2nd International Student Conference

"Modern Analytical Chemistry"

Book of Abstracts

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Charles University, Prague

2nd International Student Conference

"Modern Analytical Chemistry"

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Programme

Monday 26

9.00
Vilém Guryča[a,b], Jiří Michálek[b], Věra Pacáková[a]
[a]*Department of Analytical Chemistry, Faculty of Sciences, Charles University, Prague and* [b]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague*
Hydrophilic polymer systems in capillary electrophoretic separations

9.30
Jan Srbek[a], Jan Eickhoff[b], Karsten Kraiczek[b], Tom van de Goor[c], Pavel Coufal[a]
[a]*Department of Analytical Chemistry, Faculty of Science, Charles University, Prague,* [b]*Agilent Technologies, Hewlett-Packard, Waldbronn, Germany,* [c]*Agilent Technologies, Santa Clara, California, USA*
Chip based nano-LC/MS/MS analysis of real biological samples

10.00
Jan Grafnetter[a], Pavel Coufal[a], Eva Tesařová[b], Jana Suchánková[a], Zuzana Bosáková[a]
[a]*Departments of Analytical Chemistry and* [b]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague*
Reproducibility of preparation of butyl methacrylate monolithic capillary columns

10.30
Veronika Šímová, Petr Bezdička, Janka Hradilová, David Hradil, Eva Kotulanová, Tomáš Grygar
Academic Laboratory of Materials Research of Paintings (ALMA) - joint workplace of the Institute of Inorganic Chemistry AS CR in Řež and Academy of Fine Arts in Prague, Czech Republic
Mineralogical identification of painting layers in artworks by X-ray powder microdiffraction

11.00
Veronika Šolínová, Václav Kašička, Dušan Koval, Jan Hlaváček and Tomislav Barth
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague
Analysis, Separation and Investigation of Structure-Mobility Relationships of Biopeptides by Capillary Zone Electrophoresis

Lunch

13.30
J. Blaško
Chemical Institute, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
A new capillary column with a gradient film thickness of stationary phase

14.00
Ž. Frčková
Chemical Institute, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Very fast GC with ultrafast cooling of a column

14.30
H. Jurdáková
Chemical Institute, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
Determination of BTEX in water samples by gas chromatography with direct aqueous injection

15.00
Jan Fischer[a], Joseph Wang[b], Jiří Barek[c]
[a]*UNESCO Laboratory of Environmental Electrochemistry, Department of Analytical Chemistry, Charles University, Prague and* [b]*Departments of Chemical and Materials Engineering and Chemistry and Biochemistry, Biodesign Institute, Arizona State University, Tempe, USA*
Capillary electrophoresis microchips with amperometric detection for separation of nitrophenolic pollutants

15.30
Zuzana Hoherčáková, František Opekar
Charles University, Faculty of Science, Department of Analytical Chemistry, Prague
Contactless conductivity detector for FIA - Determination of total inorganic carbon

Dinner in student club „Chladič“ (from 18 o´clock)
(Chemical departments building)

Tuesday 27

9.00
Jan Kratzer[a,b], Jiří Dědina[a] and Petr Rychlovský[b]
[a]Academy of Sciences of the Czech Republic, Institute of Analytical Chemistry, Prague, Czech Republic and [b]Department of Analytical Chemistry, Charles University, Faculty of Science, Prague, Czech Republic
Hydride preconcentration at the quartz surface for AAS: why should an analytical chemist use it?

