

## Description of the main research directions investigated by the institute

The institute consists of five scientific departments with the following research directions:

### DEPARTMENT OF BIOANALYTICAL INSTRUMENTATION

Development of instrumentation and protocols for analyses of proteins, glycans, DNA, and metabolites down to the single-cell level using:

- capillary separations
- mass spectrometry coupling
- miniaturization and microfluidics
- optical detection based on nanostructures

### DEPARTMENT OF ELECTROMIGRATION METHODS

Basic research of electromigration in liquid media including:

- preconcentration and separation of samples with complex matrices
- theory to understand and predict the properties of separation systems
- methodology to reach maximum separation efficiency and sensitivity
- microextraction techniques based on phase interfaces

### DEPARTMENT OF ENVIRONMENTAL ANALYTICAL CHEMISTRY

Research and development of new techniques for preconcentration, separation, and detection of trace amounts of gases, aerosols, and nanoparticles in the environment:

- chemical composition, health effects, and sources of atmospheric aerosols
- toxicity and impact of nanoparticles on the environment
- the origin and fate of gaseous compounds and pollutants in the air

### DEPARTMENT OF FLUID PHASE SEPARATIONS

Theory, methodology, and development of fluid phase separation methods, including:

- divergent flow separations
- near- and supercritical water for surface modifications of fused silica and glass substrates for analytical applications
- chromatography column technology– synthesis of monolithic columns
- modification of fused silica capillaries for electrophoretic separations of unicellular organisms
- extractions of biological materials with compressed fluids

### DEPARTMENT OF TRACE ELEMENT ANALYSIS

All aspects of method development, applications, and mechanistic studies on:

- generation, concentration, and atomization of volatile compounds for analytical atomic spectrometry
- speciation analysis of toxicologically important metabolites of arsenic
- novel approaches to preconcentrating volatile compounds in AAS and AFS
- chemical and photochemical generation of volatile compounds of transition metals

## Research activity and characterisation of the main scientific results

The main research areas resolved in recent five years were:

- (i) Development of hydride atomization for AAS based on DBD plasma
- (ii) Preconcentration of volatile species by collection in atomizers for AAS
- (iii) Further development of hydride generation - AFS
- (iv) Spectrometric methods for trace elemental and speciation analysis in biological and environmental matrices
- (v) Generation of volatile compounds of transition metals
- (vi) Application of unique features of advanced methods of As speciation analysis based on hydride generation

### The research of the team was supported by 8 grant projects in recent 5 years:

"Development of novel approaches to atomization and preconcentration of volatile species for atomic absorption and atomic fluorescence spectrometry"; ASCR internal program for international collaboration support, project M200311202, 2013-2015 (**PI J. Dědina**).

"Mechanisms of toxicity of biofuel particulate emissions", Czech Science Foundation, Project P503/13/1438, 2013-2015, (team members **T. Matoušek, J. Dědina**).

"Generation and preconcentration of volatile compounds for atomic absorption and atomic fluorescence spectrometry"; Czech Science Foundation, Project 14-23532S, 2014-2016 (**PI J. Dědina**).

"Advanced speciation analysis of arsenic in difficult biological matrices; Ministry of Education, Youth and Sports of the CR", Kontakt II LH15174, 2016-2017 (**PI T. Matoušek**).

"Elucidation of dissociation mechanisms of a dielectric barrier discharge for volatile species"; Deutsche Forschungsgemeinschaft, DFG FR 1192/27-1, 2016-2019 (**cooperation partner J. Kratzer**).

"Hydride atomizers for atomic absorption and atomic fluorescence spectrometry - new horizons"; Czech Science Foundation, Project 17-04329S, 2017-2019 (**PI J. Dědina**).

"Advanced approaches to volatile species generation with spectrometric detection: ultratrace determination and speciation analysis of Cd, Hg, As and Se"; Czech Science Foundation, Project 18-01116S, 2018-2020, principal investigator (**PI J. Kratzer**).

"Novel analytical approaches for determination of transition metals based on volatile species generation and atomic fluorescence spectrometry"; Czech Science Foundation, Project 19-17604Y, 2019-2021 (**PI S. Musil**).

#### **(i) Development of hydride atomization for AAS based on DBD plasma**

The dielectric barrier discharge (DBD) is an example of a miniaturized atmospheric pressure plasma source. Its energy can be used either for dissociation or soft ionization of analyte molecules. DBD can serve as a powerful atomization and excitation tool suited for trace element analysis when dissociation of analyte molecules occurs, thus, being compatible with atomic spectroscopic detectors including AAS, AFS and optical emission spectrometry (OES).

