due to the need of sample evaporation. This work was aimed at the development of a derivatization method for saccharides in aqueous samples without water evaporation, which was achieved by silylation using a mixture of HMDS:TMCS:PYR (hexamethyldisilazane:trimethylchlorosilane:pyridine) in a large excess to the aqueous sample at 70 °C.

Acknowledgments

Work was supported by the Slovak Research and Development Agency under the contract no. APVV-0416-10, and by Comenius University in Bratislava (grant no. UK/227/2013).

References

[1] Šašinská M., Šagát T.: *Pediatria.* Košice, Satus 1998. (In Slovak.)
[2] Oefner P., Scherr H.: *Adv. Electrophoresis* **7** (1994), 157–220.
[3] Barsuhn K., Kotarskin S.F.: *J. Chomatogr. A* **546** (1991), 273–287.
[4] Churms C.S.: Sugar Derivatives Chromatography. In: *Encyclopedia of Separation Science*. Academic Press 2000.
[5] Black E.G., Fox A.: *J. Chromatogr. A* **720** (1996), 51–60.
[6] Fox A., Morgan S., Gilbart J.: *Analysis of Carbohydrates by GLC and MS*. Boca Raton, CRC Press 1989
[7] Fox A., Korar P.M., Steinberg P.A.: *GC and GC-MS.* Academic Press 2000.

The Comparison of Kinetic Constants of Different Sequence Motifs Digested by Trypsin

Tereza Šlechtová[a,*], Květa Kalíková[a], Martin Gilar[b], Eva Tesařová[a]

[a] *Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague 2, Czech Republic* ✉ *tereza.slechtova@natur.cuni.cz*
[b] *Waters Corporation, 34 Maple St., Milford, MA 01757, USA*

Keywords
digestion
HPLC
kinetics
peptide structure
trypsin

Trypsin is the most widely used protease for protein digestion in proteomic research. The speed of proteolytic digestion is affected by many factors, such as pH, temperature, presence of organic solvents, enzyme autolysis or the substrate tertiary structure [1]. Trypsin cleaves peptide bonds with high specificity after lysine (K) and arginine (R) residues, except when followed by proline (P) [2]. However, "missed cleavage" peptides are often observed when the motifs KK, RR, KR, or RK are present in the sequence. These motifs are cleaved with greatly reduced speed. Glutamate or aspartate acidic residues (E or D) present near the cleavage site can also reduce the proteolysis speed [3]. These acidic amino acids can form salt bridges with the basic arginine and lysine, competing with the complementary aspartic acid at the bottom of trypsin's active site. Consequently, motifs such as KD, KE, DK, and EK (and analogical with arginine) are also source of missed cleavage peptides. Digestion kinetics of these motifs is not well known.

Furthermore, due to the different structure of two possible cleavage sites (lysine and arginine), the strength of the bond with enzyme's active site is not the same. As a result, different rules for digestion at lysine and arginine residues have been observed [4]. Therefore, it can be predicted that the kinetic parameters of enzymatic reactions will be affected differently by the presence of acidic amino acids.

In this study, porcine trypsin and eight synthetic peptides with lysine cleavage site were studied in order to determine the changes of tryptic digestion rate in the

Table 1
Experimentally observed kinetic constants of selected peptides with following sequence: LYAA[X]LYAVR. [X] represents sequence motif consisting of 1–3 amino acids which includes tryptic cleavage site. Abbreviations: D = aspartic acid, E = glutamic acid, K = lysine, T = threonine.

Cleavage site sequence motif	Kinetic constant [10^{-3} s^{-1}]
[K]	9.74
[KE]	8.58
[EK]	4.41
[KTE]	9.07
[KD]	2.68
[DK]	0.14
[KTD]	7.75
[DTK]	2.69

presence of acidic residues. Peptide digests were analyzed by reversed-phase HPLC method with UV detection. The results (Tab. 1) showed that the presence of acidic amino acid residues near the cleavage site decreases the peptide digestion speed. It was experimentally observed that the aspartic acid is more potent inhibitor than glutamic acid; this may be related to its higher acidity (pK_a of side chain is 3.65 and 4.25, respectively). The kinetic constants were lowered more significantly with acidic residues located before than after the cleavage site. To the lesser extent these trends are also true for motifs when the acidic amino acid is separated from the cleavage site by another amino acid. To our knowledge these results are the first direct measurement of proteolytic digestion kinetics of selected missed cleavage motifs. The results are applicable in proteomic research and potentially for development of modified enzymes.

Acknowledgments

The financial support of the project Kontakt LH 11018 and the long-term research plan of the Ministry of Education of the Czech Republic, MSM0021620857, are gratefully acknowledged.

References

[1] Siepen J. A., Keevil E. J., Knight D., Hubbard S. J.: *J. Proteome Res.* **6** (2007), 399–408.
[2] He Y., Zhong W. , Yeung E. S.: *J. Chromatogr. B* **782** (2002), 331–341.
[3] Yen C., Russell S., Mendoza A.M., Meyer-Arendt K., Sun S., Cios J.J., Ahn N.G., Resing K.A.: *Anal. Chem.* **78** (2006), 1071–1084.
[4] Craik C.S., Largman C., Fletcher T., Roczniak S., Barr P.J., Fletterick R.J., Rutter W.J.: *Science* **228** (1985), 291–297.

Occurrence and Determination of Volatile Fatty Acids in Landfill Leachate and Gas

Marta Wasielewska[a,*], Anna Banel[b], Bogdan Zygmunt[a]

[a] *Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland* ✉ *marta.chemanal@gmail.com*
[b] *Norwegian Institute for Air Research, 2027 Kjeller, Norway*

Keywords
landfill leachate
malodorous compounds
volatile fatty acids

In view of continuous human activity, the amount of environmental pollution is rapidly growing. Volatile organic compounds, including short chain volatile fatty acids, play a significant role in the process of anaerobic biodegradation of organic matter. The main sources are municipal waste landfills and sewage treatment plants. However, volatile fatty acids formation can occur in industrial food processing (including dairy products, food product preservation, alcohols) and are in living organisms (mainly in ruminant stomachs). Some volatile fatty acids, in particular acetic, propionic and butyric acids, are partially responsible for unpleasant smell around sewage treatment plants and landfills [1].

An 'young' landfills large amounts of easily biodegradable organic matter present undergo rapid anaerobic fermentation, giving simple fatty acids [2]. Acidic fermentation is accelerated by high humidity and high water content [3]. In the initial process called the acid phase (acidogenic), up to 95% organic matter content is co reverted into volatile fatty acids [1]. As a result pH drops to low value decreases and some metals can be released [4]. An intensive growth of acetic bacteria, severe consumption of organic substrates and biogenic compounds occur at this phase. The leachates are rapidly loaded with carbon compounds, which results in the high numerical increase in the chemical oxygen demand indicator [5]. As the landfill matures, the methane (methanogenic) phase dominates. The pH of the waste is stabilized with the use of bacterial compounds from the volatile fatty acids group as a source of easily assimilated carbon [6]. After reaching a neutral pH a transition into the appropriate degradation phase occurs, which is

characterized by complex interactions between microorganisms [5]. Methane is produced with the involvement of methane bacteria. A sharp increase in biogas production has been observed, with a corresponding decrease in the concentration of organic carbon compounds. With the consumption of soluble substrates (carboxylic acids), the rate of methane production slowly decreases and carboxylic acids are consumed as fast as they are produced [5]. A slow humification of organic matter in waste also occurs [7]. Sewage treatment, based on the activated sludge method, is an example of volatile fatty acids playing a positive role in the environment. Short chain carbyxolic acids, or simple organic compounds, present in sewage are an endogenous source of energy and carbon for microorganisms responsible for purification processes. The content of volatile fatty acids at various steps of waste water treatment or biogas formation must be monitored; volatile fatty acids either a total content of acids or rather individual acid are determined.

It is worth recalling that there are various means of determining organic acid in different kinds of samples. The main of them are separating techniques such as gas or liquid chromatography or capillary electrophoresis must be applied.

Gas chromatography is usually a technique of choice. The mobile phase in GC is a neutral gas, which is environmentally safe. The use of capillary columns which is generally the case reduces the consumption of carrier gas, even by as much as two orders of magnitude. In comparison with packed columns, the capillary column usually contains less solid phase than a packed column, and it has a longer lifetime.

Liquid chromatography, which is generally more harmful to the environment, is superior to GC in that it can be used to determine volatile and non-volatile acids simultaneously [8, 9]. The amounts of solvents consumed can be drastically reduced by using columns of the small diameter packed. Another way of making LC greener is to use the mobile phases of more friendly to the environment, e.g., instead of methanol or acetonitril-ethanol [9]. Poorer elution properties of ethanol can be improved by, say, raising the temperature [10]. Work is in progress on the application of water as a mobile phase at temperatures and pressures approaching those of the supercritical state, which dramatically changes its properties [10]. Because of certain specific properties of supercritical fluids, chromatography using supercritical fluids is treated as a separate chromatographic technique.

Capillary electrophoresis is a technique requiring very small sample volumes, frequently nL or even of pL in some cases. Because of low consumption of regents and solvents and the small sample volume, capillary electrophoresis can be regarded as the greenest among the separation techniques.

Despite considerable progress in development of sample preparation techniques for analysis and many possibilities in the selection of a final determination technique, the determination of organic acids is often a real challenge for some analytical tasks. The problems stem from the difficulty of collecting a representative sample, the variety of sample matrices and significant differences in

individual analyte concentrations. Often the total procedure for the determination of SCCA compounds in various real samples is complex and multi-stage. Particular steps, as all procedure, require validation. Sample preparation techniques that have found wide application in the determination of SCCAs are generally based on: liquid extraction, solid extraction or gas extraction. The analytes are transferred from the sample (the primary matrix) to the receiving medium (secondary matrix), which should have a simple and clearly defined chemical composition [5, 6].

Special attention is put to the tendency to make the whole analytical procedure environmentally friendly and a lot is done with this respect at the sample preparation step. The techniques obey green analytical principles and present-day research efforts to make the step increasingly green. Monitoring of the leachate for the content of volatile fatty acids can help determine the processes occurring in waste and possible effect of the leachate on the environment in the case of some leakage. Even if present at low concentrations, volatile fatty acids can be a nuisance to people.

Acknowledgments

This work was financially supported by the National Science Center in the framework of the 'PRELUDIUM' (Decision No.: DEC-2012/05/N/ST4/02026). This work was also supported by the European Social Fund, the State Budget and the Pomorskie Voivodeship Budget according to the Operational Programme Human Capital, Priority VIII, Action 8.2, Under-action 8.2.2: 'Regional Innovation Strategy' within the system project of the Pomorskie Voivodeship 'InnoDoktorant – Scholarships for PhD students, Vth edition'.

References

[1] Manni G., Caron F.: *J. Chromatogr. A* **690** (1995), 237–242.
[2] Himanen M., Latva-Kala K., Itavaara M., Hanninen K.: *J. Environ. Qual.* **35** (2006), 516–521.
[3] Baziramakenga R., Simard R.R.: *J. Environ. Qual.* **27** (1998), 557–562.
[4] Abalosa M., Bayona J.M.: *J. Chromatogr. A* **891** (2000), 287–294.
[5] Yang M.H., Choong Y.M.: *Food Chem.* **75** (2001), 101–108.
[6] Acidity and individual acids. In: *Wine Analysis*. Taipei, Research Institute for Wines, Taiwan Tobacco & Wine Monopoly Bureau 1992, p. 111.
[7] Silva C.F., Batista L.R., Abreu L.M., Dias E.S., Schwan R.F.: *Food Microbiol*. **25** (2008), 951–957.
[8] Yang W.Z., Laarman A., He M.L., Liu Q.: *Anim. Feed Sci. Technol.* **148** (2009), 227–240.
[9] Speight S.M., Harmon D.L.: *Anim. Feed Sci. Technol.* **155** (2010), 156–162.
[10] Siedlecka E.M., Kumirska J., Ossowski T., Glamowski P., Gołębiowski M., Gajdus J., Kaczyński Z., Stepnowski P.: *Pol. J. Environ. Stud.* **17** (2008), 351–356.

Comparison of Separation Amino Acid Enantiomers on Teicoplanin and Teicoplanin Aglycone

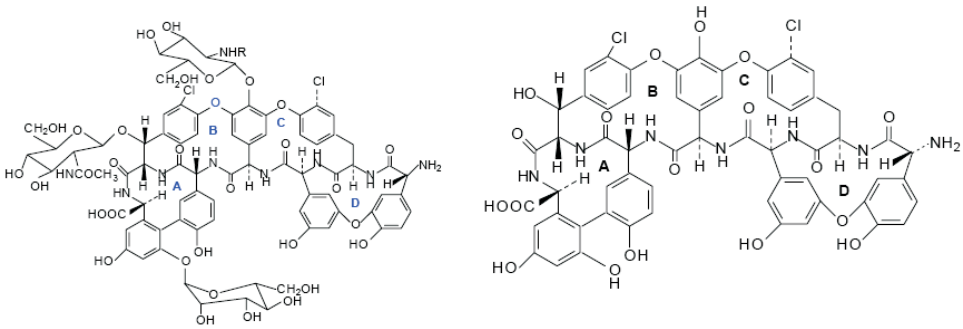
Zuzana Deáková[a,*], Zdeňka Ďuračková[b], Jozef Lehotay[a]

[a] *Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia* ✉ *zuzana.deakova@stuba.sk*
[b] *Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, 811 08 Bratislava, Slovakia*

Keywords
amino acids
electrochemical detection
HPLC
teicoplanin
teicoplanin aglycone

Homocysteine is an intermediate in methionine metabolism. Under normal conditions, intracellular concentration of homocysteine is kept low. Most of it is converted to cysteine or methionine under enzymatic control [1]. However, elevated levels of plasma/serum homocysteine have been recognized as an independent risk factor for cardiovascular diseases like atherosclerosis and venous thrombosis. Relationship of cysteine to cardiovascular disease risk is not well established [2], but it is an important indicator for several clinical disorders like cystinosis and cystinuria [1]. Non-chiral separations of these sulfur-containing amino acids are preferred. Until now, the chiral separation of DL-homocysteine has been just noticed by capillary electrophoresis with cyclodextrins [3]. In addition, several works for separation of cysteine and/or methionine enantiomers have been reported by using cyclodextrin, crown-ether or macrocyclic antibiotic.

Macrocyclic antibiotics as chiral selectors for liquid chromatography were first introduced by Armstrong in 1994 [4] and they can be used for separation of wide range of chiral compounds. Among of all glycopeptides teicoplanin chiral stationary phases are preferred for separation of amino acid enantiomers. Teicoplanin is natural glycopeptide produced by bacteria Actinoplanes teichomyceticus. Removing the sugar moieties from teicoplanin the teicoplanin aglycone was produced that has complementary selectivity to teicoplanin [5] (Fig. 1).



(1) (2)

Fig. 1. Structures of (1) teicoplanin, and (2) teicoplanin aglycone

In this work, the HPLC separations of amino acid enantiomers on teicoplanin and teicoplanin aglycone were compared. The commercially available chiral columns (ASTEC) were used (250×4.6 mm, i.d.). The standard of DL-amino acids (racemates with concentration 0.1 mg/mL) was dissolved in water and injected into the column. They were detected by electrochemical detector (Coulochem II, ESA) composed of guard cell Model 5020 (ESA) and analytical cell Model 5010A (ESA). Firstly, the separation of amino acid enantiomers on teicoplanin was carried out. Enantiomers of homocysteine and methionine were separated, except of cysteine. The resolution between D- and L-enantiomers of homocysteine was 0.8, and methionine 1.4. Secondly, amino acid racemates were injected into teicoplanin aglycone under exact same mobile phase conditions. Enantiomers of all amino acids were separated. Enantiomeric resolutions of homocysteine were 3.1, methionine 2.9, and cysteine 1.4. The time required for analysis was less than ten minutes for both columns. Better enantiomeric separation of selected sulfur--amino acids was obtained by using teicoplanin aglycone.

Acknowledgments

This work was supported by the VEGA projects n. 1/1133/11 and 1/0164/11.

References

[1] Toyooka T.: *J. Chromatogr. A* **877** (2009), 3318–3330.
[2] Rafii M., Elango R., Courtney-Martin G., House J.D., Fisher L., Pencharz P.B.: *Anal. Biochem.* **371** (2007), 71–81.
[3] Kim I.J., Park S.J., Kim H.J.: *J. Chromatogr. A* **877** (2000), 217–223.
[4] Armstrong D.W., Tang Y., Chen S., Zhou Y., Bagwill Ch., Chen J.R.: *Anal. Chem.* **66** (1994), 1473–1484.
[5] *Chirobiotic Handbook: A Guide to Using Macrocyclic Glycopeptide Bonded Phases for Chiral Separations.* 5th ed. USA, Advanced Separation Technologies 2004.

Vibrational and Electronic Circular Dichroism Study of Bilirubin Interactions with Model Membranes and Serum Albumin

Pavlína Novotná[a,*], Marie Urbanová[b]

[a] *Department of Analytical Chemistry, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic* ✉ *Pavlina.Novotna@vscht.cz*
[b] *Department of Physics and Measurements, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic*

Keywords
bilirubin
circular dichroism
model membranes
serum albumin

Bilirubin, the end product of the hem catabolism in the human body, is formed at a rate of about 300 mg per day and usually is effectively excreted from the body. Although, this pigment has been known for more than a century, its exact effects are still under debate. It was found, that it fulfills both positive and negative functions in the human organism [1–4]. In the pathologic state, bilirubin may accumulate and at higher concentrations its negative effects may turn up. During hyperbilirubinemia of newborns, the deposition of unconjugated bilirubin in the central nervous system is the major factor causing bilirubin encephalopathy [2, 4–6]. One of the main negative effects of bilirubin is its impact on nerve cell membranes and membrane proteins which may result in cell apoptosis [7–10]. These neurotoxic effects of bilirubin are thought to be caused by its enantio-discrimination on the membrane which results into membrane disruption or damage [5].