9.30
V. Vrkoslav[a], I. Jelínek[a] and J. Dian[b]
[a]Department of Analytical Chemistry, Charles University, Prague, Faculty of Sciences, Prague, Czech Republic and [b]Department of Chemical Physics and Optics, Charles University Prague, Faculty of Mathematics and Physics, Prague, Czech Republic
Porous silicon sensor of chemical species based on simultaneous measurements of luminescence quenching and luminescence decay time shortening

10.00
V. Červený, Z. Válková and P. Rychlovský
Charles University, Faculty of Science, Department of Analytical Chemistry, Prague, Czech Republic
Electrochemical hydride generation as a derivatization step for HPLC - QFAAS determination of selected arsenic species

10.30

Tereza Vařilová[a], **Hans-Gerd Janssen**[b]

[a]*Department of Analytical Chemistry, Charles University, Faculty of Science, Prague 2, 128 40 , Czech Republic*

[b]*Unilever R&D Vlaardingen, Food Research Centre, Advanced Measurements &Imaging, Olivier van Noortlaan 120, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands*

Study of whey proteins aggregation by multi-dimensional size exclusion chromatography

HYDROPHILIC POLYMER SYSTEMS IN CAPILLARY ELECTROPHORETIC SEPARATIONS

Vilém Guryča [a,b], Jiří Michálek [b], Věra Pacáková [a]

[a] *Dept. Anal. Chemistry, Faculty of Sciences, Charles University, Prague*
[b] *Inst. Macromolecular Chemistry, CAS, Prague*

Hydrophilic polymer systems (various types of column coatings, porous monoliths) are gathering great significance in numerous analytical applications.
The capillary electrochromatography (CEC) in porous acrylamide media has been employed for separations of oligosaccharides [1]. The polymerization feed consists of a copolymerization pair acrylamide + crosslinker (these compounds form a rigid polymer backbone) and hydrophilic ligand such as *N*-[Tris(hydroxymethyl)methyl]acrylamide.
From chromatographical point of view it is possible to alter separation capacity of various compounds by copolymerization of suitable separation ligands [2]; however; the "blank" acrylamide matrix was capable to fairly resolve branched glycan forms in hydrophilic interaction mode even without any other polar ligand embodied. Furthermore; the "blank" acrylamide network formed with more rigid crosslinker warrants the maximum efficiency of separations (routinely up to 350 000 plates per meter for tagged oligosaccharides), resolution of the branched glycan isomers and the highest permeabilities of columns.
The porous structure of continuous rods was investigated as well. Evidently, there is only monomodal distribution of pore sizes with its mean value about $r \approx 35$nm (at specific surface area $S_p \approx 74 m^2/g$) which is not passable for electrochromatography purposes . Thus the macroporosity in the network must be set up by a reswelling process in solvents [3]. Indeed, the electron microscopy under aqueous conditions revealed the network, placed in good solvents, restructuralizes into a spongeous shape. After swelling, communicating pores are formed with the total porosity about $P_s \approx 69\%$.

We are indebted to *National Institute of Health - USA; Indiana Genomic Initiative, Indiana University - USA; Josef Hlávka Foundation - Czech Rep.; Fund Mobility, Charles University - Czech Rep.;* and *Academy of Sciences of the Czech Rep., project AVOZ40500505* for financial support.

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CHIP BASED NANO-LC/MS/MS ANALYSIS OF REAL BIOLOGICAL SAMPLES

Jan Srbek [a], Jan Eickhoff [b], Karsten Kraiczek [b], Tom van de Goor [c], Pavel Coufal [a]

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Proteomics is one of the life sciences, development of which grows incredibly at present. Many other life science areas support this accelerated progress of proteomics developing tools for separation, identification, determination and analysis of peptides and proteins in real biological samples. This study illustrates use of a polymer microfluidic device, i.e. chip, for gradient liquid chromatography in reversed-phase mode under eluent flow rates of nanoliters per minute. The use of this microfludic device in combination with a face seal rotary valve enables effective pre-concentration, separation, electro-spraying, mass spectrometry analysis and identification of real biological samples of proteins and peptides. This study shows some features of the use of chip for analysis and identification of proteins. The work is focused on analysis of tryptic digest of rice proteins isolated from the rice sample. The performance of the chip is tested by reversed-phase gradient separation of rice tryptic digest at a flow rate of 300 nL/min. The use of the microfluidic device enables nano-LC gradient separation and MS/MS identification of rice proteins from the real rice sample.