Atomization of 4 hydride forming elements, namely Se [5], As [8], Sb [21] and Sn [36] in a planar DBD atomizer was optimized for AAS detection during the evaluated period while Bi was studied already in 2014. Subsequently, the performance of a novel DBD atomizer was compared to that of a conventional externally heated quartz tube (QTA) for each element.

Interestingly, the hydride forming elements investigated can be divided into two groups: As, Se and Sb represent elements which hydrides can be easily and efficiently atomized in the DBD providing high sensitivity and low LODs ( $\approx 0.2 \text{ ng mL}^{-1}$ ) while atomization of Sn and Bi hydrides is challenging reaching significantly worse sensitivity and impaired LODs ( $>1 \text{ ng mL}^{-1}$ ) even under the best atomization conditions. As a consequence, the performance of the DBD is comparable to that of a QTA for As, Se and Sb in terms of sensitivity and resistance to interferences. On the contrary, the QTA overpowers the DBD in atomization of Sn hydride significantly. To understand this, the effects of a DBD power supply source configuration (sinusoidal versus square-wave high voltage function modulation) and electrode attachment (glued versus sputtered) on As and Sn signals in AAS were studied [25, 36]. These effects were found negligible for As [25] while square-wave generator allowed to reach 2.5 times higher signals for Sn compared to sinusoidal generator [36]. Nevertheless, sensitivity for Sn detection by AAS in a DBD atomizer was 10 times worse compared to the QTA even in the optimized experimental arrangement. This research has been completely done by our team. Square wave modulated power supply sources for DBD atomizers employed in [25, 36] were provided by Leibniz Institute for Analytical Sciences (ISAS), Germany.

Mechanistic studies were subsequently performed focused on the atomization processes of Bi [6], As [23, 32], Se [24] and Sn [39] hydrides in DBD atomizers. A variety of spectroscopic techniques including ICP-MS, radioactive indicators, direct analysis in real time (DART) coupled to Orbitrap-MS, spatio- and temporally resolved OES was employed. Significant differences in behavior among the analytes were found. Strong interaction of Bi with the inner surface of the DBD atomizer was confirmed with  $>90\%$  of Bi deposited in the optical arm [6]. This fraction was only around 30% for Se indicating longer life-time of Se free atoms [24]. Temporally and spatially resolved OES measurements have shown that  $\text{AsH}_3$  is atomized and excited in the DBD atomizer across the whole discharge channel which explains its good sensitivity in AAS measurements [23, 32]. Analogous OES measurements have revealed that the major part of Sn is atomized close to the walls of the atomizer. Thus, the probability of the decay of analyte atoms by deposition at the glass surface of the DBD atomizer increases [39]. All the experiments presented in [6, 24] were planned and carried out by TEA team including those measured abroad. Members of prestigious laboratory (NRC Canada) provided access to their facilities, which are not available in TEA lab. Results presented in [23, 32, 39] were completely reached in the laboratory abroad (ISAS, Germany) using their unique equipment for spatiotemporally resolved OES for investigation of DBD atomizers under experimental conditions optimized previously by our team. TEA team member participated in OES measurements. Data evaluation was performed by ISAS team while their interpretation was done by both teams.

Additional experiments by means of two photon absorption laser induced fluorescence (TALIF) have shown spatial distribution of oxygen and hydrogen radicals (H radicals) in the discharge [15,16]. The latter ones are present homogeneously all over the length of the DBD optical arm and their concentration exceeds by several orders of magnitude the typical analyte hydride concentration indicating radical mechanism of hydride atomization. We also used TALIF to bring after four decades for the first time a conclusive proof of the existence of H radical population in QTA thus confirming unambiguously the radical theory of hydride atomization in this atomizer [33]. The superb power of TALIF to quantify distribution of H radicals opens a way to an elegant optimization of hydride atomization also in other atomizers [33]. All TALIF measurements were performed together with colleagues from Department of Physical Electronics, Faculty of Science, Masaryk University. The idea to employ TALIF to determine O and H radicals in DBD and in QTA was developed by our team. TEA team members also participated in TALIF measurements.

Our vast experience with DBD atomizers reaching from method development to fundamental studies was utilized in the authorship (20% contribution) of a review article invited by *Spectrochimica Acta Part B* [12].

### **(ii) Preconcentration of volatile species by collection in atomizers for AAS**

Preconcentration of volatile species from the gaseous phase prior their detection is an elegant way to decrease detection limits of spectrometric detectors, especially AAS. The most common approach is in situ trapping of hydrides in graphite furnaces.