Bilirubin belongs to the group of linear tetrapyrrolic pigments, but its structure is non-planar. Its rigid planar dipyrrole units form a helical structure, which may have either P- or M-sense of helicity. However, in homogenous solution bilirubin exists as a racemate because the racemic barrier between P- and M-form is very low [11–14]. As it is supposed that the P- or M-form of bilirubin interacts preferentially with the membranes, it is convenient to study these systems with methods enabling the observations of the enantiomeric excess [15–18].

Because of this, chiroptical spectroscopic techniques, vibrational and electronic circular dichroism were employed for the studies of bilirubin with model membranes. This combination of techniques enabled us to study the bilirubin systems at different conditions: concentrations, pH, temperature, and also for different model membrane composition. Large unilamellar vesicles composed of different phospholipids and sphingolipids were used as a bilayer membrane model, while micelles composed of surfactants were used as a monolayer membrane model.

While both the spectroscopies provided the information about the bilirubin which interacted with model membrane, the vibrational circular dichroism spectroscopy also helped to identify the lipid functional groups, which were significantly influenced by the interaction. In some cases the bilirubin was found to penetrate inside the membrane. We found, that the composition of the membrane influenced the enantioselectivity of the bilirubin interaction and also the penetration into the membrane. The obtained results also showed that the interaction is governed not only by the membrane composition but also by the physicochemical conditions.

However, bilirubin is usually supposed not to be dangerous to health as it is transported in the organism by the serum albumin transport protein to which bilirubin has a very high binding constant ($K_a = 9\times10^{-7}$ L mol^{-1}) [1, 17, 19, 20]. Since serum albumin is the main transport protein of bilirubin in the mammalian body, its influence on the bilirubin-model membranes interaction was explored. Fig. 1 shows the results obtained by the electronic circular dichroism spectroscopy for

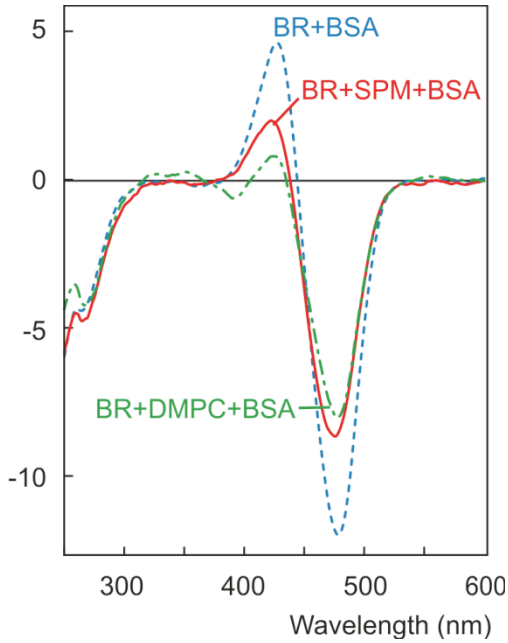


Fig. 1. Electronic circular dichroism spectra of bilirubin with bovine serum albumin (dashed line), bilirubin with bovine serum albumin and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine liposomes (dot-and-dash line) and of bilirubin with bovine serum albumin sphingomyelin liposomes (solid line). The concentrations were: 1×10^{-5} mol L^{-1} for bilirubin, 1×10^{-5} mol L^{-1} for bovine serum albumin, and 1×10^{3} mol L^{-1} for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine or sphingomyelin.

the titration of bilirubin solutions with liposomes by the serum albumin (bovine). These results suggest that although the binding constant of bilirubin to albumin is high, the bilirubin which is bound to the liposomes in the P-form is not released from the liposomes after the addition of serum albumin. It is also evident, that the spectral pattern for the M-form of bilirubin bounded to bovine serum albumin is strongly influenced by the presence of liposomes. Similar results were obtained also for human serum albumin.

In this study, the vibrational circular dichroism spectroscopy was employed for the first time to study the interaction of bilirubin with model membranes. The information about functional groups that participated in the interaction was obtained complemented by the electronic circular dichroism results which showed the bilirubin conformation and the influence of the presence of serum albumin.

Acknowledgments

Financial support from Specific University Research (MSMT No. 21/2012 and No. 20/2013), and by the Grant Agency of the Czech Republic (P206/11/0836, P208/11/0105) is gratefully acknowledged.

References

[1] Vítek L.: *Bilirubin a interní choroby: význam pro kliniku a praxi.* Praha, Grada 2009.
[2] Vitek L., Ostrow J. D.: *Curr. Pharm. Design* **15** (2009), 2869–2883.
[3] Vitek L., Sedlackova L., Branny P., Ruml T.: *Chem. Listy* **97** (2002), 24–28.
[4] Voet D., Voet J. G.: *Biochemistry, 4th ed.* New York, Wiley 2011, p. 1056.
[5] Bernardini C., D'Arrigo P., Elemento G., Mancini G., Servi S., Sorrenti A.: *Chirality* **21** (2009), 87–91.
[6] Brito M. A., Brites D., Butterfield D. A.: *Brain Res.* **1026** (2004), 33–43.
[7] Brites D.: *Front. Pharmacol.* **3** (2012), 1–27.
[8] Brites D., Brito M. A., Matos D., Silva R. F. M.: *J. Hepatol.* **34** (2001), 181–181.
[9] Brito M. A., Brondino C. D., Moura J. J. G., Brites D.: *Arch. Biochem. Biophys.* **387** (2001), 57–65.
[10] Brito M. A., Silva R., Tiribelli C., Brites D.: Eur. *J. Clin. Invest.* **30** (2000), 239–247.
[11] Boiadjiev S. E., Lightner D. A.: *Chirality* **9** (1997), 604–615.
[12] Boiadjiev S. E., Lightner D. A.: *Tetrahedron-Asymmetry* **10** (1999), 607–655.
[13] Boiadjiev S. E., Person R. V., Lightner D. A.: *Tetrahedron-Asymmetry* **4** (1993), 491–510.
[14] Boiadjiev S. E., Person R. V., Puzicha G., Knobler C., Maverick E., Trueblood K. N., Lightner D. A.: *J. Am. Chem. Soc.* **114** (1992), 10123–10133.
[15] Urbanova M., Malon P. In: *Analytical Methods in Supramolecular Chemistry.* Schalley C. (edit.). Weinheim, Wiley 2007, p. 265–304.
[16] Goncharova I., Urbanova M.: *Tetrahedron-Asymmetry* **18** (2007), 2061–2068.
[17] Goncharova I., Urbanova M.: *Anal. Bioanal. Chem.* **392** (2008), 1355–1365.
[18] Goncharova I., Urbanova M.: *Anal. Biochem.* **392** (2009), 28–36.
[19] Brodersen R.: *Crc. Cr. Rev. Cl. Lab. Sc.* **11** (1979), 305–399.
[20] Brodersen R.: *J. Biol. Chem.* **254** (1979), 2364–2369.

3-Monochloropropane-1,2-diol in Infant Foods and Human Breast Milk: Determination by GC-MS

Renata Jędrkiewicz*, Magdalena Kupska, Justyna Gromadzka, Jacek Namieśnik

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland ✉ *rjedrkiewicz@gmail.com*

Keywords
3-monochloropropane-1,2-diol
GC-MS
infant foods
human breast milk

3-Monochloropropane-1,2-diol is one of the food borne contaminants called chloropropanols. It was first discovered by Velisek et al. in 1978 in hydrolysed vegetable protein used in soy sauce production [1]. Because of the fact that its carcinogenicity and genotoxicity were reported intensively throughout decades, the experts of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EC Scientific Committee on Food established in 2001 a maximum tolerable daily intake (TDI) for 3-monochloropropane-1,2-diol of 2 μg/kg body weight per day for hydrolysed vegetable protein and soy sauce [2]. From chemical point of view, 3-monochloropropane-1,2-diol is a glycerol chlorohydrin, the product of stereospecific reaction resulting in *R*- and *S*- enantiomers. The above limit applies to racemate [3].

3-Monochloropropane-1,2-diol is formed during acid hydrolysys (in soy sauce production), heat processing and from its esters of palmitic, oleic and stearic fatty acids [4]. The mechanism of the first two pathways is quite well known regarding precursors and conditions, however the release of 3-monochloropropane--1,2-diol from its bound form has not been fully understood up to now. Also, the level of release of free 3-monochloropropane-1,2-diol in gastrointenstinal tract needs further investigation because the lack of in vivo research in this field forces the 100% hydrolysis assumption [5].

The research regarding absorption, metabolism and toxicity of 3-monochloropropane-1,2-diol has been carried out for over thirty years and last until now. Studies on rodents proved that 3-monochloropropane-1,2-diol exhibits damaging effect on urinary tract and antifertility effect. Research towards

mutagenic activity gave positive results in vitro, however studies *in vivo* did not prove this effect. No clinical studies on human beings have been reported, that is why the aspect of toxicology of 3-monochloropropane-1,2-diol still needs further investigation [6].

Over the last decade plenty of analytical methods was developed in order to determine 3-monochloropropane-1,2-diol in its free and bound form in various foodstuffs with the use of GC-FID [7], GC-ECD [8], LC-TOFMS [9] and finally GC-MS [10], which is now widely used in laboratories. 3-Monochloropropane-1,2-diol was determined apart from soy sauce in bread, crackers, biscuits, malt-derived products, smoked food [4], edible oils and fats [11] and lipid fractions of infant foods and human breast milk [12, 13]. The presence of 3-monochloropropane--1,2-diol in the last two groups emerged as a serious problem, however there are very few literature data regarding 3-monochloropropane-1,2-diol determination in this matrices.

The aim of this research was the determination of 3-monochloropropane--1,2-diol in ten samples of infant foods from various producers and three samples of human breast milk collected during different periods after childbirth. Applied analytical approach was presented in Figure 1. The lipid fractions from above samples were isolated with the use of Roese-Gottlieb method. 3-Monochloropropane-1,2-diol present in isolated fractions was released from its bound form during acid hydrolysys and alkaline transesterification with sodium methoxide

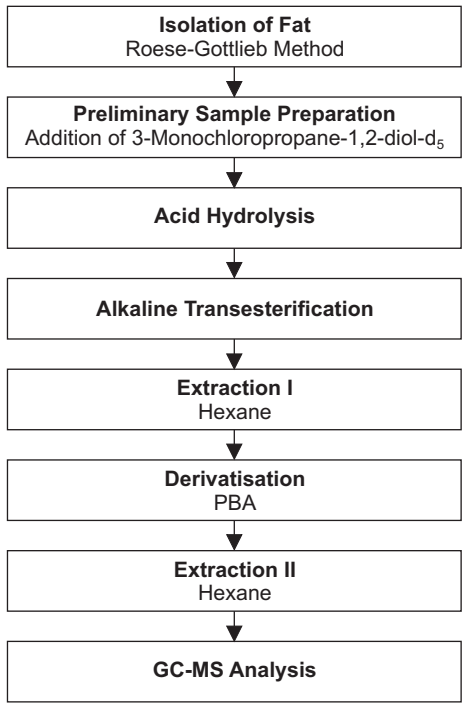


Fig. 1. The scheme of analytical approach applied in presented research.

and derivatised with PBA. Obtained derivative was analysed with the use of GC-MS and quantify by internal standard (3-monochloropropane-1,2-diol-d_5) method. Applied method allowed quite rapid analysis of many samples, however serious disadvantage appeared because samples contaminated the ion source in GC-MS and in constant use for this kind of samples it has to be cleaned at least once a week with the aim of sufficient detector sensitivity.

3-Monochloropropane-1,2-diol was determined in all of the analysed samples. The concentrations were proportional to the amount of fat which foodstuffs contained. The level of free and bound 3-monochloropropane-1,2-diol ranged from 20 μg/kg to 2000 μg/kg. This amount may be considered as dangerous for infants because the small body weight of infants together with regular intake of this kind of food (including human breast milk) may cause exceeding the tolerable daily intake set for 3-monochloropropane-1,2-diol.

The research presented in this paper proved that this field needs urgent further investigation regarding possible precursors and mitigating the formation of 3-monochloropropane-1,2-diol in fats and oils used for infant foods production. Also, the mechanism of bioavailability of 3-monochloropropane-1,2-diol regarding its release to biological fluids as human breast milk needs additional studies because it still remains not fully understood. Last but not least, the equipment problems proved the need of the development of new analytical approaches, using advanced sample preparation step, for example solid phase microextraction and headspace analysis, which is a challenge for analytical chemists.

Acknowledgments

This research was financially supported by the National Centre for Research and Development within LIDER project (grant No 11/171/L-3/11/NCBR/2012).

References

[1] Velišek J., Davídek J., Hajslova J., Kubelka V., Janíček G., Mánková G.: *Z. Lebens. Unters. Forsch.* **167** (1978), 241–244.
[2] *Chloropropanols in Food. An Analysis of the Public Health Risk.* Technical Report Series No.15. Food Standards Australia New Zeland, October 2003.
[3] Hamlet C.G., Sadd P.A., Crews C., Velišek J., Baxter D.E.: *Food. Addit. Contam.* **19** (2002), 619–637.
[4] Baer I., de la Calle B., Taylor P.: *Anal. Bioanal. Chem.* **396** (2010), 443–456.
[5] Bakhiya N., Abraham K., Gürtler R., Appel K.E., Lampen A.: *Mol. Nutr. Food Res.* **55** (2011), 1–13.
[6] JECFA (Joint FAO/WHO Expert Committee on Food Additives). 3-Chloro-1,2 propanediol. *WHO Food Add. Ser.* **48** (2002) 401–432.
[7] Plantinga W.J., van Toorn W.G., van der Stegen G.H.D.: *J. Chromatogr. A* **555** (1991), 311–314.
[8] Spyres G.: *J. Chromatogr. A* **638** (1993), 71–74.
[9] Haines T., Adlaf K.J., Pierceall R.M., Lee I., Venkitasubramanian P., Collison M.W.: *J. Am. Oil Chem. Soc.* **88** (2011), 1–14.
[10] Divinová V., Svejkovská B., Doležal M., Velišek J.: *Czech J. Food Sci.* **22** (2004), 182–189.
[11] Zelinkova Z., Svejkovská B., Velišek J., Doležal M.: *Food Addit. Contam.* **23** (2006), 1290–1298.
[12] Zelinkova Z., Doležal M., Velišek J.: *Eur. Food Res. Technol.* **228** (2009), 571–578.
[13] Velišek J., Zelinkova Z., Novotny O., Schurek J., Hajslova J., Doležal M.: *Food Addit. Contam.* **25** (2007), 669–676.

Voltammetric Determination of Caffeine at Carbon Paste-Based Electrodes. An Initial Study with Unmodified Carbon Paste.

Iveta Ksandrová*, Lucie Kosová, Matěj Stočes, Ivan Švancara

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic ✉ *ebeline@centrum.cz*

Keywords
caffeine
cyclic voltammetry
square-wave voltammetry
unmodified carbon paste electrode

Basic electrochemical and electroanalytical characterization of caffeine, a naturally occurring xanthine-related alkaloid with well-known properties of a stimulant drug, has been performed when using unmodified carbon paste electrode made of mineral oil in combination with cyclic voltammetry and later also square-wave voltammetry.

The electrode oxidation of the target substance was studied first in a set of Britton-Robinson buffers (with pH = 1–12) at a scan rate of 50 mV s^{-1} finding out that the signal of interest, appearing at about +1.50 V vs. ref. (Ag/AgCl/3M KCl), had gradually decreased with the lowered acidity of the solution, disappearing completely beyond pH > 7. Thus, a series of mineral acids of different concentration was tested next in order to define the optimal pH as well as the acid as such. Among HCl, HNO_3, $HClO_4$, and H_2SO_4 in a concentration range of 0.01–1.0 mol L^{-1}, highly concentrated sulphuric acid, ca. 0.5M H_2SO_4 gave the most satisfactory results with respect to the overall peak height and despite somewhat deteriorated base-line.

The following investigations were focused on optimization of some key instrumental parameters, such as the scan rate, pulse amplitude, and square-wave voltammetry-ramp frequency. Also, the conditioning of the electrode due to the scanning up to highly positive potentials was checked, showing that a periodical application i.e., after each scan of a negative potential had a beneficial effect upon

the resultant response. Then, a conditioning at 1.0 V for 60 s was included into the otherwise simple procedure for electroanalytical determination of caffeine in aqueous solutions.

In the final stage of this study, some model samples (e.g., Coca-Cola®, energy drinks, tea or coffee) are to be analysed and the whole method verified and evaluated with respect to its electroanalytical performance and usefulness in practice.

Acknowledgments

Supports of the Ministry of Education, Youth and Sports of the Czech Republic are gratefully acknowledged.

Hydrophilic Interaction Chromatography on the Diol-Based Columns

PETR JANÁS*, PAVEL JANDERA, TOMÁŠ HÁJEK

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 572, 532 10 Pardubice, Czech Republic ✉ *petrjanas@seznam.cz*

Keywords
diol bonded phases
flavonoids
hydrophilic interaction chromatography
phenolic acids

The principle of the hydrophilic interaction chromatography (HILIC), which is a useful method for separation of small polar compounds, consists in adsorption of water from organic/aqueous mobile phase (often with buffer additives), where the concentration of water is relatively low (the range of content of water is around 2–20%) [1], on the surface of polar stationary phase, where it creates a diffuse layer. The retention is based on the combination of two major mechanisms, partition into the water-rich layer and adsorption onto the surface of a polar stationary phase [2].