REPRODUCIBILITY OF PREPARATION OF BUTYL METHACRYLATE MONOLITHIC CAPILLARY COLUMNS

Jan Grafnetter [a], Pavel Coufal [a], Eva Tesařová [b], Jana Suchánková[a], Zuzana Bosáková[a]

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Keywords: Monoliths; Butyl methacrylate; CLC; Reproducibility

The radical polymerization thermally initiated with azobisisobutyronitrile (AIBN) was applied to preparation of butyl methacrylate monolithic columns in 320 μm i.d. fused silica capillaries. The butyl methacrylate capillary columns obtained by this preparation procedure can then be used in capillary liquid chromatography (CLC) as separation columns for reversed-phase elution mode. The optimized mixture of functional monomer, crosslinker, porogen solvent and initiator containing 17.8% of butyl methacrylate, 21.8% of ethylene dimethacrylate, 18.0% of 1,4-butanediol, 42.0% of 1-propanol and 0.4% AIBN was used as the polymerization mixture. In this work, the run-to-run and column-to-column repeatability and mixture-to-mixture reproducibility of the preparation procedure of butyl methacrylate monolithic columns for CLC were investigated. The prepared columns were characterized with the common chromatographic properties and after that repeatability and reproducibility of retention times, retention factors, peak asymmetry factors, column efficiencies and resolutions of seven test analytes were evaluated as relative standard deviations.

MINERALOGICAL IDENTIFICATION OF PAINTING LAYERS IN ARTWORKS BY X-RAY POWDER MICRODIFFRACTION

Veronika Šímová, Petr Bezdička, Janka Hradilová, David Hradil, Eva Kotulanová, Tomáš Grygar

Academic Laboratory of Materials Research of Paintings (ALMA) - joint workplace of the Institute of Inorganic Chemistry AS CR in Rez and Academy of Fine Arts in Prague, Czech Republic. E-mail: veronika@iic.cas.cz

Identification of inorganic and organic compounds in colour layers helps in dating and restoring of historical paintings. X-ray powder microdiffraction (micro-XPRD) extends the possibilities of traditional optical microscopy and SEM/EDX used in microanalysis of mineral pigments.
Laboratory diffractometers with monocapillary primary optics and x,y,z-stage are now available for a routine analysis of 0.1 mm large samples such as heterogeneities in fragments and colour layers in cross-sections with a roughly flat surface. No special devices, e.g. synchrotron and area detector, are required. The advantage of X-ray microdiffraction is its non-destructive nature and no need of sample pre-treatment. Sample after microdiffraction can hence be used for other analyses or archivation.
As an example, phase identification of painting layers of art works in fragments, canvas, wall paintings and polychromes on sandstone are shown.

Keywords: micro-XPRD, mineral pigments, paintings

ANALYSIS, SEPARATION AND INVESTIGATION OF STRUCTURE-MOBILITY RELATIONSHIPS OF BIOPEPTIDES BY CAPILLARY ZONE ELECTROPHORESIS