However, hydride trapping in metal and quartz atomizers has been reported as well. We have developed procedures for in atomizer preconcentration of Au [1] and Ag [2], i.e. elements that do not form gaseous hydrides, but “volatile” nanoparticles. Experimental conditions for chemical generation of volatile Au and Ag species were firstly optimized together with their subsequent on-line atomization for AAS detection. Secondly, trapping of volatile Au species [1] and Ag species [2], respectively, in quartz and sapphire atomizers followed by analyte volatilization were optimized. Sapphire atomizer was found to be a better alternative to the quartz atomizer regarding in situ trapping of volatile Au and Ag species since it is chemically more resistant to inner surface devitrification caused by high aerosol carryover from the generator having also higher temperature durability allowing thus also for higher volatilization temperature values if desired.

Efficient preconcentration of analyte hydrides directly in the DBD atomizer prior to AAS detection was found feasible. This novel approach based on in situ trapping of hydrides in planar DBD atomizers was pioneered at our laboratory being firstly reported by our team. The trapping and volatilization processes are controlled only by a presence and absence of O<sub>2</sub> in the discharge gas, respectively. This makes the procedure simple and operator friendly. Addition of low oxygen flow rates to the discharge Ar gas ( $\approx$  a few mL min<sup>-1</sup> O<sub>2</sub>) results in quantitative analyte retention. After a given preconcentration period, O<sub>2</sub> flow is switched off, while the DBD discharge as well as the hydride generation of blank is kept running. Trapped analyte species are released by the Ar discharge that contains H<sub>2</sub> evolved from hydride generation of blank. Complete trapping and subsequent volatilization were reached for As [8] and Sb [21] resulting in 100% preconcentration efficiency. In the case of Se [29], 70% preconcentration efficiency was reached due to incomplete analyte volatilization as proven clearly by radioactive indicator. LOD can be improved by an order of magnitude down to 0.01 ng mL<sup>-1</sup> with 300 s preconcentration period compared to the mode without preconcentration. The results presented here under the research area (ii) have been completely reached by our team.

### **(iii) Further development of hydride generation - AFS**

In order to go ahead with development of instrumentation and methodology of hydride generation - atomic fluorescence spectrometry (HG-AFS), a detailed optimization of relevant experimental parameters of two hydride atomizers for AFS: flame-in-gas-shield atomizer (FIGS) with a two-channel shielding unit and a standard atomizer for AFS, miniature diffusion flame (MDF), was performed employing arsine as the model hydride. Analytical characteristics of both atomizers were compared. Sensitivity obtained with FIGS was approximately twice higher than with MDF. The additional advantage of the FIGS atomizer is significantly lower flame emission resulting in a better signal to noise ratio. Extremely low LODs were found: 3.8 pg mL<sup>-1</sup> and 1.0 pg mL<sup>-1</sup>, respectively, for MDF and FIGS atomizers [3]. This research has been completely done at TEA. The optimized HG-AFS parameters were used in our followed-up investigations [7, 11, 20, 31] described in research area (vi).

**(iv) Spectrometric methods for trace elemental and speciation analysis in biological and environmental matrices**

Our team participated in collaborative research, in which we were responsible for development of optimum measurement techniques for trace-elemental and speciation analysis, providing the state-of-the-art capabilities in the analytical services according to demands of other research groups. Generally, our team performed the trace elemental analysis, while the partner teams provided relevant samples, performed the final interpretation of the data and prepared the manuscripts. The co-operations included teams within the biomedically-oriented research CAS institutes located in the same campus in Prague – Krč as well as other CAS institutes, universities and also a team within IAC. The subjects included very diverse fields and analysis of bioelements as well as different toxic species. The important co-operations along are summarized below.

Our team participated in a grant project (13-01438S Mechanisms of toxicity of biofuel particulate emissions) together with another department (ENV) of our institute. We performed ICP-MS analyses of metals in provided samples of aerosol leachates in simulated lung fluids to assess their bioaccessible fractions in atmospheric aerosols [10], an important issue with respect to air pollution in the urban environment. Also, for a study comparing emissions from conventional fuels and alternative biofuels, we analyzed total contents of 11 metals by ICP-MS in provided samples of mineralized engine emissions, fuels, and lubricating oils [37].