In this work, the effect of content of buffered water in the mobile phase on the retention of phenolic acids and flavonoid antioxidants was investigated on an YMC-Triart Diol-HILIC column, which is based on organic/inorganic hybrid silica particles with bonded-diol phases. The retention was measured over the full mobile phase composition range-from 2 to 95 percent of buffered water (10 mM CH_3COONH_4, 0.1% HCOOH) in acetonitrile. The column showed both reversed phase and aqueous normal phase (HILIC) mechanism and this dual retention mechanism was successfully described by a four parameter equation [3]:

$$\log k = a + m_{RP}\,\varphi_{H_2O} - m_{HILIC}\log\left(1 + b\,\varphi_{H_2O}\right) \qquad (1)$$

where a is the logarithm of the retention factor in pure less polar solvent, in this case in acetonitrile. Parameters m_{HILIC}/m_{RP} characterize the rate of decreasing/increasing retention with increasing volume fraction of buffered water in the mobile phase. All plots of retention factor k dependence on the content of water showed 'U-shape' [4]. The HILIC retention mechanism predominated in the range

of 2% to ~ 50% of buffered water in mobile phase and with the content of buffered water in mobile phase more than ~50%, the RP mechanism controlled the retention. The volume fraction of water, φ_{min}, corresponding to the minimum of the U-turn, indicates the transition between the HILIC and RP modes. The volume fraction of water, $\varphi_{k=0.5}$, where the retention of the phenolic acids and flavonoids in the HILIC mode is still sufficient, was subtracted from the plot, too.

The separation selectivity was investigated by calculating the separation factors, α, which signify the relation of the retention factors of phenolic acids to the retention factor of 4-hydroxyphenylacetic acid and the retention factors of flavonoids to the retention factor of flavone.

The next part of the work was focused on the investigation of the structural correlations of the retention of phenolic acids and flavonoids using Abraham LFER model. The LFER model employs multiple correlations between the retention (log *k*) and so-called solvatochromic parameters, characterizing the solubility and solvation of the solute and the stationary phase [5–7]:

$$\log k = \log k_0 + m' V_x + s' \pi + a' \alpha + b' \beta \quad (2)$$

where V_x, π, α, and β are the structural descriptors characterizing the sample: the volume of the solute, V_x; dipole-dipole interactions, π; hydrogen bonding acidity, α; and hydrogen bonding basicity, β. The coefficients *m*', *s*', *a*', and *b*' of Eq. (2) characterize selective non-polar, dipoledipole and hydrogen-bonding interactions [8]. The parameters were obtained as a result of multilinear regression analysis fitting the experimental retention factors *k*, to the LFER model described by Eq. (2) and are shown in Table 1.

Table 1
The parameters log k_0, *m'*, s', *a'* and *b'* of phenolic acids and flavonoids on YMC Triart Diol-Hilic column.

Compounds	Mode	φ_{H_2O}	log k_0	*m*'	*s*'	*a*'	*b*'	R^2
Phenolic acids	HILIC	0.10	1.08	–2.40	0.46	–0.23	2.08	0.91
		0.08	1.23	–2.43	0.47	–0.21	2.09	0.91
		0.06	1.41	–2.48	0.53	–0.27	2.04	0.93
		0.04	1.56	–2.43	0.55	–0.30	1.95	0.92
		0.02	1.76	–2.08	0.54	–0.33	1.51	0.92
	RP	0.95	–1.59	1.68	–0.38	0.20	–0.56	0.89
		0.90	–1.13	0.93	–0.25	0.02	–0.19	0.90
Flavonoids	HILIC	0.06	–0.25	–1.00	0.23	–0.06	0.95	0.75
		0.04	–0.12	–1.01	0.22	–0.05	0.97	0.73
		0.02	0.04	–1.09	0.24	–0.01	1.04	0.72
	RP	0.95	–0.92	1.61	0.13	–0.38	–1.30	0.97
		0.90	–0.89	1.23	0.12	–0.29	–1.03	0.97
		0.80	–0.94	0.72	0.13	–0.20	–0.66	0.95
		0.70	–0.96	0.38	0.11	–0.18	–0.35	0.92

Experimental data were compared with the data, measured on a Luna HILIC column, which contains crosslinked oxyethylene-hydroxy phases and a LiChrospher 100 Diol column with bonded glycerol groups [9]. The relative retention was the highest on YMC-Triart Diol-HILIC column in both HILIC and RP modes, except for the phenolic acids in RP mode. All three columns showed dual retention mechanism with U-shape of the dependence of the retention factor on the volume fraction of water. The YMC-Triart Diol-HILIC column showed the widest HILIC range in comparison to other columns for phenolic acids and the LiChrospher Diol column showed the widest HILIC range for flavonoids. The example of the U-plot (*p*-hydroxybenzoic acid) for all three columns is shown in Fig. 1.

The separation factors of phenolic acids were the highest in the HILIC mode on YMC-Triart Diol-HILIC, and the lowest in the RP mode. The separation factors of flavonoids were the highest on the LiChrospher column in both modes, HILIC and RP and the lowest on the Luna HILIC column in both modes.

Fitting the experimental data with Abraham LFER model was the most successful for flavonoids in the RP mode. For phenolic acids was the experimental fitting as similar in HILIC as in RP mode. The retention of phenolic acids and flavonoids is most affected by the parameter *m*', which characterizes contribution of the size of the molecule to the retention and *b*', which characterizes contribution of proton-donor interactions.

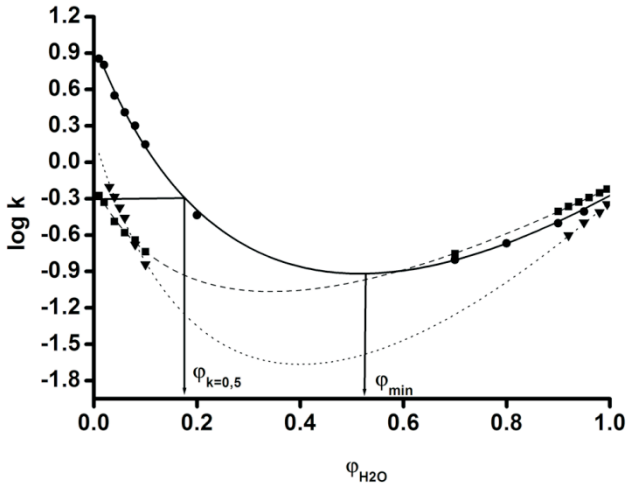


Fig. 1. Effect of the mobile phase composition on the retention of *p*-hydroxybenzoic acid on the YMC-Triart Diol-HILIC column (solid line) and comparison to the Luna HILIC (dash line) and LiChrospher (dot line) columns for the same compound. φ_{H_2O} is the volume fraction of buffered water in the mobile phase, φ_{min} corresponds to the volume fraction of buffered water, which indicates the transition between HILIC and RP modes. $\varphi_{k\,=\,0.5}$ corresponds to the volume fraction of buffered water, where the retention of compounds is still sufficient. Points are experimental data, line is the best-fit plot of Eq. (1).

As a result of the comparison, Ymc Triart Diol HILIC showed better separation selectivity for phenolic acids and higher relative retention in the HILIC mode and wider range of volume fraction of buffered water in the mobile phase, where the HILIC mechanism predominates.

Acknowledgments

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

References

[1] Alpert, A. J.: *J. Sep. Sci.* **29** (1990), 1784–1821.
[2] Soukup, J., Jandera, P.: *J. Chromatogr. A* **1245** (2012), 98–108.
[3] Jandera, P.: *Anal. Chim. Acta* **692** (2011), 1–25.
[4] Pesek, J. J., Matyska, M. T., Larrabee, S.: *J. Sep. Sci.* **30** (2007), 637–647.
[5] Tan, L. C., Carr, P. W., Abraham, M. H.: *J. Chromatogr. A* **752** (1996), 1–18.
[6] Du, C. M., Valko, K., Bevan, C., Reynolds, D., Abraham, M. H.: *J. Chromatogr. Sci.* **38** (2000), 503–511.
[7] Reta, M., Carr, P. W., Sadek, P. C., Rutan, S. C.: *Anal. Chem.* **71** (1999), 3484–3496.
[8] Jandera, P., Hajek, T., Skerikova, V., Soukup, J.: *J. Sep. Sci.* **33** (2010), 841–852.
[9] Jandera, P., Hajek, T.: *J. Sep. Sci.* **32** (2009), 3603–3619.

Multi-Analyte Method for the Determination of Organohalogen Contaminants in Dust

Darina Lankova*, Kamila Kalachova, Ondrej Lacina,
Jana Pulkrabova, Jana Hajslova

Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague,
Technicka 3, 166 28 Prague 6, Czech Republic ✉ *darina.lankova@vscht.cz*

Keywords
brominated flame retardants
dust
GC-MS/MS
perfluoroalkyl substances
UPLC-MS/MS

Indoor contamination by organohalogen pollutants originated mainly from consumer products has been recognized as a an issue of concern. The indoor air and dust, in addition to food, are the major sources of exposure for the general population (specifically children), which can induce various adverse health effects. High production volume chemicals such as perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs), the latter represented mainly by polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs) and tetrabromobisphenol A (TBBPA), are obviously the key sources of indoor pollution. As regards to PFASs, due to their unique characteristics such as chemical inertness, stability, hydrophobicity and lipophobicity, they are used in a variety of industrial and consumer applications while BFRs are used to reduce the flammability of treated materials [1–2]. To get more information about health risks associated with dust intake, analytical data are needed. Since BFRs and PFAS represent largely differing groups of chemicals, a sample preparation protocol that combines the determination of BFRs and PFASs has not yet been investigated. Moreover, the determination of each group in dust has to be done separately [3–6], thus requiring a higher sample amount (> 1 g). Considering that only a small amount of a sample (< 0.1 g) is often available, miniaturized analytical approach had to be implemented. For our purpose, we took an inspiration from matrix solid phase dispersion (MSPD). This analytical strategy enables simultaneous

disruption and extraction of solid and semi-solid samples. Matrix solid phase dispersion permits complete fractionation of the sample matrix components as well as the ability to selectively elute a single compound or several classes of compounds from the same sample [7].

The main aims of our study were to adopt an analytical procedure based on MSPD for the simultaneous analysis of BFRs and PFASs in dust (the amount of sample < 0.1 g) and to apply a newly developed multi-analyte method for the examination of real dust samples. Two fractions were obtained: (*i*) hexane fraction A (16 congeners of PBDEs, 3 isomers of HBCDs and 7 novel BFRs), and (*ii*) fraction B (18 PFASs, 3 brominated phenols and TBBPA). Both fractions were analysed by different instrumental techniques, gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) and ultra-performance liquid chromatography hyphenated with tandem mass spectrometry (UPLC-MS/MS). Due to the elution of LC-amenable HBCDs, *N*-ethylperfluoro-1-octanesulfonamide (Et-FOSA) and *N*-methylperfluoro-1-octanesulfonamide (Me-FOSA) in the fraction A, the final extract was analysed beside GC-MS/MS also with UPLC-MS/MS. The fraction B was measured only by UPLC-MS/MS. All GC-MS/MS experiments were performed using a gas chromatograph Agilent 7890A GC coupled to a triple quadrupole mass spectrometer Agilent 7000B MS operated both in the electron ionization and negative chemical ionization mode (details and parameters of method [8]). For the UPLC-MS/MS analysis the Acquity Ultra-Perfomance LC system (Waters) hyphenated with a tandem quadrupole mass spectrometer Xevo TQ-S (Waters) with electrospray in negative ionisation mode was used (details and parameters of method [9]).

To demonstrate the applicability of the implemented analytical method, the validation experiments were performed on the standard reference material 2585 (SRM 2585, US National Institute of Standards and Technology). The recoveries (%) and repeatabilities (expressed as relative standard deviations, RSD, %) of the entire method for PBDEs were calculated from six replicate analyses of the SRM 2585 (Tab. 1). The concentrations of TBBPA and HBCDs in the SRM 2585 were compared with previous studies [10–11]. For the other analytes (PFASs, brominated phenols, and novel BFRs), which are not certified in the SRM 2585, the performance characteristics were determined by the analysis of the artificially contaminated 'blank' dust sample with a mixture of analytes at two different concentration levels based on a regular occurrence of PFASs and BFRs in dust. The recoveries of these compounds at both concentration levels ranged from 81 to 122% (RSD < 19%). The limits of quantification were for PFASs in the range of 0.25–1.00 ng/g, for brominated phenols in the range of 2.5–25.0 ng/g and for novel BFRs in the range of 1–50 ng/g.

Finally, 52 dust samples obtained within the collaboration with the Karolinska Institutet in Stockholm in 2011 from Sweden were analysed. The high variability between concentrations of PFASs and BFRs represented by HBCD isomers, TBBPA, PBDEs in various dust samples was observed (Fig. 1). The concentrations

Table 1
Verification of trueness of generated data. Concentrations (ng/g) of selected polybrominated diphenyl ethers (BDE), isomers of hexabromocyclododecane (HBCD), and etrabromobisphenol A (TBBPA) in Standard Reference Material 2585 (US National Institute of Standards and Technology). *SD* = standard deviation, *n* = 6.

Analyte	Measured		Certified/published		Ref.
	Mean	SD	Mean	SD	
BDE 28	40.2	4.8	46.9	4.4	SRM 2585
BDE 47	467	53	497	46	SRM 2585
BDE 49	53.3	6.9	53.5	4.2	SRM 2585
BDE 85	41.3	3.7	43.8	1.6	SRM 2585
BDE 99	825	73	892	53	SRM 2585
BDE 100	144	13	145	11	SRM 2585
BDE 153	119	12	119	1	SRM 2585
BDE 154	66.1	7.1	83.5	2.0	SRM 2585
BDE 183	44.6	8.7	43.0	3.5	SRM 2585
BDE 203	30.1	3.0	36.7	6.4	SRM 2585
BDE 206	219	25	271	42	SRM 2585
BDE 209	2903	576	2510	190	SRM 2585
α-HBCD	23.0	3.0	20.0	3.1	[10]
β-HBCD	4.0	1.0	4.3	0.5	[10]
γ-HBCD	112	22	121	19	[10]
TBBPA	215	74	245	7	[11]

of PFASs in all 52 dust samples were in the range of 7.1–1630.0 ng/g and 67.8–165828.0 ng/g for BFRs. The most abundant analytes were PFOA (in 100% of samples, median 12.4 ng/g), PFOS (in 100% of samples, median 7.7 ng/g) and BDE 47 (in 100% of samples, median 11.8 ng/g) followed by HBCDs (in 90% of samples, median expressed for the sum of three isomers, 634 ng/g), BDE 99 (in 94% of samples, median 9.3 ng/g), BDE 209 (in 87% of samples, median 168 ng/g).

In conclusion, within this study, the new analytical approach for the simultaneous determination of 27 BFRs and 18 PFASs in dust is presented. The benefits of the procedure based on MSPD are: (*i*) under optimized conditions, only a small amount of dust (< 0.1 g) can be used for the analysis, and (*ii*) extraction time is relatively short, what allows the preparation approx. 20 samples per day. Finally, the analysis of the above organohalogen compounds in 52 samples of household dust showed large variability of contamination pattern. The relationship between analytical and health data obtained on exposed humans will be investigated

Acknowledgments

The financial support from the specific university research (MSMT No 20/2013) is acknowledged.

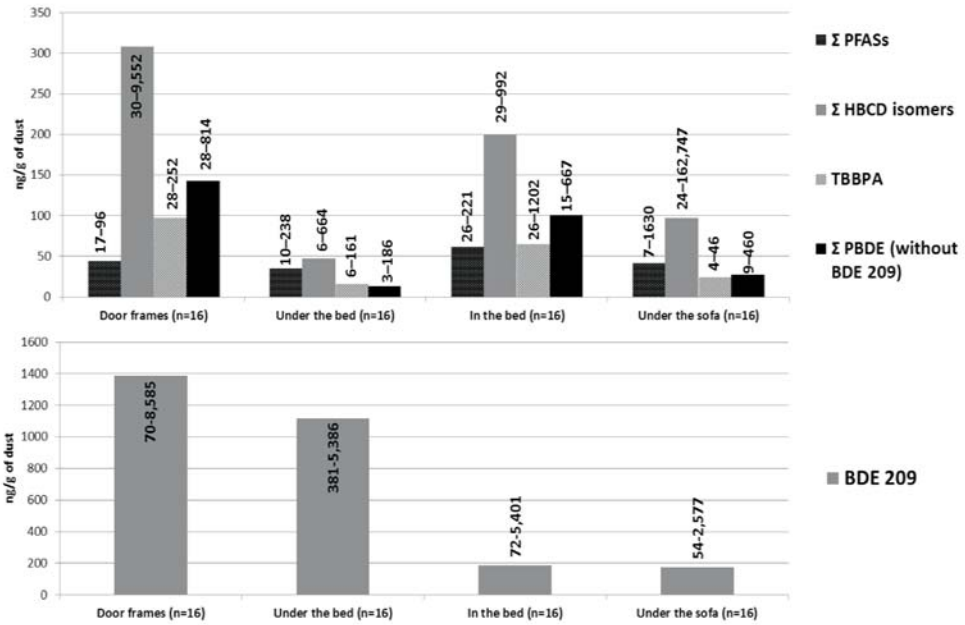


Fig. 1. Median concentrations (ng/g of dust) of sum of perfluoroalkyl substances (PFASs), sum of hexabromocyclododecane (HBCD) isomers, tetrabromobisphenol A (TBBPA), sum of polybrominated diphenyl ethers (PBDEs; without BDE 209), and BDE 209 in various sampling sites. The charts labels indicate the range of maximum and minimum concentrations.