Veronika Šolínová, Václav Kašička, Dušan Koval, Jan Hlaváček and Tomislav Barth

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Capillary zone electrophoresis (CZE) has been applied to the analysis, separation and physicochemical characterization of synthetic biopeptides and their derivatives and fragments, such as insect oostatic peptides (IOPs), enkephalins (ENKs) and dalargin (DLR). IOPs are antigonadotropic insect hormones with specific effect on the reproduction of the flies and are considered as a potential new type of biologically degradable insecticides. ENKs and DLR play significant roles in mediating stress and abatement of pain and are involved in temperature control, feeding behavior, respiration and are used for the treatment of some mental illnesses. Peptides were analyzed in three acidic background electrolytes (BGEs) (100 mM H_3PO_4, 50 mM Tris, pH 2.25; 100 mM and 50 mM iminodiacetic acid, pH 2.30 and pH 2.40) and in an alkaline BGE (40 mM Tris, 40 mM Tricine, pH 8.1). The analyses were carried out in CZE analyzer (Beckman-Coulter), in uncoated fused silica capillary, total/effective length 400/300 mm, ID/OD 75/360 μm, with separation voltage 10 kV, at constant temperature 25°C, using UV-absorption detector at 206 nm. Peptides were dissolved in water or BGEs in the concentration range 0.05-0.5 $mg.ml^{-1}$.
In addition to the purity degree determination of peptides, the effective electrophoretic mobility and effective charge of analyzed peptides were determined [1, 2]. Several semiempirical models of the dependence of effective mobility of peptides on the ratio of their effective charge and relative molecular mass were tested to describe the electromigration behavior of analyzed peptides, particularly the shape of their molecules. None of models was found to be quite unambiguously applicable for the whole set of peptides differing in size and charge, but a good correlation of some models was found for the subsets of homologous peptides. The logarithmic plot of the dependence of the ratio, m_{ef}/q, on M_r, ($\log (m_{ef}/q) \sim k \log M_r$), suggested by Cross and Garnham [3], was found as a suitable tool for prediction of the shape of peptide molecules since in this way the exponent k in the above non-logarithmic relation can be easily determined as a slope of the logarithmic plot. From the k values, related to the shape of the electrophoretically migrating particles, the probable secondary structure of peptide molecules in solution can be estimated.

The work was supported by GACR, grants Nos. 203/03/0716, 203/04/0098, 203/05/2539, and by the Research Project Z40550506 of ASCR.

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A NEW CAPILLARY COLUMN WITH A GRADIENT FILM THICKNESS OF STATIONARY PHASE

J. Blaško

Chemical Institute, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovakia

Gas chromatography started with packed columns. Nowadays, most of gas chromatographic separations (roughly 95 %) are performed on capillary columns. These kinds of columns, compared to packed ones, give much better resolution in shorter analysis time. The basic characteristics of the separations are resolution, speed and sample capacity.

The film thickness plays an important role in the number of plates required for a given separation, solute elution temperatures, and the available efficiency of the column. Increasing the film thickness will increase both column capacity and solute retention.

The aim of this work was to prepare a new type of chromatographic capillary column with gradient film thickness of stationary phase to improve efficiency in comparison with commercial columns.

Key words: Gradient film thickness, Efficiency, Sample capacity

VERY FAST GC WITH ULTRAFAST COOLING OF A COLUMN

Ž. Frčková

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An overview is given of existing methods to minimize an analysis time in gas chromatography (GC) being a subject of many publications in the scientific literature. The features of a system of the tubes for fast temperature-programmed and very fast cooling down gas chromatography have been evaluated. This study develops and applies a system of tubes for very fast heating up and ultrafast cooling down of capillary column. The experiments were carried out using the prepared system of metal tubes – heating/cooling module (HC – M), the capillary column is placed inside the prepared HC - M, which can be heated by the temperature of GC oven and cooled by cooling medium. The prepared HC - M assembly can generate temperature rates up to 330 °C.min^{-1} and can be cooled down 6 000 °C.min^{-1}. The system is prepared for following measure in a time few second. The prepared module of tubes showed a good separation reproducibility component identification through the relative retention times and quantitative determination through peak areas, it is comparable with conventional GC. The prepared HC - M can be applied to the commercial gas chromatograph.

Keywords: Fast temperature programming; Very fast chromatography; Ultrafast cooling

DETERMINATION OF BTEX IN WATER SAMPLES BY GAS CHROMATOGRAPHY WITH DIRECT AQUEOUS INJECTION

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A simple method of solventless extraction of volatile organic compounds (benzene, toluene, ethylbenzene and xylenes) from aqueous samples was developed. This method allows direct injection of large volume of water sample into a gas chromatograph using the sorption capacity of the sorbent Chromosorb P NAW applied directly in the injection port of gas chromatograph. The system prevent water penetration into a column, keep it adsorbed on its surface until the analytes are stripped into a column, and the residual water is purging using split flow. The limit of detection ranging from 0,6 for benzene to 1,1 $\mu g.l^{-1}$ for *o*-xylene and limit of quantification ranging 2,0 - 3,6 $\mu g.l^{-1}$ are lower that those reached by gas chromatography with flame ionization detection and direct aqueous injection before.

Keywords: DAI; Large volume; determination BTEX; GC-FID

CAPILLARY ELECTROPHORESIS MICROCHIPS WITH AMPEROMETRIC DETECTION FOR SEPARATION OF NITROPHENOLIC POLLUTANTS

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Microfabrication has the potential to revolutionize chemical and biological analysis, just as it has revolutionized electronics and computing. A miniaturized analytical system for separating and detecting toxic nitrophenolic compounds, based on the coupling of a micromachined capillary electrophoresis (CE) chip with a glassy carbon detector will be described. Nitrophenols, coming from pesticide degradation products, car exhausts, and industrial wastes are listed as priority pollutions by the US Environmental Protection Agency. They are potential cancinogens, teratogens, and mutagens. Therefore, their separation and determination is of importance in environmental analysis.

The described integrated microsystem offers a rapid (120 s) simultaneous measurement of five priority nitrophenolic pollutants (2-nitrohenol, 3-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol). There compounds can be detected down to the 1×10^{-5} M level, with linearity up to the 1×10^{-4} M level. The stable response observed for repetitive injections (R.S.D. = 3.2 %, n = 8) reflects the negligible surface passivation due to the injection of extremely small sample volumes. Tested nitrophenols were separated using 15 mM phosphate buffer pH 7.2 and 1.3 mM α-cyklodextrin as running solution on 77 mm long capillary by applying 3 kV separation voltage and –0.7 V vs. Ag /AgCl wire detection potential. Applicability to ground water samples will be demonstrated.

While the concept of miniaturized separation/detection systems is presented here within the framework of nitrophenolic contaminants, such devices should be attractive for field monitoring of other classes of priority contaminants. The development of fast-responding miniaturized systems with negligible waste production holds particular promise for meeting the requirements of field 'Green Analytical Chemistry'.

This work was financially supported by EPA. J.F. was partly supported by The Grant Agency of the Charles University (Project 332/2005).

CONTACTLESS CONDUCTIVITY DETECTOR FOR FIA. DETERMINATION OF TOTAL INORGANIC CARBON

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New, very simple, contactless conductivity detectors have been designed for determination in flow injection analysis combined with gas diffusion separation (GD-FIA). The cells consists of two insulated wire electrodes punctured through the walls of teflon tubing at a close mutual distance and placed either across the tube or along the tube axis, so that the detection occurs within the space limited by the two electrodes. As the thickness of the dielectric layer is significantly smaller than that in common contactless conductivity cells used in CE, the a.c. current flowing between the electrodes is much higher and the measuring sensitivity is enhanced. The newly designed contactless conductometric cells were applied to the direct determination of total inorganic carbon (TIC) in model water samples, it exhibited a linear current response up to 0.1 mmol.l^{-1} $NaHCO_3$ with a detection limit of 0.6 and 6 □mol.l^{-1} for detector with axially placed and across the tube electrodes. The RSD was generally 4.1 and 4 %, respectively, at a throughput of 15 samples per hour. The effects of operating frequency, insulator layer thickness and detector cell geometry on the detection sensitivity were discussed in detail.

HYDRIDE PRECONCENTRATION AT THE QUARTZ SURFACE FOR AAS: WHY SHOULD AN ANALYTICAL CHEMIST USE IT?

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Hydride generation[1] coupled to atomic absorption detector is a suitable method for Sb, As, Bi and Se determination. Analytical chemists are often required to determine these elements even at (ultra)trace levels because of their importance from the toxicological or biochemical point of view. Using hydride generation analyte can be easily separated from the matrix. Moreover, it can be preconcentrated either in a special collection device or directly in the atomizer, so that the detection limit can be decreased by several orders of magnitude. Quartz surface was found to be a promising material for hydride trapping recently[2,3] because quartz atomizers are predominantly used for hydride atomization. Thus, quartz preconcentration devices could be simply interfaced to the atomizer or trapping could be done directly in the atomizer (*in-situ*). Compared to special quartz preconcentration devices, the *in-situ* trapping results in better repeatability and less labour is involved. The aim of the present work was to develop a simple and non-expensive analytical method for determination of these elements at low ppt levels.