References

[1] Alaee M., Arias P., Sjödin A., Bergman Å.: *Environ. Int.* **29** (2003), 683–689.
[2] Lehmler H.: *Chemosphere* **58** (2005), 1471–1496.
[3] Król S., Zabiegala B., Namieśnik J.: *Talanta* **93** (2012), 117.
[4] Huber S., Haug L.S., Schlabach M.: *Chemosphere* **84** (2011), 1686–1693.
[5] Goosey E., Harrad S.: *Environ. Int.* **37** (2011), 86–92.
[6] Shoeib M., Harner T., Wilford B.H., Jones K.C., Zhu J.: *Environ. Sci. Technol.* **39** (2005), 6599–6606.
[7] Barker S.A.: *J. Chrom. A* **885** (2000), 115–127.
[8] Kalachova K., Cajka T., Sandy C., Hajslova J., Pulkrabova J.: *Talanta* **105** (2013), 109–116.
[9] Lankova D., Kockovska M., Lacina O., Kalachova K., Pulkrabova J., Hajslova J.: *Anal. Bioanal. Chem.*, in press, DOI: 10.1007/s00216-013-7076-x.
[10] Harrad S., Abdallah M.A.E., Covaci A.: *Environ. Int.* **35** (2009): 573–579.
[11] Abdallah M.A.E.: *Investigating the Sources and Magnitude of Human Exposure to Halogenated Organic Pollutants using Advanced Methods for Environmental Analysis. A Thesis for the Degree of Doctor of Philosophy.* The University of Birmingham 2009. On-line: http://etheses.bham.ac.uk/546/1/abullah10PhD.pdf (accessed July 8, 2013).

A Conductivity Probe for Determination of the Carbon Dioxide Tension at the Oxygenator Exhaust Outlet during Extracorporeal Membrane Oxygenation

Bastiaan van der Weerd*, Frank-Michael Matysik, Rudolf Bierl

Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg
Universitätsstrasse 31, 93053 Regensburg, Germany ✉ *bastiaan.vanderweerd@hs-regensburg.de*

Keywords
carbon dioxide sensor
conductivity probe
extracorporeal membrane oxygenation
gas sensor
oxygenator

A veno-venous extracorporeal membrane oxygenation is a mature clinical treatment for an acute respiratory distress syndrome. Extracorporeal membrane oxygenation is applied to patients with severe lung failure. It can be understood as an artificial lung realizing oxygenation and carbon dioxide elimination. During a veno-venous extracorporeal membrane oxygenation venous blood is pumped through a membrane oxygenator before the oxygenated blood flows back into the patient's vein [1]. There is a need for reliable, accurate and instant determinations of the arterial blood carbon dioxide tension p_aCO_2 to guarantee a physiological therapy. The current state of the art is the manual collection of blood samples followed by a separate determination of p_aCO_2 by means of a blood gas analyzer. This approach delays the optimal regulation of the system and suffers from many unwanted manual steps [2]. A well known method for an indirect determination of the p_aCO_2 is the analysis of the partial pressure of carbon dioxide pCO_2 in the exhaust gas outlet from the membrane oxygenator [3].

The common technique to measure the p_aCO_2 in blood samples is the use of Severinghaus-type electrochemical carbon dioxide sensors. In this potentiometric sensor the separation of the sample from the internal electrolyte of the sensor is ensured by the implementation of a polymer membrane. This membrane is permeable to carbon dioxide gas but not to ions and water. In the internal electrolyte the pCO_2 determines the pH, which is measured by means of an integrated pH electrode [4].

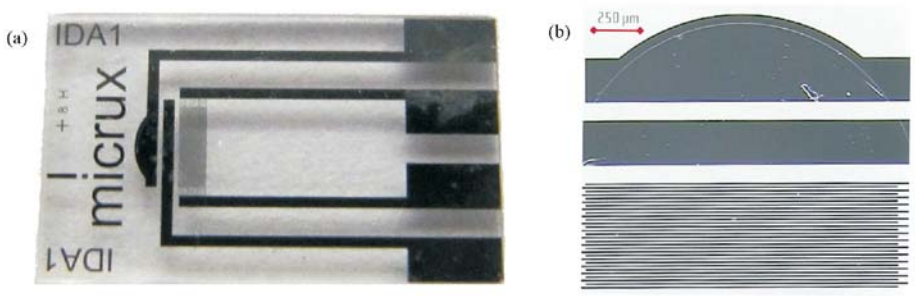


Fig. 1. Photographs of the platinum thin-film sensor: (a) general chip layout, (b) enlarged view of the interdigitated structure.

A new concept for the determination of pCO_2 in the exhausted gas volume was studied. The electrochemical detection is based on a commercial thin-film microelectrode as illustrated in Fig. 1. Interdigitated platinum electrodes are structured on a 750 µm Pyrex substrate (10×6 mm), in detail 15 pairs of 10 µm width, separated by gaps of 10 µm, form the interdigital structure. Furthermore, there are two large electrodes, which could be used as reference and auxiliary electrodes. The whole chip is protected by a SU-8 layer. The key idea of the present sensor concept is, to have a membraneless device which measures the pCO_2 dependent conductivity in a thin film of water in direct contact with the gas phase. There is no need for a separating membrane as the measured medium in the oxygenator exhaust gas analysis consists only of oxygen, carbon dioxide and water vapor. The fact to have a sensor without any membrane is promising in terms of attractive response characteristics.

An impedance-phase analyzer was utilized for the investigation of the electrochemical characteristics of the sensor. Impedance spectra were measured to study the frequency behavior of the electrochemical sensor. It was found that an optimal measuring frequency is in the range of 10 kHz for the conductivity range of interest (2–20 µS/cm) [5]. The sensor was mounted into various of flow cells with different geometries and materials. The performance was characterized using the gas test bench. For preliminary studies the microelectrode was completely immersed into the electrolyte solution. Signal stability and reproducibility, calibration curves and response characteristics were studied. The effect of film thickness on the sensor characteristics was investigated.

References

[1] Müller T., Lubnow M., Bein T., Philipp A., Pfeifer M.: *Intensivmedizin und Notfallmedizin* **46** (2009), 109–119.
[2] Kristiansen F., Hogetveit J.O., Pedersen T.H.: *Perfusion* **21** (2006), 21–26.
[3] Potger K.C., McMillan M., Southwell J., Dano H., O'Shaughnessy K.: *J. Extra-Corpor. Technol.* **35** (2003), 218–223.
[4] Zosel J., Oelßer W., Decker M., Gerlach G., Guth U.: *Meas. Sci. Technol.* **22** (2011), 072001.
[5] Timmer B., Sparreboom W., Olthuis W., Bergveld P., van den Berg A.: *Lab Chip.* **2** (2002), 121–124.

The Influence of Mobile Phase pH on the Analysis of Oligonucleotides by RP-HPLC

Zahra Safaei*, Sylwia Studzinska, Boguslaw Buszewski

Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarin St., 87-100 Toruń, Poland ✉ *Z.safaei86@yahoo.com*

Keywords
effect of pH
oligonucleotide
RP-HPLC
stationary phase

Chromatography is the most common technique used in bio-analysis. Reversed phase high performance liquid chromatography is widespread used for analysis of small and large biomolecules. The advantages of this technique are high efficiency and the ability to distinguish between compounds that are chemically very similar. Oligonucleotides have a wide range of application in research, genetic testing, and forensics, They have a great therapeutical potential like gen therapy. Oligonucleotides as drugs have been studied extensively for some disease such as virus-associated illness, AIDS, Alzheimer, cardiovascular disorders, and cancer. Oligonucleotides are short sequence of DNA or RNA molecules, build of 20 or more bases, although they can be synthesized up to 160–200. Retention of oligonucleotides in RP-HPLC depends significantly on the pH and concentration of mobile phase buffer, as well as on selectivity and efficiency of stationary phase.

The main aim of present investigation was the study of oligonucleotides retention on various stationary phases for RP-HPLC. Three different packing materials were used during the chromatographic studies, namely alkyl-amide, cholesterol, and alkyl-phosphate. Isocratic and gradient elution have been studied during present investigation. Special attention was paid to the pH of mobile phase buffer and the structure of stationary phase. They have great impact interaction taking place during the retention mechanism. Research was carried out in four different pH (in a range from 4 to 7) and various concentration of mobile phase buffer (in a range from 30 to 75 mM). The retention of oligonucleotides was greater for mobile phase in pH = 4 in comparison with pH = 7. This is a consequence of

electrostatic and hydrophobic interaction. In pH equal 7 and 6 hydrophobic interactions were dominating and in pH 5 and 4 electrostatic interactions were the most influential.

Acknowledgments

I would like to express my deepest appreciation to Polish Chemical Society that provided me the possibility to study in Poland. Furthermore I would also like to express the deepest appreciation to my Professor Boguslaw Buszewski. In addition express my gratitude to my supervisor Sylwia Studzińska for the useful comments, remarks and engagement through the learning process of this PhD thesis.

Comparison of UV-Photochemical and Chemical Volatile Compounds Generation for the Determination of Selenium by AAS

MARCELA RYBÍNOVÁ*, VÁCLAV ČERVENÝ, PETR RYCHLOVSKÝ

Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *rybinova.marcela@seznam.cz*

Keywords
AAS
chemical generation
selenium
UV-photochemical generation
volatile compounds

Selenium is one of the elements for which there is a thin border between their essential and toxic concentration in environmental system; therefore it is necessary to pay attention to their determination. Presented study was focused on the determination of selenium in aqueous samples using two methods of its volatile compounds generation. First, UV-photochemical generation, newly emerging technique in the field of analytical chemistry, was used and then conventional chemical generation was applied for comparison. Atomic absorption spectrometry with the externally heated quartz tube (QF-AAS) was chosen for the detection with both approaches.

One of the key steps for the determination of an analyte by QF-AAS is conversion of nonvolatile precursors (inorganic selenium(IV)) from the condensed phase to volatile species. Principle of transformation is different for mentioned methods. To be specific, in the case of UV-photochemical generation, volatile compounds are formed by the effect of UV irradiation in the presence of a low molecular weight organic acid (formic, acetic, propionic or malonic) [1]. Reaction mechanism remains the subject of discussion because of the complicated nature of photoreactions [2]. In contrast to UV-photochemical generation, chemical generation is based on reaction with a reducing agent, most commonly sodium borohydride, with the involvement of high-purity mineral acid (generation according to 'hydrogen transfer theory'). That could be considered as an advantage that no sodium borohydride is needed for UV-photochemical generation,

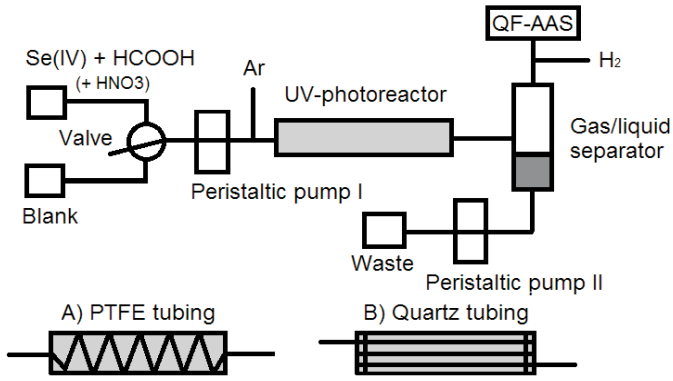


Fig. 1. Scheme of the experimental setup for UV-photochemical generation: (A) UV photoreactor-PTFE tubing wrapped around UV lamp, (B) UV photoreactor-quartz tube attached to the surface of UV lamp.

because this agent is expensive and relatively unstable [2]. Additionally, it can be prepared only in a limited purity, which increases the potential for contamination of the sample.

For UV-photochemical generation, attention was first paid to the construction of the photoreactor. It was realized by attaching reaction coil to the surface of low-pressure Hg vapor UV lamp (20 W, 254 nm). The subject of interest was mainly the material and the length of the coil. PTFE and quartz tubes of different diameters were tested. A scheme of used arrangement is illustrated in Fig. 1. Optimum experimental conditions for UV-photochemical volatile compounds generation using formic acid were found after the completion of the apparatus. Formic acid was chosen for the study as a representative of simple organic acids. Following parameters were optimized: the sample flow rate, the carrier gas flow rate as well as the auxiliary hydrogen flow, the concentration of formic acid or the concentration of additives. Analyte response was significantly increased by adding nitric acid. With the instrumental setup and the optimum analytical conditions, a detection limit of 32 ng L^{-1} Se(IV) with a repeatability of 1.9 % (RSD, $n = 10$) was obtained.

Similarly, optimum experimental conditions for more frequently used chemical generation were studied (a schematic of this system is illustrated in Figure 2). Some of the tested parameters were the same, e.g., the sample flow rate or the carrier gas flow rate, others were logically different. Namely the concentration and the flow rate of the reducing agent sodium borohydride or the concentration of hydrochloric acid, which was required for acidification of the reaction mixture. A detection limit of 105 ng L^{-1} Se(IV) with a repeatability of 1.3 % (RSD, $n = 10$) was achieved by chemical volatile compounds generation.

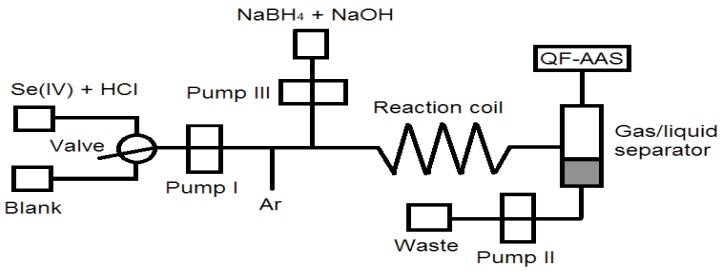


Fig. 2. Scheme of the experimental setup for chemical generation.

Consequently, the generation efficiency was investigated. It was determined by comparing of slope of calibration curves of UV-photochemical and reference chemical generation. Although in chemical generation the individual efficiencies (efficiencies for formation, gas-liquid separation and analyte transport) are known to be essentially quantitative and the response can thus be used as a relative benchmark for performance [1], higher sensitivity was achieved using UV-photochemical generation. In the near future, the determination of the generation efficiency and identification of potential losses of analyte in an apparatus would be verified using radiometric measurements with isotope ^{75}Se.

As the obtained results show, UV-photochemical generation is a useful alternative to the conventional chemical generation technique. Not only because of its low detection limits obtained but also because of high sensitivity (comparison of slopes of calibration curves).

Acknowledgments

This work was supported by MSMT (project No. MSM 0021620857), Charles University in Prague (project SVV267215 and project UNCE 204025/2012).

References

[1] Guo, X., Sturgeon, R.E., Mester, Z., Gardner, G.J.: *Anal. Chem.* **75** (2003), 2092–2099.
[2] He, Y., Hou, X., Zheng, Ch., Sturgeon, R.E.: *Anal. Bioanal.. Chem.* **388** (2007), 769–774.

Analysis of BTEX in Water Samples by Large Volume Injection Gas Chromatography

Juraj Adamuščin*, Peter Podolec, Alexandra Hengerics Szabó, Róbert Kubinec

Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovakia ✉ *adamuscin@fns.uniba.sk*

Keywords
BTEX
gas chromatography
large volume injection
water sample

Solventless sample preparation techniques for environmental samples which allow elimination of liquid solvents in the analytical procedures and reduction of sample preparation time are permanently in development [1]. One of the methods avoiding sample preparation is direct aqueous injection [2–4]. Direct aqueous injection presumes the analysis of volatile organic compounds in water samples by direct injection of water samples into a chromatographic column. The elimination of sample pre-treatment and pre-concentration steps helps to minimize the losses of volatile analytes as well as the possibility of sample contamination. The major disadvantage of this method is that water is not compatible with the stationary phases of capillary columns and with the flame ionization detector of the gas chromatograph [5–7].

The volatile aromatic compounds benzene, toluene, ethylbenzene, *ortho*-, *meta*-, *para*-xylene (BTEX) are fuel components commonly found in ground water contamination. The analysis of BTEX in aqueous samples is usually achieved by purge-and-trap gas chromatography (PTI-GC) [8]. In order to determine water samples with concentration of BTEX on trace levels without any pretreatment, it is necessary to inject large volumes of water samples (0.1–1.0 mL) [5], but in the meantime prevent the entering of large amounts of water into the capillary column of the gas chromatograph. The upper limit for direct aqueous injection acceptable in capillary GC is 10 μL [6].

The aim of this work was the development of a new simple method which enables direct injection of large volumes of water samples into a gas chromatograph. This method uses the sorption capacity of the sorbent Chromosorb P NAW

applied directly in the injection port of gas chromatograph to prevent water from penetrating into a column, by keeping it adsorbed on its surface until the analytes are stripped into a column, and the residual water is purged using split flow.

The experimental parameters as injection port temperature, water sample injection volume and concentration of analytes in relationship to stripping efficiency of analytes in time as well as linearity of calibration dependences and limits of determination and quantification were investigated.

A new solventless method was developed employing Chromosorb P NAW in the liner of injection port as adsorbent material to retain water in the injection port while the BTEX are stripped into the column. This arrangement enables direct injection of large volumes of water samples by using capillary gas chromatography for the determination of volatile organic compounds BTEX. The developed large volume direct aqueous injection method is suitable for the analysis of BTEX in drinking and river water samples concerning the reached detection and quantification limits. The main advantages of this method lie mainly in facility, rapidity (no enrichment or pre-treatment steps are required) and lower price of analysis in comparison with other methods (purge-and-trap, SPME).

Acknowledgments

This publication is the result of the project implementation: 'Amplification of the Centre of Excellence on Green Chemistry Methods and Processes (CEGreenII)' ITMS: 26240120025 supported by the Research & Development Operational Programme funded by the ERDF. Work was also supported by the Slovak Research and Development Agency under the contract nos. APVV-0416-10 and APVV-00415-11 and by Comenius University in Bratislava (grant no. UK/229/2013).

References

[1] Namiesnik J., Wardencki W.: *J. High Resol. Chromatogr.* **23** (2000), 297–303.
[2] Gurka D.F., Pyle S.P., Titus R.: *Anal. Chem.* **64** (1992), 1749–1752.
[3] Zwank L., Schmidt T.C., Haderlein S.B., Berg M.: *Environ. Sci. Technol.* **36** (2002), 2054–2059.
[4] Golfinopoulos S.K., Lekkas T.D., Nikolaou A.D.: *Chemosphere* **45** (2001), 275–284.
[5] Straková M., Matisová E.: *Chem. Listy* **91** (1997), 330–341.
[6] Kuráň P., Soják L.: *J. Chromatogr. A* **733** (1996), 119–141.
[7] Sung N.J., Johnson S.I., Zlatkis A., Middleditch B.S.: *J. High Resol. Chromatogr. Chromatogr. Commun.* (1988), 409–410.
[8] Baltussen E., Cramers C.A., Sandra P.J.F.: *Anal. Bioanal. Chem.* **373** (2002), 3–22.

Overall Complexation: A Usefull Description of Dual-Cyclodextrin Separation Systems in Capillary Electrophoresis

LUDMILA MÜLLEROVÁ*, PAVEL DUBSKÝ, BOHUSLAV GAŠ

Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *ludmila.mullerova@natur.cuni.cz*

Keywords
capillary electrophoresis
cyclodextrins
dual selector system
model of electromigration

In capillary electrophoresis, addition of a selector to the background electrolyte is often used in order to achieve enantioseparation, separation of neutral analytes or to improve separation of charged analytes. Several mathematical models have been derived in order to understand mechanism of the separation and also to help in finding optimal separation conditions [1]. Electromigration behavior of an analyte in background electrolyte containing one selector is characterized by two complexation parameters: equilibrium constant of analyte's complexation with the selector and electrophoretic mobility of the formed complex [2]. Models have been also derived describing the so-called 'dual selector systems' in which the analyte undergoes complexation with two selectors [3]. The main drawback of dual models is their higher complexity in comparison with the single models. With two independent variables (concentrations of two selectors) it is more difficult to optimize the separation or even get insight into the separation mechanism.

In our group, we have recently shown that any mixture of selectors interacting with an analyte can be regarded as a single selector as long as the molar ratio of all the mixture constituents remains constant and these criteria are met: (i) the complexation is much faster than the electrophoretic movement, (ii) only 1:1 (analyte:selector) complexation occurs (no mixed complexes are formed), (iii) it can be assumed that concentration of free selector is equal to its analytical concentration [4, 5]. In such a case, the migration behavior of an analyte is characterized by 'overall complexation parameters': overall complexation constant and overall

mobility of complex. The overall complexation parameters can be measured experimentally (in a similar way as parameters of complexation with a single selector) or calculated when the mixture composition and parameters of complexation with the individual selectors are known.

In this work, we choose a dual selector system as the simplest selector mixture to verify the model experimentally. Affinity capillary electrophoresis was used to measure complexation parameters of three neutral cyclodextrins (β-cyclodextrin, dimethyl-β-cyclodextrin, maltosyl-β-cyclodextrin) with two model, fully charged analytes (ibuprofen and flurbiprofen). Although flurbiprofen and ibuprofen are chiral compounds, chiral separation was not in our scope of interest and we performed achiral separation of the two individual analytes. Consequently, seven dual systems (four mixtures of dimethyl-β-cyclodextrin and β-cyclodextrin and three mixtures of dimethyl-β-cyclodextrin and maltosyl-β-cyclodextrin differing in molar ratio of the two cyclodextrins used) were prepared and overall parameters of complexation of analytes with these mixtures were measured by affinity capillary electrophoresis. Simultaneously, these parameters were calculated according to our model using the complexation parameters with the individual selectors. Agreement between calculated and measured values was quite satisfying: difference between calculated and measured values did not exceed 10% in case of overall complexation constants and 3% in case of overall complex mobilities.

Capability of the calculated overall complexation parameters to predict separation abilities of dual systems without the need to perform 'wet experiments' was also examined: using the calculated parameters we plotted dependencies of selectivity (ratio of effective mobilities of the analytes being separated) on total concentration of the mixture. These theoretical curves were compared with experimental selectivities and appeared to well characterize the shapes of selecticity dependencies. It was also shown, as illustrated in Fig. 1, that selectivity of a mixture at particular concentration does not have to lie 'between' selectivities that are induced by the individual selectors at the same concentration.

This result was rather counterintuitive as the overall complexation constant of a mixture has always a value between complexation constants of the individual selectors, and also value of overall mobility of complex always lies between values of mobilities of complexes with the individual selectors. However, according to our model, the overall mobility of complex (mobility of analyte at infinite concentration of the selector mixture) changes from mobility of complex with the first selector to that with the second selector, but it does not change linearly (it is caused by the fact, that overall complex mobility does not depend only on mobilities of complexes with the individual selectors, but also on the overall complexation constant). Therefore, if complexes of the two separated analytes with the first selector have same mobility and complexes of analytes with the second selector also have same mobility, but the mobilities of complexes with the first selector differ from mobilities of complexes with the second selector (which

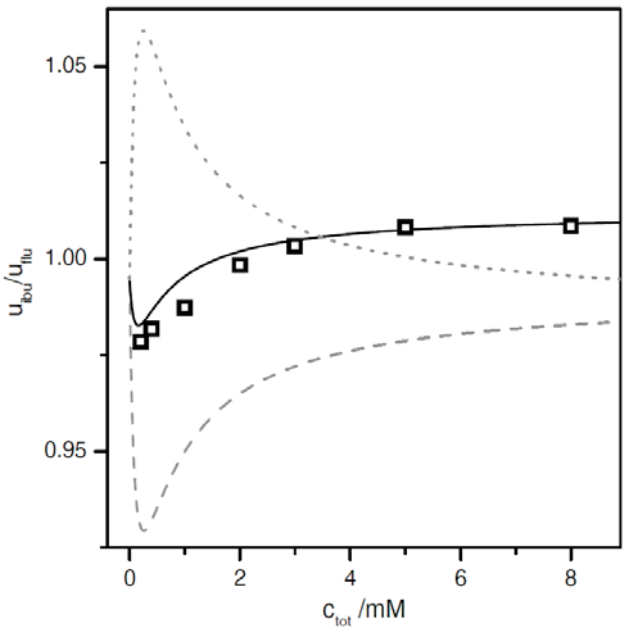


Fig. 1. Dependences of selectivity ((ratio of effective mobilities) of ibuprofen and flurbiprofen on total concentration of dual-selector mixture composed of β-cyclodextrin and dimethyl-β-cyclodextrin (molar fraction of β-cyclodextrin in the mixture 0.6) predicted using calculated overall complexation parameters (solid line); experimental selecticities in background electrolytes containing this mixture (squares); dependency of selectivity on concentration of the individual selectors (dash line: β-cyclodextrin; dot line: dimethyl-β-cyclodextrin) drawn according to the individual complexation parameters.

often happens in practice), in a mixture, the overall mobilities of complexes of the separated analytes may differ significantly. This phenomenon causes the observed counterintuitive shapes of selectivity dependencies and might be very useful in separation practice.

Acknowledgments

This work was financially supported by the Grant Agency of the Charles University, grant number 669412, by the Grant Agency of the Czech Republic, grant number P206/12/P630, and by long-term research plan of the Ministry of Education of the Czech Republic, MSM 0021620857.

References

[1] Jáč P., Scriba G. K. E.: *J. Sep. Sci.* **36** (2013), 52–74.
[2] Wren S. A. C., Rowe R. C.: *J. Chromatogr.* **609** (1992), 363–367.
[3] Scriba G. K. E.: *J. Pharm. Biomed. Anal.* **27** (2001), 373–399.
[4] Dubský P., Svobodová J., Gaš B.: *J. Chromatogr. B* **875** (2008), 30–34.
[5] Dubský P., Svobodová J., Tesařová E., Gaš B.: *Electrophoresis* **31** (2010), 1435–1441.

Chromatographic Analysis of Oligonucleotides

Lidia Pietrzak*, Sylwia Studzińska, Bogusław Buszewski

Department of the Environmental Chemistry & Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarin St., 87 100 Toruń, Poland ✉ pietrzak.lidia@wp.pl

Keywords
antisense therapy
ion-pair chromatography
oligonucleotides
stationary phase

Oligonucleotides are short, single-stranded fragments of nucleic acids, which are the most important organic compounds on Earth. The basic building block of oligonucleotides is called nucleotide and consists of three parts: nitrogenous base, pentose sugar and phosphate residues [1]. Synthetic oligonucleotides are used in many branches of science, especially in medicine [2]. Determine the structure of human genome allowed the development of a new generation of drugs, called antisense oligonucleotides. Antisense therapy is based on concept of inhibition of protein synthesis by synthetic oligomers with a well-defined sequence [3].

The oligonucleotides used in antisense therapy are produced in fully automated synthesizers [4]. No process is 100% efficient, therefore there is a high demand for research on purification, separation and determination of short fragments of nucleic acids. The main challenge in the field of antisense oligonucleotides is the development of analytical methods that provide good selectivity. Ion pair chromatography is commonly used in the analysis of oligonucleotides [5].

The main aim of our research was the study of retention and separation of the eight oligonucleotides based on ion-pair liquid chromatography. Four columns: octadecyl (SG-C18), alkylamide (SG-AP), cholesterol (SG-CHOL) and alkyl-phosphate (SG-PC) has been tested. Octadecyl column is the most commonly used in the determination of studied compounds. However, in some cases this phase does not provide satisfactory resolution of oligonucleotides, depending on the composition of the sample. For this reason, the goal of the study was to test variety HPLC columns and choose the best one for analysis of studied compounds. Three

columns of mixed nature (hydrophilic-hydrophobic) were chosen. The impact of the type and concentration of ion-pair reagent on the retention of oligonucleotides has also been examined. Three kinds of ion-pair reagents have been tested: triethylammonium acetate, dimethylbutylammonium acetate, and combination 1,1,1,3,3,3-hexafluor-2-propanol and triethylammonia. All of ion-pairing agents used in the study are particularly attractive because they are compatible with MS coupling to HPLC [6]. Results obtained during the investigation have proved that retention mechanism of oligonucleotides depends on the chemical structure of the ion-pair reagent and the polar groups in the structure of the stationary phase. Attempt to separate mixtures of oligonucleotides, showed that the most effective in the chromatographic analysis of tested compounds are alkylamid and octadecyl stationary phases. Due to the presence of polar groups in the SG-AP, retention time of oligonucleotides is shorter, but the separation of the analytes is similar to SG-C18.

Acknowledgments

The authors are grateful to Foundation for Polish Science for Parent-Bridge programme, cofinanced from European Union, Regional Development Fund. Financial support was provided also by the National Science Center (Cracow, Poland) under grant No. 2011/01/D/ST4/04142.

References

[1] Blackburn G.M.: *Nucleic Acid in Chemistry and Biology*. 3rd Ed. London, The Royal Society of Chemistry 2006.
[2] Stec W.J., Koziołkiewicz M.: *Kosmos* **19** (2000)**,** 489–501. (In Polish.)
[3] Mielicki J.: Oligonukleotydy antysensowe. In: *Na pograniczu chemii i biologii. Tom III.* Wydawnictwo Naukowe UAM 1999. (In Polish.)
[4] Okafo G., Levin G.S., Elder D.: *Chromatography Today* **3** (2011), 4–8.
[5] McGinnis A.C., Chen B., Brtlett M.G.: *J. Chromatogr. B* **883–884** (2012), 76–94.
[6] Bonilla J.V., Srivatsa G.S.: *Handbook of Analysis of Oligonucleotides and Related Products*. CRC Press 2011.

Simultaneous Determination of Caffeine, Caramel III, and Riboflavin in Energy Drinks by Synchronous Fluorescence Technique

Diana Markechová*, Ľudovít Žiak, Jana Sádecká

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovakia ✉ *diana.markechova@stuba.sk*

Keywords
caffeine
caramel
food
partial least squares
riboflavin
synchronous fluorescence spectrometry

Caffeinated energy drinks have become a modern day phenomenon during the past two decades. The ingredients, that are used in soft drinks, are regulated by the US Food and Drug Administration and the national laws and regulations. In the European Union there is no upper limit of caffeine being set. European Union regulation [1] establishes that beverages containing caffeine in a proportion in excess of 150 mg L^{-1} must provide the message 'High Caffeine Content'. Energy drinks typically contain caffeine, sugar, taurine, guarana, and B vitamins. Although the actual caffeine content for many energy drinks is not easily identified on product packaging or via the Internet, it can exceed 500 mg in some cans or bottles. Energy drink can contain Class III caramel. In the legislation, there is no maximum concentration value for caramels in energy drinks. However the presence of an minor Class III component (4-/5-methylimidazole) in most foods and beverages can be hazardous to humans because of toxicity [2]. Riboflavin (vitamin B2) is often included in energy drinks because it may contribute to the maintenance of mental function. So far, no references are available for the simultaneous determination of caffeine, caramel, and riboflavin by fluorescence spectroscopy.

In this paper, a multivariate method based on partial least squares (PLS) calibration is proposed for the simultaneous determination of caffeine, caramel, and riboflavin in caffeinated drinks, using the synchronous fluorescence properties of these compounds and standard addition method.

Energy drink samples of the available brands were obtained from local market and stored at room temperature until submitted to the analysis. Fluorescence spectra were recorded using a Perkin-Elmer LS 50 Luminescence spectrometer equipped with a Xenon lamp. Excitation and emission slits were both set at 5 nm. Scan speed was 200 nm min^{-1}. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the excitation wavelength range 200–500 nm, with constant wavelength differences $\Delta\lambda$ between them. Spectra were recorded for $\Delta\lambda$ interval from 10 to 100 nm, in steps of 10 nm. The spectrometer was connected to a computer supplied with FL Data Manager Software (Perkin-Elmer) for spectral acquisition. The HPLC method described by González et al. [3] was used as reference method.

Based on preliminary HPLC results for caffeine, caramel and riboflavin concentrations in the drink samples, sixteen calibration and sixteen prediction samples were prepared for energy drink, containing different amounts of energy drink (calibration, 5–60 μL; prediction, 10–75 μL), caffeine (calibration, 0.2–4.2 mg L^{-1}; prediction, 0.5–3.0 mg L^{-1}), Class III caramel (calibration, 0.25–5.25 mg L^{-1}; prediction, 0.5–4.5 mg L^{-1}), and riboflavin (calibration, 0.007–0.054 mg L^{-1}; prediction, 0.008–0.048 mg L^{-1}).

All the solutions were measured at $\Delta\lambda$ = 90 nm from 200 nm to 500 nm. The spectral regions between 250 and 500 nm for energy drinks were selected for the PLS, because these intervals contain the maximum spectral information for the mixture components.

The PLS model was developed in the PLS1 mode (i.e., PLS was run for each compound separately) and PLS2 mode (i.e., PLS was run for all compounds simultaneously) using calibration/prediction dataset. The calibration, cross-validation and prediction characteristics indicated suitability of the proposed PLS methods for simultaneous determination of caffeine, Class III caramel and riboflavin, e.g., R^2Pred values in all cases are very close to 1, which is an indication of similarity between predicted and known values. Considering caffeine, the calibration, cross-validation and prediction characteristics are almost the same for both PLS1 and PLS2 models. Similarly no significant differences are observed between the results obtained in PLS1 and PLS2 mode for Class III caramel. On the other hand, PLS2 is more suitable for riboflavin. Considering all three analytes, the best model (the lowest errors) were obtained for riboflavin. In addition the calibration and cross-validation characteristics (*RMSEC*, *RMSECV*, R^2 Cal, R^2 CV) are better for caffeine than those for Class III caramel, however prediction (SEP, Pred Bias, R^2 Pred) is better for Class III caramel.

A simple multivariate calibration spectrofluorimetric method using standard addition constitutes has been developed for the determination of caffeine, caramel and riboflavin in drinks. It involves measurement of the synchronous fluorescence spectra at $\Delta\lambda$ = 90 nm in 200–500 nm region. The spectral data are then processed using a partial least squares calibration designed with a series of drink samples in which the analyte concentrations were determined by a HPLC method.

The statistical characteristics for the calibration and prediction in calibration/prediction sets of samples were excellent. Furthermore, the developed procedure provided comparable results with those obtained by the HPLC reference method.

Acknowledgments

This research was supported by 'Program na podporu mladých výskumných pracovníkov STU v Bratislave'.

References

[1] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union,* L304/18, 1863.
[2] Cunha S. C., Barrado A. I., Faria M. A., Fernandes J. O.: *J. Food Compos. Anal.* **24** (2011) , 609–614.
[3] González M., Gallego M., Valcárcel M.: *Anal. Chem*. **75** (2003), 685–693.

Comparison of Enantioseparation Potential of Chiral Stationary Phases Based on Immobilized Polysaccharides in Reversed Phase Mode

RADIM GERYK*, DENISA PLECITÁ, KVĚTA KALÍKOVÁ, EVA TESAŘOVÁ

Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *radim.geryk@natur.cuni.cz*

Keywords
chiral separation
high performance liquid chromatography
immobilized polysaccharides

Chirality is an unique phenomena affecting human life in many aspects. Molecules that are not identical to their mirror images are kinds of stereoisomers called enantiomers. Enantiomers have identical physical properties but could differ in biological activity. Ignorance of pharmaceutical and toxicological differences of the individual enantiomer forms can cause disastrous consequences. As an example can serve chiral compound called thalidomide which was discovered as a sedative to help pregnant women with affects of morning sickness. While *R*-enantiomer had required pharmacological effect, the *S*-enantiomer caused fetal malformations.

High performance liquid chromatography has become a powerful technique for the development of enantioselective separations of chiral drugs and has a significant impact for pharmaceutical, food and agrochemical industries. Nowadays, chiral stationary phases based on polysaccharides (amylose, cellulose) have proven to be one of the most useful tools for separation of a wide range of chiral compounds. Immobilized polysaccharide-derived chiral stationary phases are a new type of chromatographic columns, which demonstrate better performance in the areas of enantioselectivity, efficiency and chiral stationary phase-solvent compatibility [1, 2]. Studied chiral stationary phases have already found applications under normal phase mode, while the reversed phase mode has not been fully studied yet. The aim of our work was to investigate the separation potential of the chiral stationary phases based on derivatized polysaccharides in the reversed phase mode, since this mode is more suitable for enantioseparation of biological

active compounds and is preferred due its better compatibility with MS detection. We compared three chiral stationary phases that differed in the type of derivatization group or in the nature of the glycosidic linkage of the polysaccharide derivatives.