In principle, analyte hydride is collected at the heated quartz surface using O_2 excess over H_2 in the carrier gas (H_2 formed in hydride generator decreases trapping efficiency). In the next step, trapped analyte is revolatilized in an atmosphere with a stoichiometric H_2 excess over O_2. Two auxiliary gas channels for H_2 and O_2 make possible to change the gas composition in the trapping device/atomizer.

Conditions for *in-situ* trapping of Sb[4] and Bi hydrides and subsequent analyte revolatilization in commercial quartz atomizers were found (100% preconcentration efficiency, LOD ~ units of $ng.l^{-1}$ for 5 min preconconcentration). This novel approach is also promising for As.

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POROUS SILICON SENSOR OF CHEMICAL SPECIES BASED ON SIMULTANEOUS MEASUREMENTS OF LUMINESCENCE QUENCHING AND LUMINESCENCE DECAY TIME SHORTENING

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Porous silicon is nanostructured silicon-based material that exhibits bright visible room temperature photoluminescence. This photoluminescence strongly depends on the presence of chemical compounds in its environment. The change of photoluminescence properties in the presence of analytes in gas phase is primarily connected with the adsorption of the molecules in the porous matrix. Critical role in the interaction of analyte with the porous silicon plays the chemical composition of porous silicon surface. Freshly prepared porous silicon is terminated with Si-H bonds, which are not stable due to slow oxidation. Enhancement of operational stability of luminescence from porous silicon can be achieved by replacement of Si-H bonds by Si-O or Si-C bonds.

Photoluminescence characteristics of porous silicon – intensity and decay time – were used for detection of organic molecules in gas phase. We present a systematic study of photoluminescence intensity decrease and decay time shortening of porous silicon for standard and surface modified porous silicon samples in the presence of precisely controlled concentrations of organic analytes in gas phase. Surface modification was performed either by wet oxidation in H_2O_2 or hydrosilylation with methyl-10-undecenoate. We studied photoluminescence responses for linear alcohols from methanol to n-hexanol, propanol and butanol isomers, hexane, toluene, p-xylene, chloroform and water. The presence of analyte in porous matrix affects photoluminescence intensity and photoluminescence decay time in different ways. Observed photoluminescence quenching and decay time shortening for the studied groups of chemical compounds and porous silicon surfaces are presented. Selectivity improvement of experimental setup based on dual detection towards various types of organic species is discussed.

ELECTROCHEMICAL HYDRIDE GENERATION AS A DERIVATIZATION STEP FOR HPLC - QFAAS DETERMINATION OF SELECTED ARSENIC SPECIES

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The technique of electrochemical (electrolytic) hydride generation (EcHG) was utilized as a derivatization step for HPLC-EcHG-QFAAS determination of most frequently analyzed arsenic species. There were inorganic forms of arsenic (As(III) and As(V)) and one of their products of human metabolism (dimethylarsinate, DMAA) analyzed with this coupled (hyphenation) technique.
An anion-exchange column Supelco LC-SAX1 and phosphate buffer (pH=7.5; 25.0 $mmol \cdot l^{-1}$) as mobile phase were selected for separation of used arsenic compounds. Laboratory made thin layer flow-through cell with lead wire cathode served as the hydride generator. The manifold connecting the electrolytic hydride generator with HPLC separation unit and detector - atomic absorption spectrometer with quartz furnace atomizer (QFAAS) were constructed and tested. This coupled technique was optimized to attain the maximum sensitivity and to minimize the LOD and LOQ for arsenic speciation. The influence of relevant experimental parameters (electrolytic generation current, carrier gas flow rate, catholyte flow rate, mobile phase flow rate, injected sample value) on the analytical signal was studied. Attained results were compared with results obtained by the technique of the chemical hydride generation (CHG) using $NaBH_4$ solution as the reducing agent. Some real surface water samples from Prague were analyzed.
The attained LOD for tested inorganic arsenic compounds with electrolytic hydride generation as the derivatization technique are comparable with results attained by the technique of the chemical generation. The most significant success of EcHG for speciation analysis is of an one order lower LOD for organic arsenic compounds than by CHG. Moreover the sensitivities of determination of DMAA and both used inorganic arsenic compounds were comparable.

Acknowledgment: *The authors thank The Grant Agency of the ASCR (project: A400310507/2005) for the financial support*

STUDY OF WHEY PROTEINS AGGREGATION BY MULTI-DIMENSIONAL SIZE EXCLUSION CHROMATOGRAPHY

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In the development of foods with improved structures and structure stability, a better understanding of how food ingredient composition and processing affect the ultimate properties of the food is crucial. Whey proteins are an important source of functional ingredients in many formulated foods because of their high nutritional value and functionality [1]. The major whey proteins are α-lactalbumin (ALA) and β-lactoglobulin (BLG). Soluble aggregates of polymerized whey proteins are formed after heat-treatment gelation.

For studying the composition of the mixed aggregates formed during heat treatment of mixed protein systems we have developed a two-dimensional size exclusion chromatography (SEC) set-up. In the first dimension a wide pore SEC column is used to separate aggregates from remaining native and denatured single protein molecules. In the second dimension a narrower pore-size SEC column is used to separate single ALA from BLG molecules and determine the ratio of these two proteins. A crucial step in the procedure described above is conversion step in which the aggregates are converted back into the single molecules. Two options for achieving this task were studied: 1) A refolding buffer was used to convert the molecules back into the native folded state. We investigated two possibilities - 'refolding by dilution' mode as well as 'SEC refolding'. 2) Better results were obtained with the denaturation approach. Here the aggregates isolated from the first dimension were treated with an SDS and mercaptoethanol containing buffer. After heating in boiling water full denaturation was obtained.

Our results are in good agreement with the levels reported in literature [2,3]. In comparison with the methods used for the ratio determination in those literature reports (SDS-PAGE) our two-dimensional method was much faster and less laborious.

Acknowledgment: This work was done in Unilever in The Netherlands and it was financially supported by European Union - Marie Curie grant QLK1-CT2001-60028.

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PREPARATION AND CHARACTERISATION OF MOLECULARLY IMPRINTED MONOLITHIC COLUMNS

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Molecularly imprinted polymers have received much attention in various fields because of their high selectivity for target molecules. It has been recognized as a promising technique, where the molecule to be detected is added to a reaction mixture of a cross-linker, a solvent, an initiator and a functional monomer that possesses a functional groups capable of interacting with the target molecule. Binding sites in the resultant polymers involve functional groups, which are constructed according to the shape and chemical properties of the target molecules. After removal of the target molecules, these specific binding sites exhibit high selectivity and affinity for the template molecules.
Monolithic HPLC stationary phases based on molecular imprinting were prepared by *in situ* polymerization. The template compound (L-tosylphenylalanine), the functional monomer (methacrylic acid), the cross-linker (ethylene glycol dimethacrylate), the initiator (azobisisobutyronitrile or hydroxydimethylacetophenone) and a porogen mixture (dodecanol with cyclohexanol or toluene) were placed in glass columns (150 x 3 mm i.d.) and heated or irradiated to induce polymerization.
Newly prepared monolithic columns were tested on HPLC separations of enantiomer pairs of derivatized amino acids and a standard mixture of hydrophobic solutes. The structure of the monoliths was investigated by scanning electron microscopy and nitrogen/mercury porosimetry. The dependences of the polymer morphology and the chromatographic behavior on the composition of the polymerization mixture and on the polymerization conditions were also investigated.

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