The tested columns CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC are based on tris-(3,5-dimethylphenylcarbamate) of amylose, tris-(3,5-dimethylphenylcarbamate) of cellulose and tris-(3,5-dichlorophenylcarbamate) of cellulose, respectively. The set of diverse chiral compounds including acidic, neutral and basic ones was tested to characterize and understand the principles of chiral recognition. This facilitates the development of new separation methods and accelerate their optimization. In the frame of the separation procedure the various types of mobile phases were tested. The acidic mobile phases were used for acidic analytes to suppress the ionization because charged analytes cannot interact efficiently with chiral stationary phases [3]. Basic analytes were separated in suitable basic buffer systems or in the acidic mobile phases with considerable amount of chaotropic reagent (KPF_6, $NaClO_4$) which forms an ion pair with the positively charged analyte. The influences of the type and the amount of organic modifier (acetonitrile, methanol) on chromatographic parameters were evaluated. The retention times of the analytes decrease with increasing the amount of organic eluent. Additionally, the same amount of acetonitrile gives a shorter retention than an equivalent amount of methanol. The effect of column temperature on enantioselectivity and resolution of the enantiomers was also studied. The chromatographic parameters of different pharmaceuticals obtained in reversed phase separation systems on three different chiral stationary phases were compared. The reversed phase mode was proved to be suitable for separation of the majority of tested compounds. The results showed that both the polysaccharide type and substituent affect the separation behavior.

Acknowledgments

The Grant Agency of the Charles University in Prague, project No. 356411, the Ministry of Education, Youth and Sports of the Czech Republic, project Kontakt LH11018, and the long-term project MSM0021620857 are gratefully acknowledged for the financial support.

References

[1] Zhang T., Nguyen D., Franco P.: *J. Chromatogr. A* **1217** (2010), 1048–1055.
[2] Zhang T., Nguyen D., Franco P., Murakami T., Ohnishi A., Kurosawa H.: *Anal. Chim. Acta* **557** (2006), 221–228.
[3] Tachibana K., Ohnishi K.: *J. Chromatogr. A* **906** (2001), 127–154.

Voltammetric Determination of Selected Aminonaphthalenes at Different Electrode Surfaces

Jaroslava Zavazalova[a,*], Mariana Emilia Ghica[b], Jiri Barek[a], Karolina Peckova[a], Christopher M. A. Brett[b]

[a] *UNESCO Laboratory of Environmental Electrochemistry & University Research Centre 'Supramolecular Chemistry', Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic*
✉ *jaroslava.zavazalova@natur.cuni.cz*
[b] *Department of Chemistry, Faculty of Science and Technology, University of Coimbra, Rua Larga, 3004-535 Coimbra, Portugal*

Keywords
aminonaphthalenes
differential pulse voltammetry
electrochemical impedance spectroscopy
glassy carbon electrode

Aminonaphthalenes are amino derivatives of polycyclic aromatic hydrocarbons. They are significant pollutants of working and living environments and may have carcinogenic, mutagenic, and teratogenic effects. 2-Aminonaphthalene is a proven human carcinogen [1], and for 1-aminonaphthalene mutagenic effects have been verified [1, 2].

Because the amino group on the aromatic skeleton can easily undergo electrochemical oxidation, it is possible to use modern electroanalytical detection methods as a suitable tool for monitoring amino derivatives of polycyclic aromatic hydrocarbons in environmental and biological matrices. Previously, the behaviour of aminonaphthalenes at boron doped diamond electrode has been studied by batch voltammetric methods and high performance liquid chromatography with amperometric detection [3]. In this study, a differential pulse voltammetric method was optimized for the determination of 1-aminonaphthalene and 2-aminonaphthalene using bare or modified glassy carbon electrode as working electrode.

First, the influence of parameters of differential pulse voltammetric scan (such as pulse time, potential step, and scan rate) on the response of 1-aminonaphthalene was investigated at glassy carbon electrodes. Then, the effect of pH on the

analyte's peak current and potential was measured in Britton-Robinson buffer with pH values ranging from 2.0 to 12.0. Under the optimized conditions, the analytical parameters were determined for both compounds. Further, the effect of modification of the glassy carbon surface with Nafion permselective membrane and multiwalled carbon nanotubes was studied and compared to different types of bare carbon-based materials, such as carbon film and boron doped diamond. Electrochemical impedance spectroscopy was used to acquire information about the electrochemical processes occurring at the electrode surface in the presence of the two compounds.

Finally, simultaneous determination of the analytes was performed by differential pulse voltammetry. The practical applicability of the proposed method will be demonstrated by the determination of 1-aminonaphthalene and 2-aminonaphthalene in model samples of river water.

Acknowledgments

Financial support from Fundação para a Ciência e a Tecnologia (FCT), Portugal PTDC/QUI-QUI/116091/2009, POCH, POFC-QREN (co-financed by FSE and European Community FEDER funds through the program COMPETE and FCT project PEst-C/EME/UI0285/2013) is gratefully acknowledged. M.E.G. thanks FCT for a postdoctoral fellowship SFRH/BPD/36930/2007. The research was also supported by the Grant Agency of the Czech Republic (Projects P206/12/G151) and Grant Agency of the Charles University in Prague (Project GAUK 684213). J.Z. thanks to Specific University Research (SVV267215); the Faculty of Science, Charles University in Prague (project STARS); and the European Commission (grant under the Erasmus student exchange programme) for financial support and thanks to prof. C. M. A. Brett (University of Coimbra) for the opportunity to work in his research group.

References

[1] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Vol. 99: *Some aromatic amines, organic dyes, and related exposures.* Lyon 2010.
[2] Cheung Y., Lewis D.F.V., Ridd T.I., Gray T.J.B., Ioannides C.: *Toxicology* **118** (1997), 115–127.
[3] Zavazalova J., Dejmkova H., Barek J., Peckova K.: *Electroanalysis* **25** (2013), 253–262.

Utilization of Ambient Mass Spectrometry for Saffron Authentication

HANA NOVOTNÁ*, ONDŘEJ LACINA, JOSEP RUBERT, JANA HAJŠLOVÁ

Department of Food Chemistry and Analysis, Institute of Chemical Technology Prague, Technicka 3, 116 28 Prague 6, Czech Republic ✉ *novotnah@vscht.cz*

Keywords
authenticity
Direct Analysis in Real Time-MS
fingerprinting/profiling
saffron

Saffron (*Crocus sativus* L.) is a traditional, highly valuable spice, due to its coloring properties, alluring aroma, and pleasant bitter taste. Since the saffron is the most expensive spice in the world (up to 20 €/g), it is susceptible to adulteration like addition of other plant materials or artificial colorants. A wide range of analytical approaches is currently used for saffron authentication. These methods are mostly based on the spectroscopic techniques, volatile compounds analysis, microscopy and/or molecular biology. Chemical approaches for authentication or characterization of saffron are mostly limited to a few analytes or classes of analytes [1]. Non-target screening of compositional characteristics represents a promising alternative to the target analysis. Among the currently very popular 'omics' fingerprinting strategies, metabolomics has been demonstrated as a challenging option for a comprehensive characterization of small molecules occurring in plants, and thus, it can be promising tool for authentication purposes [2].

This study concerns with the development and evaluation of the ambient mass spectrometry technique employing Direct Analysis in Real Time coupled with high resolution Mass Spectrometer with Orbitrap mass analyzer (DART-OrbitrapMS). This technique requires little or no sample preparation and enables the recording of mass spectra without prior separation [2].

Within this study, 41 samples coming mainly from the Czech and Spanish market were analyzed. Two of them were obtained from a saffron bank in Spain as the reference standard of high-quality La Mancha saffron. Among the tested extraction solvents, an ethanol-water mixture (70/30, v/v) was chosen as the best option for the experiments. Extracts were then transferred using sampling rods to the optimized position in front of the DART gun exit. The extract was then

Table 1
List of compounds typical for saffron identified in DART-OrbitrapMS spectra.

m/z value	Formula of ion	Tentative identification	Ion type	Bias (Δppm)
139.1115	$C_9H_{15}O$	β-Isophorone	$[M+H]^+$	–1.45
151.1116	$C_{10}H_{15}O$	Safranal	$[M+H]^+$	–1.07
		HTCC	$[M-H_2O+H]^+$	
155.1065	$C_9H_{15}O_2$	2-Hydroxyisophorone	$[M+H]^+$	–0.94
169.1222	$C_{10}H_{17}O_2$	HTCC	$[M+H]^+$	–0.87
329.1752	$C_{20}H_{25}O_4$	Crocetin	$[M+H]^+$	–0.87
331.1757	$C_{16}H_{27}O_7$	Pikcocrocin	$[M+H]^+$	–1.57
348.2013	$C_{16}H_{30}O_7N$	Picrocrocin	$[M+NH_4]^+$	–1.06

desorbed from the surface of the glass rod for 30 s by hot helium, creating ions outside the instrument, during which time the spectral data were recorded. Samples were measured in the positive ionization mode and obtained data were evaluated using Principal Component Analysis (PCA).

As we used the high-resolution mass spectrometer, accurate mass spectra were obtained and thus the calculation of elemental composition of ions was possible. In the spectra of saffron extracts, many of the ions belonging to compounds typical for saffron were observed. These compounds are listed in Table I.

Crocetin ($C_{20}H_{24}O_4$, 8,8′-diapo-Ψ,Ψ′-carotenedioic acid; together with its glycosides) is the most important compound responsible for the red colour of saffron, picrocrocin (4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is considered to be the most important contributor to saffron's bitter taste, while safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) together with HTCC (4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde), isophorone (3,5,5-trimethyl-2-cyclohexene-1-one) and other similar compounds are responsible for the alluring aroma of saffron [1, 3].

From the measured spectra, intensive ions were selected and used as variables in PCA. From the obtained PCA scores plot, some clusters of the samples were evident. For example, origin protected samples (localities La Mancha and Aragón, Spain) were very close to the reference samples; with higher intensities of the markers typical for saffron. On the other hand, also some outliers were observed in the PCA scores plot; they had opposite position in the scores plot than the reference samples. These samples had lower intensities of the saffron typical markers and, therefore, they were probably adulterated. After the exclusion of the outliers, it was also possible to differentiate between samples originating from different countries. The examples of the spectra of a reference sample and one of the suspicious samples are shown in the Figure 1. As can be seen from this figure, no typical saffron marker is present in the suspicious sample spectrum.

It can be concluded that the developed method was suitable for rapid evaluation of saffron authenticity and quality.

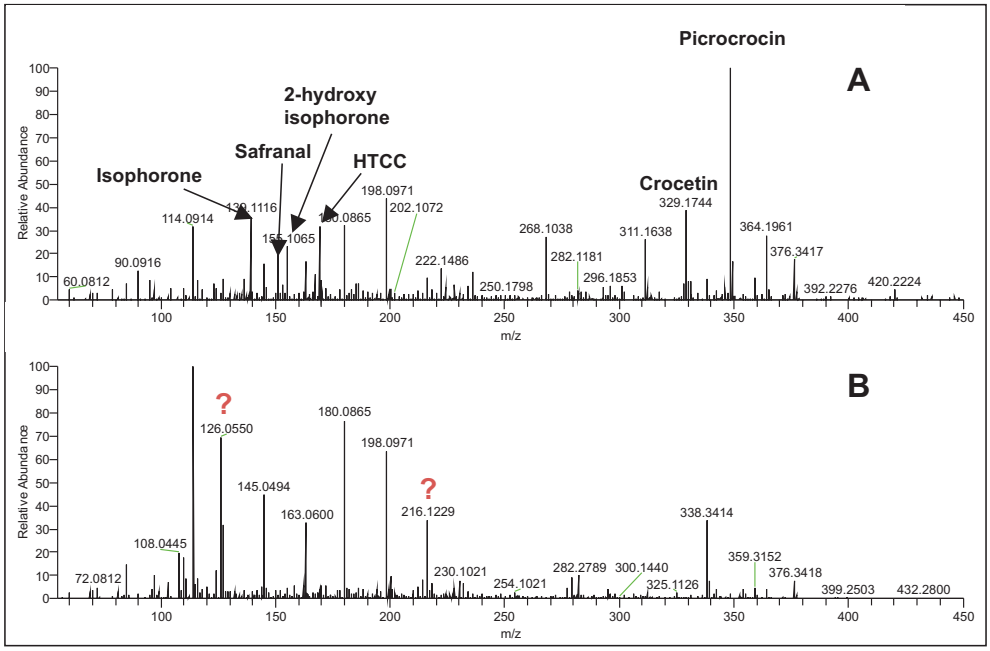


Fig. 1. Comparison of the DART(+)-MS spectra of (A) reference saffron (Spain, La Mancha) with (B) the suspicious sample (bought in Czech market, unknown country of origin).

Acknowledgments

This study was carried out within the project COST, FA1101, LD12035, and supported by The Ministry of Education, Youth and Sports, Czech Republic, the project MSM 6046137305 and specific university research (MSMT no. 21/2013).

References

[1] Sánchez A.M., Maggi L., Carmona M., Alonso G.L.: Authentication of Saffron Spice (*Crocus sativus* L.). In: *Progress in Authentication of Food and Wine.* Ebeler S.E., Takeoka G.R., Winterhalter P. (edits). Washington, American Chemical Society 2011, p. 309–331.

[2] Wishart D.S:. *Trends Food Sci Technol.* **19** (2008), 482–493.

[3] Melnyk J.P., Wang S., Marcone M.F.: *Food Res. Int.* **43** (2010), 1981–1989.

Different Types of Frits in Packed Capillary Columns

MARTIN FRANC*, PAVEL COUFAL

Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *martinfrancx@gmail.com*

Keywords
capillary chromatography
frit
monolith
slurry packing

Of all the various methods of capillary column preparation, slurry packing [1] is the easiest one. Columns can be prepared in a few hours, no special equipment is required, and even HPLC chips can be prepared this way [2]. The resulting column performance is influenced by several parameters set during the packing, such as packing pressure or use of ultrasound, as well as by parameters set before the packing process. Among them are for instance type of capillary and solvent selection. Another important parameter is the material of an outlet frit. Such a frit has to be manufactured prior to packing and will hold the stationary phase particles inside the column. Several types of frits are available, each with its advantages and disadvantages. We have selected six different types and evaluated their influence on the resulting column performance.

Frits were prepared according to Fig. 1. Type A frit consisted of a bit of glass wool pushed into the capillary and secured by cementing a smaller, 75 μm i.d./-280 μm o.d. capillary inside. Type B glass wool frit was prepared by pushing a bit of glass wool into the capillary and secured by a PEEK union. Type B monolithic frit was prepared out of styrene-divinyl benzene polymer of approximately 1 mm length. Type C frits were prepared by inserting a frit (a piece of glass wool, stainless steel frit of 0.030" thickness and 1/16" diameter or titanium frit of 0.040" thickness and 1/16" diameter) into a PEEK union and connecting it to the capillary.

The packing process was based on our earlier work [3]. Columns were packed with Nucleosil C18 stationary phase, particle size 5 μm. Column bodies were made out of 320 μm i.d. polyimide coated fused silica capillaries of 15 cm length. Packing pressure was 300 bar, packing time was 30 minutes. Columns were tested with

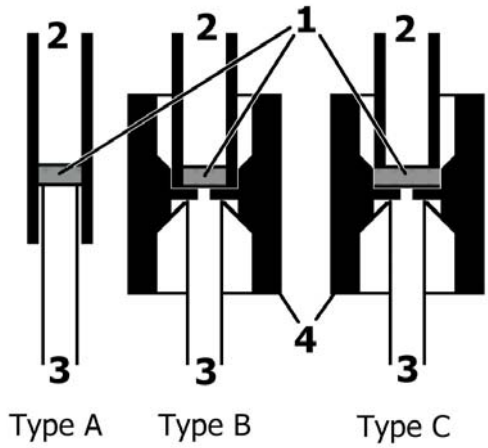


Fig. 1. Frit diagrams: (1) frit, (2) 320 μm i.d. capillary (column body), (3) 75 μm i.d. capillary (to detector), (4) PEEK union.

a mixture of phenol and toluene, with thiourea used as an unretained compound (1 mg/mL each).

Van Deemter curves of the resulting columns are shown in Fig. 2. Titanium and stainless steel frit had detrimental effect on the column efficiency and all the peaks exhibited a strong tailing. Their only advantage is extremely easy preparation. The monolithic frit performed mediocre at best. Both the column efficiency and peak shape were better than with metal frits, but not as good as with the glass wool frits. In addition, the preparation of monolithic frit is difficult and takes a lot of time. Best results, both in column efficiency and peak shape, were obtained with the type C glass wool frit. Although the type A had comparable performance, type C is much more versatile, it can be used with virtually any type of capillary and instrument and it can be prepared almost instantly.

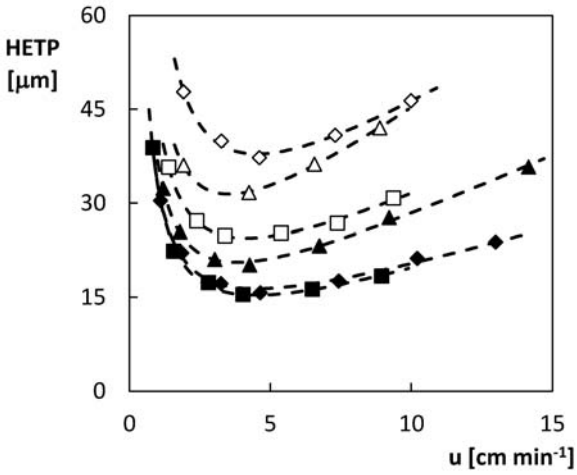


Fig. 2. Van Deemter curves based on different types of frits: ◇ type A glass wool frit, ▲ type B glass wool frit, ■ type C glass wool frit, ◆ stainless steel frit, △ titanium frit, □ monolithic frit.

Acknowledgments

The projects 349511 and SVV 267215 of the Grant Agency of the Charles University in Prague, and Research Project MSM 0021620857 of the Ministry of Education, Youth and Sports of the Czech Republic are acknowledged for the financial support.

References

[1] Ehlert S., Rösler T., Tallarek U.: *J. Sep. Sci.* **31** (2008), 1719–1728.
[2] Jung S., Ehlert S., Mora J.A., Kraiczek K., Dittmann M., Rozing G.P., Tallarek U.: *J. Chromatogr. A* **1216** (2009), 264–273.
[3] Franc M., Vojta J., Sobotníková J., Coufal P., Bosáková Z.: *Chem. Pap.*, in press, DOI: 10.2478/s11696-013-0422-9

Analysis of Pesticide Residues in Fruits and Vegetables Using QuEChERS Sample Preparation Method and Gas Chromatography with Electron Capture Detection

Jolanta Stocka*, Marek Biziuk

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Narutowicza Str. 11/12, 80-233 Gdansk, Poland ✉ *jolanta.fenik@gmail.com*

Keywords
fruits
GC-ECD
pesticides
QuEChERS
vegetables

Pesticides are a numerous and diverse group of chemical compounds. They are some of the most toxic substances contaminating the environment, which are used to eliminate pests in agriculture and the household. They enable the quantities and quality of crops and food to be controlled, and help to limit the many human diseases transmitted by insect or rodent vectors. Despite their many merits, however, pesticides are some of the most toxic, environmentally stable and mobile substances in the environment. Their non-rational use has a deleterious effect on humans and the environment; their presence in food is particularly dangerous. With properties like environmental stability, the ability to bioaccumulate and toxicity, pesticides in the human body may place it at greater risk of disease and poisoning [1].

Despite considerable progress in the development of methods for preparing samples for analysis and for the final determination of analytes, the analysis of pesticides in biological samples continues to present challenges to analysts. A number of problems crop up in the analysis of pesticide residues: one is the complexity and diversity of matrices in biological materials; another is the low concentration of pesticide in samples of fruit and vegetables. Target analytes must, therefore, be isolated from matrices and then be enrichment before the final determination can be undertaken. The complete procedure for determining

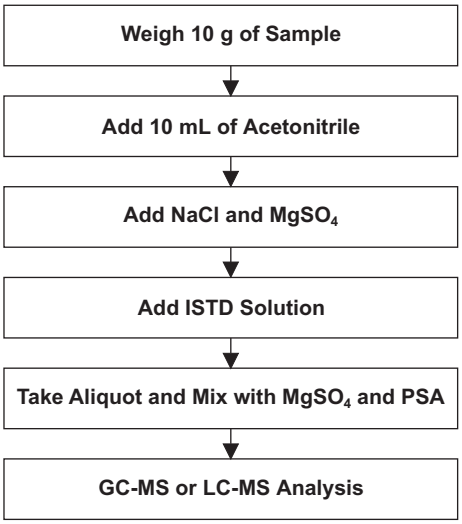


Fig. 1. Steps in the QuEChERS procedure of sample preparation for the determination of pesticide residue in fruit and vegetables.

pesticide residues in biological materials is complex and consists of several stages, which are summarized follow:

- preparation of samples for analysis,
- isolation (extraction) of pesticides from samples,
- extract clean up,
- identification and determination of analytes.

Approaches are being sought to develop pesticide determination techniques that are quick, simple, cheap, effective and safe. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) [2–7] is one such method. QuEChERS is highly effective sample preparation technique for the pesticide multiresidue analysis in various sample matrices. This method is based on a salting-out extraction with a solvent (mainly acetonitrile) followed by a solid phase extraction (SPE) and was developed by Anastassiades et al. [8]. The original QuEChERS method is based on a number of stages (Fig. 1). The QuEChERS approach is very flexible and it serves as a template for modification [9].

QuEChERS sample preparation was used for the determination of eight pesticides residues in forty samples of fruits and vegetables collected from markets in Gdansk. Pesticides standards with a minimum of 98% purity, were purchased from Sigma Aldrich. Stock solutions of individual standards (10 mg/L) were prepared in acetonitrile. The calibration standards solutions contained the eight pesticides in concentrations ranging from 0.05 to 1.00 mg/L. The fruits and vegetables samples were prepared with QuEChERS method. For extraction, 10 g sample were transferred into a polypropylene centrifuge tube, 10 mL acetonitrile were added and the solution was mixed using a Vortex mixer for 1 min then, 4 g anhydrous $MgSO_4$ and 1 g NaCl were added and solution was mixed again for 1 min. The tube was centrifuged for 10 min at 1200 rpm. Then four milliliter aliquot of the upper layer was transferred to a polypropylene centrifuge tube

containing 200 mg PSA and 600 mg anhydrous $MgSO_4$. The extract was mixed using a vortex for 30 s and then centrifuged for 3 min at 3500 rpm. The clean extract was then transferred with a glass Pasteur pipette to 1.5 ml properly identified glass vials with plastic screw cap. Samples were normally analyzed by the same day and kept in the fridge when analysis was not running.

Analyses were carried out with a GC 6000 Vega Series 2 Carlo Erba gas chromatograph equipped with an electron-capture detector (ECD ^{63}Ni). The system was equipped with capillary column (Zebron ZB-5MS 30 m × 0.25 mm, 0.25 μm). Temperature of the ECD detector was at 340 °C. The total run time was 25 min and ChromCard chromatography data system was used for instrument control and data analysis. Quantification of the pesticides was by peak area using the external standard method. The validation of the analytical method was performed by the following parameters: linearity, precision and accuracy, limits of detection and quantification, and repeatability. All pesticides showed linearity in the concentration range of 0.05–1.00 mg/L, with correlation coefficients (R^2) higher than 0.995.

The results of the method validation indicate that the QuEChERS sample preparation coupled with the GC-ECD analysis is suitable for the determination of pesticides in fruits and vegetables. As for the forty samples analysed in the present study, no pesticide residue was detected in any of the samples. The QuEChERS approach is so flexible and rugged that most pesticides give excellent results when different amounts and types of solvents and salts are used for extraction and different sorbents and amounts are used in d-SPE. QuEChERS is a multi-residue method with fast sample preparation and low solvent consumption.

Acknowledgments

The investigations were financially supported by a grant (No. 2012/05/N/ST4/02019) from the Polish National Science Center. This research work was supported by the system project 'InnoDoktorant – Scholarships for PhD students, Vth edition'. Project is co-financed by the European Union in the frame of the European Social Fund.

References

[1] Fenik J., Tankiewicz M., Biziuk M.: *Trends Anal. Chem.* **30** (2011), 814–826.
[2] Thanh Dong N., Ji Eun Y., Dae Myung L., Gae-Ho L.: *Food Chem.* **110** (2008), 207–213.
[3] Lesueur C., Knittl P., Gartner M., Mentler A., Fuerhacker M.: *Food Control.* **19** (2008), 906–914.
[4] Mayer-Helm B.: *J. Chromatogr. A* **1216** (2009), 8953–8959.
[5] Lacina O., Urbanova J., Poustka J., Hajslova J.: *J. Chromatogr. A* **1217** (2010), 648–659.
[6] Gilbert-López B., García-Reyes J. F., Molina-Díaz, A.: *Talanta* **79** (2009), 109–128.
[7] Cunha S.C., Fernandes J.O., Oliveira M.B.P.P.: *J. Chromatogr. A* **1216** (2009) 8835–8844.
[8] Anastassiades M., Lehotay S. J., Štajnbaher D., Schenck F. J.: *J. AOAC International* **86** (2003), 412–431.
[9] Furlani R.P.Z.,Marcilio K.M., Leme F.M., Tfouni S.A.V.: *Food Chem.* **126** (2011), 1283–1287.

Development of Poly(methylene blue) Modified Electrode Sensor for Hydrogen Sulfide

Magda Zlámalová[a, b, *], Pavel Janda[b]

[a] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic,* ✉ *magda.zlamalova@jh-inst.cas.cz*
[b] *Department of Electrochemical Materials, J. Heyrovský Institute of Physical Chemistry of the ASCR, v. v. i., Dolejškova 2155/3, 182 23 Prague 8, Czech Republic*

Keywords
atomic force misroscopy
cyclic voltammetry
hydrogen sulfide
poly(methylene blue)

There is widespread awareness of the high toxicity of sulfide in its liberated hydrogen sulfide form, which can cause severe or fatal poisoning due to the neurotoxic effect of this gas. The gaseous hydrogen sulfide has an unpleasant odour even at a very small concentration in the air. In addition, the biological oxidation of hydrogen sulfide to sulfuric acid has severe corrosive effect on construction materials. Hydrogen sulfide is a constituent of natural gas, volcanic gases and sulfur springs. It is also a by-product of many industrial processes and in nature it is produced primarily by bacterial decomposition of sulfur containing minerals and organic materials.

In recent years, hydrogen sulfide has been extensively studied as the human endogenous messenger molecule with auspicious therapeutic effects [1]. It has been reported that hydrogen sulfide appears to be a physiologic gasotransmitter of comparable importance to nitrogen oxide and carbon monooxide [2]. Due to very low physiological and therapeutic concentrations, there has been an increasing need for more appropriate analytical methods with emphasis on their sensitivity and selectivity.

Electrochemical detection systems utilizing conductive polymers are found to be advantageous for a wide range of electroanalytical applications. Electrodes modified by suitable redox mediator solve problems usually associated with solid electrodes such as insufficient sensitivity, low electrocatalytic activity, slow

electron transfer, current and potential oscillations, or need for high overpotential on the electrode.

Methylene blue is a water-soluble phenothiazine cationic dye. It has been used not only in the spectrometric determination of sulfides, but in recent years, it has also served as a mediator of charge transfer reactions [3]. Electrochemical polymerization of monomer methylene blue leads to immobilization of a conductive polymer film on the surface of solid electrode. This film proves significant electrocatalytic activity in oxidation of sulfides, thiols and other sulfur compounds [4]. Besides strong adhesion of the polymer to the electrode surface, this system exhibits good stability in a wide range of pH [5].

This work presents the study of poly(methylene blue) mediator in electrochemical detection of sulfide anions in an aqueous medium. Cyclic voltammetry has been performed on basal plane of highly oriented pyrolytic graphite electrode modified by deposited polymeric methylene blue. Atomic force microscopy has been used for imaging nanomorphology of the poly(methylene blue) film.

Acknowledgments

This work was supported by the research project MSM0021620857, and grant project SVV267215 of Charles University in Prague.

References

[1] Predmore, B.J., Lefer, D.J., Gojon, G.: *Antioxid. Redox Signaling* **17** (2012), 119–140.
[2] Gadalla, M.M., Snyder, S.H.: *J. Neurochem.* **113** (2010), 14–26.
[3] Karyakin, A.A., Strakhova, A.K., Karyakina, E.E., Varfolomeyev, S.D., Yatsimirsky A.K.: *Bioelectrochem. Bioenerg.* **32** (1993), 35–43.
[4] Marinho, M.I.C., Cabral, M.F., Mazo, L.H.: *J. Electroanal. Chem.* **685** (2012), 8–14.
[5] Liu, J., Mu, S.: *Synthetic Mat.* **107** (1999), 159–165.

Use of Molecularly Imprinted Polymers for Determination of Biologically Active Compounds in Plant Samples

Andrea Spevak*, Katarína Hroboňová

Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia ✉ *anga.spevak@gmail.com*

Keywords
coumarins
HPLC
molecularly imprinted polymers
plant samples

Molecularly imprinted polymers are synthetic materials with artificially created sites capable of interacting preferentially with investigated analyte in the presence of other compounds. In the preparation process, molecules (template) and monomers usually form complexes by covalent or non-covalent bonds [1]. Coumarins are a group of flavor substances occurring in the free form or glycosidically linked compounds. One of the most frequent natural coumarin derivatives is umbelliferone (7-hydroxycoumarin). It is characterized by the ability to absorb ultraviolet radiation from the sun [2]. Aim of this work was to prepare and test molecularly imprinted polymer for the selective sorption of investigated group of coumarins and use of these polymers as sorbents for extraction of coumarins from plant samples. Molecularly imprinted polymer for umbelliferone was prepared by non-covalent approach. The polymers were prepared by using methacrylic acid as monomer, EGDMA as crosslinking agent, and AIBN as initiator of polymerization. The polymer prepared in chloroform as porogen was selective for the investigated group of coumarins. It was found out that the MIP-SPE technique was suitable for selective extraction of coumarins from plant samples with yields higher than 70% (RSD < 10%). Different solvents for extraction of coumarins were tested. It was found out that the best solvent was mixture methanol/water (1/1, v/v). In the plant extracts of chamomile, lavender, melilot and angelica archangelica was found out the presence of three investigated coumarins, umbelliferone, coumarin and herniarin at concentration level above limit of detection.

References

[1] Kadimalla V.B., Hunagxian J.: *Anal. Bioanal. Chem.* **380** (2004), 587–605.
[2] Kresánek J. ml., Kresánej J. st.: *Atlas liečivých rastlín a lesných plodov.* Martin, Osveta 2008. (In Slovak.)

New Fast Strategy to Determine Ethanol in Alcoholic Beverages and Overall Quality Check

Michal Stupak*, Jana Pulkrabova, Vladimir Kocourek, Jana Hajslova

Department of Food Analysis and Nutrition, Institute of Chemical Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic ✉ *Michal.Stupak@vscht.cz*

Keywords
ethanol
GC-MS
isotopic dilution
spirits
volatile compounds

Alcoholic beverages are complex mixtures mainly comprised of ethanol and water and a large number of minor compounds that may be present in the raw materials or formed during the distinct stages of the production process such as: alcohols, acids, esters, aldehyde, polyphenols, metals, aminoacids, etc. The different concentrations of these substances confer the particular characteristics to each product, affecting sensory properties appreciated by consumers such as odor, taste, and color [1].

Adulteration of spirits is a very old issue and a content of ethanol is one of the crucial elements for taxing the products. The higher ethanol content in a product means more tax for producers. It is one of the main reasons for using specific substances for adulteration. One of these substances is methanol, which is a notorious ingredient used illegally in products of imitated spirits and wines. Higher concentration of methanol in spirits is very toxic to human health[2]. To respect this fact, fast and reliable analytical methods for the assessment of quality and authenticity of spirits and raw materials used for production of spirits are needed. The overall quality check for alcoholic beverages became 'the hot topic' in the Czech Republic a last year when spirits were adulterated with methanol. During a 'methanol scandal' (from September 2012 to January 2013) 39 people died of methanol poisoning and 117 people with serious health problems were hospitalized. Methanol was added to alcoholic beverages deliberately. The Department of Food Analysis and Nutrition at the Institute of Chemical Technology Prague was involved in the solution of this crisis and in October 2012 obtained an appropriate Accreditation of the analytical method for alcoholic beverages control.

Determination of ethanol in alcoholic beverages is crucial in the spirits industry for production and calculation of taxes [1, 2]. A content of ethanol is very important to determine the quality of the drinks and compliance with regulations. Finally, it is necessary to control the maximum levels of certain substances, such as methanol, acetaldehyde, higher alcohols, esters etc., expressed in grams per hectoliter of 100% vol. alcohol according regulation EC No 110/2008.

The official pycnometry method for the determination of the ethanol content in alcoholic beverages is based on the measurement of a specific gravity, carried out after a previous distillation of the samples [3]. Pycnometry involves the accurate determination of mass and volume of the distillate to obtain its density. Although the theory of the method is very simple, the technique is time consuming and requires an experienced technician, making it expensive and susceptible to error [4]. Moreover, some components, such as aromatic compounds (e.g. higher alcohols), are retained in the distillates. This fact can affect the resulting concentration of ethanol [3]. Higher alcohols are treated as important flavor compounds. On the other hand in some studies on surrogate alcohol a number of authors attributed the possible higher toxicity of this group of illegal or home-produced alcohol to its content of higher alcohols. For example, compared to consumers of mainly licit alcohol, consumers of home-made alcohol in India have been reported to have higher rates of alcoholic liver disease [4].

Nowadays, for determination of ethanol pycnometry (mentioned above) and occasionally gas chromatography coupled to a flame ionization detector (GC-FID) is used. Gas chromatography coupled to mass spectrometry (GC-MS) is more selective but there are no articles on the use of GC-MS for determination of ethanol using isotopic dilution mass spectrometry [5].

In this work five different spirits differing in the concentration of ethanol were investigated. Two of them (hruškovice and grappa) included spirits with a high content of higher alcohols and another volatile compounds. As representative spirits with a medium content volatile compounds and higher alcohols rum (Havana) and whiskey (Grans) were selected and vodka with minimum volatile compounds were selected.

In this new approach, a sample preparation is very fast, briefly 50 μL of the sample, 100 μL of the internal standard (Ethanol-2,2,2-d3) and 10 mL of water is mixed and all collected volumes of sample, internal standard and water are weighed on the analytical balance. An Agilent 6890 gas chromatograph coupled to a mass spectrometer 5975 (Agilent Technologies, USA) was used for the GC-MS analysis of ethanol. The sample (0.5 μL) was injected onto HPINNOWax (30 m×0.25 mm i.d., 0.25 μm film thickness). Column temperature program: 40 °C (4.5 min), post run 180 °C (5 min).

Results of the content of ethanol in samples examined by GC-MS were compared to results obtained by pycnometry (Table 1). RSD (n = 6) was in the range from 0.19 to 0.67 %.

Table 1
Comparison of concentration of ethanol obtained by GC-MS and pycnometry.

Sample	Concentration of ethanol (%, *v/v*)	
	GC-MS	Pycnometry
Rum	37.7	37.5
Hruškovice	44.5	44.8
Grappa	40.1	40.4
Whiskey	40.2	40.1
Vodka	45.4	44.8

References

[1] González-Rodríguez, J., Péréz-Juan, P., Lunque de Castro, M.D.: *Talanta* **59** (2003), 691–696.
[2] Wang, M.L., Wang, J.T., Choong, Y.M.: *Food Chem.* **86** (2004), 609–615.
[3] Yarita, T., Nakajima, R., Otsuka, S., Ihara, T., Takatsu, A., Shibukawa, M.: *J. Chromatogr. A* **976** (2002), 387–391.
[4] Bereton, P., Hasnip, S.: *TrAc Trends Anal. Chem.* **22** (2003), 19–25.
[5] Palmer, P. T.: Gas Chromatography/Mass Spectrometry. In: *Encyclopedia of Analytical Chemistry.* Wiley 2006.

‘Green’ Multi-Residue Methods for the Determination of High Importance Currently Used Pesticides in Environmental Samples

Maciej Tankiewicz*, Marek Biziuk

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 G. Narutowicza Str., 80-233 Gdańsk, Poland ✉ *maciej.tankiewicz@gmail.com*

Keywords
GC-MS
multi-residue methods
sample preparation techniques
pesticides
water samples

The use of pesticides provides unquestionable benefits in increasing agricultural production to grow the quantity and quality of food needed to sustain the human population. Pesticides protect crops before and after harvest from infestation by pests and plant diseases, and help to limit many human diseases transmitted by insect or rodent vectors [1].

The wide and large number of pesticides applications has resulted in an extension beyond agricultural applications into many other parts of the environment. These compounds pose a particular threat in natural waters, as these are sources of drinking water and are essential for the maintenance of life. The various processes that pesticides undergo in the aquatic environment can cause them to be converted into substances of greater toxicity [2]. Despite their numerous merits, pesticides are some of the most toxic substances contaminating our environment, which fate and function is still largely unknown. They are not only toxic but also mobile and capable of bioaccumulation. Their presence was detected in all types of water circulating in the ecosystem, which is an easy source of exposure to life and health of all living organisms [3]. Concentrations are the highest during the spring snow melt period and when pesticides are being applied to crops. Currently, is considered one of the most dangerous and toxic pollutants, which fate and function is still largely unknown.

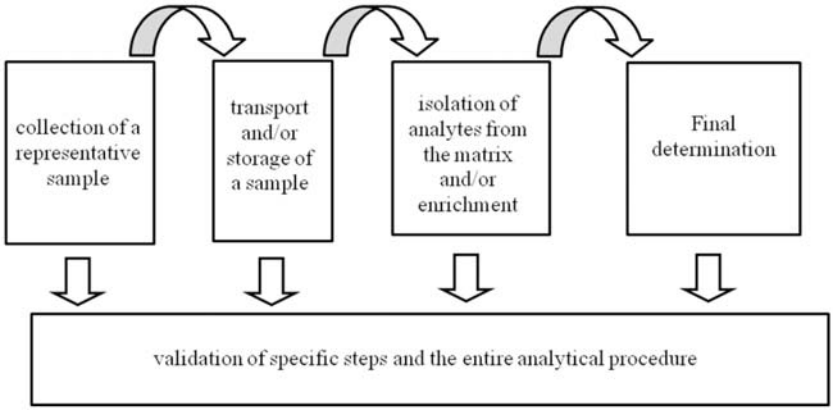


Fig. 1. The steps of determination of multiresidue of pesticides in samples characterized by complex composition of the matrix

In Europe, the monitoring of surface water and groundwater is carried out (mainly pesticides withdrawn from use, including: organochlorine pesticides, simazine, atrazine, chlorfenvinphos, chlorpyrifos, diuron, isoproturon, and trifluralin). The levels of currently used pesticides are not controlled, even though they are also suspected of carcinogenic compounds or even be carcinogenic. Moreover, used so far analytical procedures are usually laborious and time-consuming, requiring the use of large quantities of toxic and expensive solvents and are often characterized by a low coefficient of enrichment. In addition to national guidelines, international legal regulations imposed by the EU concerning the permissible level of pesticide residues in water and food, have driven the development and improvement of novel analytical techniques towards multi-residue analysis, low limits of detection and the use of small sample volumes. The improvements of existing techniques are aimed at miniaturization, automation, and the use of solvent-free techniques at the sample preparation stage, that are consistent with 'Green Chemistry' principles [4].

Nowadays, the trend is to develop analytical methods enabling a broad spectrum of analytes to be determined in a single analytical run (multiresidue methods). But the problem here is that the compounds to be determined simultaneously, often present at low concentrations, have different physicochemical properties depending on their chemical structure (e.g., polarity, solubility, volatility, and acidic/base characteristics). Figure 1 presents the steps of the multi-residue method. Such a methodology, apart from being able to determine a large number of compounds in one run, should:

- ensure maximum removal of interferents from extracts,
- give large recoveries of target compounds, high sensitivity and good precision,
- be environmentally-friendly, i.e. require the smallest possible quantities of samples and chemical reagents, especially organic solvents,
- be cheap, quick and easy to carry out.

Still is missing one and a universal procedure that would allow to determine at the same time in a quick, cheap and safe way for the environment [5].

The purpose of the research has been to develop and apply 'green' analytical methodologies to control and monitor the level of currently used pesticides from different chemical groups in aqueous samples. For extraction of analytes solid--phase microextraction and dispersive liquid-liquid microextraction have been applied and compared. For solid-phase microextraction, commonly used materials for coating fibers include: polydimethylsiloxane, polyacrylate, and also mixtures of: polydimethylsiloxane and polydivinylbenzene, carbowax and polydivinylbenzene, polydivinylbenzene and carbowax and polydimethylsiloxane were compared. For dispersive liquid-liquid microextraction, different mixtures of extraction and disperser solvents were compared and evaluated. For the identification and quantitative determination gas chromatography coupled with mass spectrometry was applied. The analytical procedures were validated and applied to natural water samples from river, lake, sea, pond, well, canal and rain collected around Glasgow region (Scotland, UK), Tricity area and central Masuria (Poland). The results of analysis of collected water samples in the study area indicate a potential risk of environmental contamination, because detected concentration levels of pesticides were higher than those established by the European Union legislation. The developed methodologies have proved to be selective, sensitive and precise for the simultaneous determination of currently used pesticides from various chemical groups. Optimized analytical methods are simpler, lower cost and less labor intensive sample preparation techniques than conventional methods. The results show that the selectivity of developed methodologies are sufficient to analyze environmental samples and can be used in the monitoring studies to control the content of selected pesticides.

Acknowledgments

This research work was financial supported by National Science Centre in Poland (decision number: DEC-2011/01/N/ST4/01977).

References

[1] Tankiewicz M., Fenik J., Kosikowska M., Biziuk M.: Pesticides. In: *Encyclopedia of Environmental Management.* Jorgensen S.E. (edit.). New York, CRC Press, Taylor & Francis 2013, p. 1963–1982.
[2] Tankiewicz M., Fenik J., Biziuk M.: *Trends Anal. Chem.* **29** (2010), 1050–1063.
[3] Tankiewicz M., Fenik J., Biziuk M.: *Talanta* **86** (2011), 8–22.
[4] Stocka J., Tankiewicz M., Biziuk M., Namieśnik J.: *Int. J. Mol. Sci.* **12** (2011), 7785–7805.
[5] Tankiewicz M., Morrison C., Biziuk M.: *Talanta* **107** (2013), 1–10.

Determination of Arsenic in the Waste Waters by Flow-Through Coulometry

Ľubomír Machyňák*, Ernest Beinrohr

Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia ✉ *l.machynak@gmail.com*

Keywords
arsenic
flow-through coulometry
waste waters

This work deals with the determination of arsenic in the waste waters by flow-through coulometry. The measurements were done on an electrochemical flow system EcaFlow® 150 GLP manufactured by Istran, s.r.o., Bratislava. The three-electrode flow cell consisted of the gold wire working electrode, the auxiliary platinum electrode and the Ag/AgCl/KCl reference electrode which was separated from the flowing solution by a membrane. As a carrier electrolyte we used a solution of 0.1 mol L^{-1} hydrochloric acid with addition of 0.1% Triton, denoted as R101s. The fast quantitative reduction of As(V) to As(III) in acidic solution was done by a solution containing potassium iodide and ascorbic acid. Ascorbic acid acts a stabilizer and potassium iodide as a reducing agent was oxidized by the acidic solution of As(V) to iodine, whereas As(V) was reduced to As(III). The obtained results shows that the employed reducing agent ensures a fast and quantitative reduction of As(V) to As(III). The procedure was validated and applied for analysis of real samples.

Characterization of Volatile Compounds in Alcoholic Beverage 'Oskurošovica'

Olga Vyviurska[a, *], Ivan Špánik[a], Antónia Janáčová[a], Solomiya Pysarevska[a, b]

[a] *Institute of Analytical Chemistry, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia* ✉ *olga.vyviurska@stuba.sk*
[b] *Department of Analytical Chemistry, Ivan Franko National University, 790 00 Lviv, Ukraine*

Keywords
alcoholic beverage
GC×GC
Oskurošovica
volatile organic compounds

Nowadays, a number of alcoholic beverages with a variety of tastes and aromatic features are accessible in the market. At the same time, in every country traditional beverages are still enjoying wide popularity in customers. For example, alcoholic distillate 'Oskurošovica' made from fruits of Service Tree (*Sorbus domestica*) is specific for Slovakia and Czech Republic region. This distillate is often high valued by experts due to their specific aroma and taste characteristics, which are defined by presence of particular organic compounds. In addition, Service Tree fruits are famous for its antioxidant effect and a few studies have been devoted to this question [1, 2].

Despite of broad possibilities that could be provided by gas chromatography technique, all investigations connected with Service Tree distillates were performed only by liquid chromatography [3]. Therefore, the purpose of this study was to fulfill a lack of information about volatile organic compounds content of Service tree distillate by comprehensive two-dimensional gas chromatography (GC×GC). The studied sample was obtained from Bosaca distillery (Slovakia). Liquid-liquid extraction procedure was applied for the preconcentration. In total, a 30 mL of sample with dissolved sodium chloride was extracted with three 10 mL portions of pentane in separation funnel. Collected extracts were concentrated using Kuderna-Danish apparatus at constant water bath temperature 43 °C to final volume 1.5 mL. A non-polar HP-PONA (50m × 0.2 mm, 0.5 μm) column and a high polar DB-FFAP (30 m × 0.25 mm, 0.25 μm) column were tested for the first dimension separation, whereas a semi-polar BPX-50 (2 m × 0.1 mm, 1 μm) column was used for the second dimension separation. The detection was carried out by

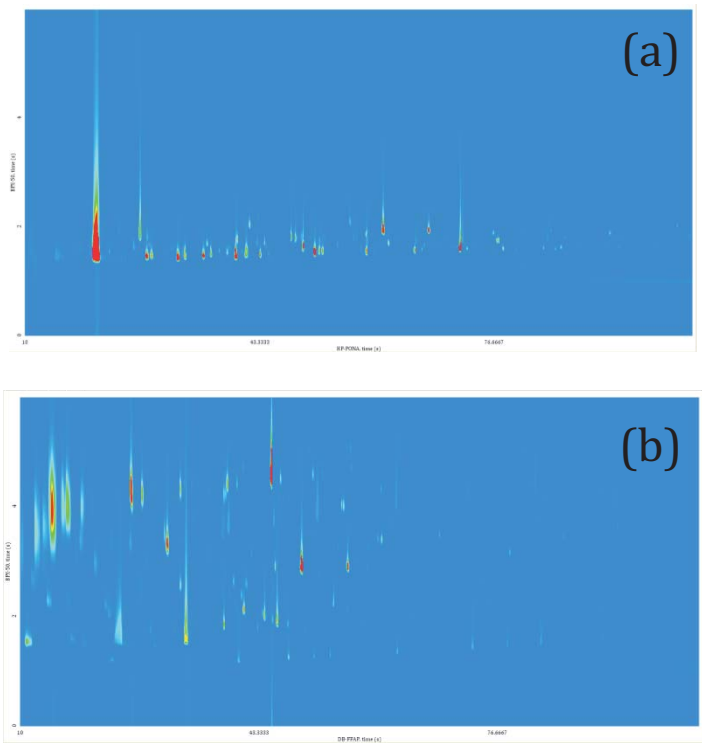


Fig. 1. GC×GC-TOF-MS chromatograms of the studied distillate obtained with the column combinations of (a) HP-PONA and BPX-50, (b) DB-FFAP and BPX-50.

time-of-flight mass spectometer (TOF-MS). The data software and GC×GC-TOF--MS data of standard compounds were applied for identification procedure.

In the case of comprehensive two-dimensional gas chromatography, an important issue is the degree of orthogonality for the selected column setup, since this factor evaluates the available retention space for compounds separation. Thus, the effectiveness of two columns combinations with different orthogonality characteristics were compared for the analysis of the studied beverage (Fig. 1). As can be seen from the data, the combination of the polar and semi-polar columns was more sufficient for the analysis of *Sorbus domestica* distilate. The similar results as to columns setup were obtained for another alcoholic beverage [4]. More than 400 compounds from various chemical classes such as esters, alcohols, organic acids, aldehydes, ketones, acetals, terpens, furans, saturated and aromatic hydrocarbons have been detected. Such complicated volatile organic compounds composition of the studied beverage is caused by a number of sources, fruit origin, conditions of fermentation process, type of distillation procedure, etc. Consequently, the found volatile organic compounds content could be further successfully exploited for search of specific compounds in the beverage.

Acknowledgments

This work was supported by EHP financial mechanisms under contract number SAV-EHP-2008-02--05.

References

[1] Termentzi A., Kefalas P., Kokkalou E.: *Food Chem.* **106** (2008), 1234–1245.
[2] Termentzi A., Zervou M., Kokkalou E.: *Food Chem.* **116** (2009), 371–381.
[3] Deák E., Gyepes A., Stefanovits-Bányai É., Dernovics M.: *Food Res. In.* **43** (2010), 2452–2455.
[4] Zhu S.: *J. Chromatogr. A* **1216** (2009), 3312–3317.

Pressure Modulator Development and Optimization for Applications in Comprehensive Gas Chromatography

MARTIN STON*, RADOMÍR ČABALA

Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic ✉ *ston@natur.cuni.cz*

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

Comprehensive two-dimensional gas chromatography (GC×GC) is a modern technique that passes each sample component through two different capillary columns. Separation is achieved by coupling a gas chromatography separation in the first and second column. The modulator is a piece of hardware that transfers effluent from the exit of the primary column to the head of the secondary column as a repetitive series of pulses [1]. The several different modulators that are commercially available can be classified into two main categories: thermal modulators and valve-based modulators. Every commercially available thermal modulator requires the consumption of large amounts of liquid cryogen coolant and gaseous working fluid. Thermal modulation produces optimal resolution, but also sacrifices some of the simplicity of conventional gas chromatography [2]. Valve-based modulators do not require additional consumables and use straightforward low-cost designs. Two main classes of valve-based modulators have been developed: flow diversion and differential flow. Pulse (fluid) pressure modulator is a simple differential flow modulator [3, 4].

A fast three-way solenoid valve is the key piece of the pulse modulator. The solenoid valve switching is timed by PC and provides distribution of a mobile phase between two transfer lines. Each transfer line ends on a T-connector. These connectors are positioned between columns and storage transfer line, which is situated between first and second column. Storage transfer line is for storage of effluent from the first analytical column. After the filling of the storage sample line with the effluent, high flow rate pulse flushes the effluent from the storage transfer line to the second analytical column [5].

The main aim of this study is construction of the pulse pressure modulator and optimization of its connection to the gas chromatograph with a flame ionization detector. Optimal conditions were found for the analysis of the mixture of selected volatile solvents. Pressure interdependences in the system and relations between dimensions of modulator capillaries and columns have been evaluated with respect to the length of the modulation period and pulse duration.

Two non-polar columns of the same stationary phase (15 m × 0.25 mm DB-5MS, 10 m × 0.15 mm CP-Sil 5 CB) were tested to compare the separation efficiency in the first dimension and the DB-5MS column was selected. The Supelcowax 10 columns of the lengths 1, 2, and 3 m were compared in the second dimension. It was found that using of 3 m length column leads to the extension of analysis, however the increase in separation efficiency in the second dimension was evident. The experimental measurements have shown that the selected parameters for the evaluation of the separation efficiency (the peak capacity and total resolution product) were inappropriate.

Gasoline, kerosene and diesel fuel were analyzed as examples of real samples. Ethanol content in a biofuel as an additive was determined in the gasoline sample.

In the present, we compare this modulator with a new prototype which is equipped with two two-way solenoid valves instead of one three-way valve. Optimal conditions for the analysis were found of the same testing mixture of selected volatile solvents.

Acknowledgments

Financial support from the Grant Agency of Charles University in Prague (GA UK-894513), the Ministry of Education, Youth and Sports of the Czech Republic, project MSM 0021620857, and SVV 2012-267215 is gratefully acknowledged.

References

[1] Cortes H.J., Winniford B., Luong J., Pursch M.: *J. Sep. Sci.* **32** (2009), 883–904.
[2] Dallüge J., Beens J., Brinkman U.A.T.: *J. Chromatogr. A* **1000** (2003), 69–108.
[3] Górecki T., Harynuk J., Panić O.: *J. Chromatogr. A* **1027** (2004), 359–379.
[4] Blumberg L.M.: *J. Chromatogr. A* **985** (2003), 29–38.
[5] Poliak M., Kochman M., Amirav A.: *J. Chromatogr. A* **1186** (2008), 189–195.

Author Index

Keyword Index

Proceedings of the 9th International Students Conference "Modern Analytical Chemistry"

Edited by Karel Nesměrák.
Published by Charles University in Prague, Faculty of Science.
Prague 2013.
1st edition – 146 pages – Number of copies: 90

ISBN 978-80-7444-023-6

ISBN 978-80-7444-023-6