Several co-operations were established with the biologically- and medically-oriented teams in the Krč campus and elsewhere:

We continued an established co-operation with the Laboratory of Molecular Biology of Bacterial Pathogenes at Institute of Microbiology of the CAS providing the ICP-MS data for quantification of the levels of intracellular  $K^+$  in cell lysates. The goal was elucidating the mechanism of actions of Adenylate cyclase toxin-hemolysin of the whooping cough agent *Bordetella pertussis* which penetrates cell membrane [9, acknowledgement in another paper].

For Laboratory of membrane transport, Institute of Physiology of the CAS, we determined the contents of Rb and K in samples of yeast extracts. The aim was to characterize transport properties of  $K^+$  transporters and to clarify their regulation at the level of biogenesis. These analyses were acknowledged in 2 research papers.

We also established a co-operation with Department of Biomathematics at Institute of Physiology of the CAS when we were invited to determine intracellular Li concentrations in HEK293 cell samples for studies dealing with mechanism of Li treatment of patients with bipolar disorder. Within this research, a prolonged exposure of live HEK293 cells to the therapeutic concentration of Li was found to result in down-regulation of  $\delta$ -opioid receptor protein level and attenuation of  $\delta$ -opioid receptor function in parallel with increased oxidative stress and increased level of lipid peroxidation products [27]. In a following study, a regulation of  $Na^+/K^+$ -ATPase in bipolar disorder and lithium therapy has been investigated using HEK293 and Jurkat cells [35].

For Synthetic Nanochemistry group of Institute of Organic Chemistry and Biochemistry of the CAS, we provided ICP-MS and ICP-MS/MS analyses of Gd and Fe. The project focused on development of hybrid nanoscale sensors that can be adjusted to directly monitor physiological species by attaching paramagnetic gadolinium complexes to nanodiamonds with nitrogen-vacancy centres through surface engineering. This analysis was acknowledged in a Nature Communications paper.

Our services were needed also in the field of geochemistry and environmental analysis. In long time continuing cooperation, we have provided HPLC-ICP-MS analyses of As speciation in natural waters for several projects of Institute of geochemistry, mineralogy and mineral resources, Faculty of Science, Charles University. The studies published in evaluated period

employing these analyses studied the transformation of As-rich (>50 wt% As) copper smelter dust over 24 months in soil [22] or microbial sulfidogenesis of arsenic in naturally contaminated wetland soil [38, acknowledgement in another research paper].

A combination of HPLC separation with post-column UV-photochemical generation (UV-PVG) and subsequent high resolution continuum-source (HR-CS) AAS detection was optimized for mercury speciation analysis. Subsequently, extraction procedure for speciation analysis of mercury in fish samples, compatible with the newly developed HPLC-UV-PVG-HR-CS-AAS was developed and verified on certified reference materials and real samples [30]. The research was done at the Department of Analytical Chemistry, Faculty of Science, Charles University. Our role was speciation analysis of Hg in certified reference materials and real samples (fish tissues) by independent method based on HPLC-ICP-MS and co-supervision of a Ph.D. student.

Two simple approaches to speciation analysis of mercury in flue gases have been developed distinguishing between elemental mercury  $\text{Hg}^0$  and oxidized form of mercury (often  $\text{HgCl}_2$ ) either in off-line or on-line measurement arrangements employing AAS detection [34]. This research was exclusively done by our team.

Service measurements were also provided to the non-research companies. Namely, we dealt with analysis of trace metal content in high purity inorganic reagents manufactured by Puralab, s.r.o, Prague, and with determination of trace metal impurities and final gold content in samples of high purity gold micro-wire, planned for production of computer chips of new generation, for Swisswire Technology AG, Prague.

#### **(v) Generation of volatile compounds of transition metals**

One of our main aims has been research conducted in the field of generation of volatile species of transition metals as an alternative sample introduction technique for analytical atomic spectrometry to standard liquid solution nebulization. Chemical generation of volatile species of Ag [2] and Au [1] followed by their in-atomizer preconcentration was already described in the research area (ii). We employed a special in-house made generator and studied chemical generation of volatile species of copper by aqueous solution of tetrahydroborate and with the addition of various modifiers [14]. Using Triton X-100 and Antifoam B as modifiers, the release and generation efficiencies were found satisfactorily high, which suggested quite efficient process of generation. A great effort was put on the experiments leading to identification of the nature of generated volatile species which was partly done in cooperation with the Institute of Microbiology of the CAS (Dr. O. Benada, 10% contribution). These experiments indicated that the generated species in the gas phase were not truly volatile but were strongly associated with a fine aerosol cogenerated and transported by a carrier gas to the detector. Copper clusters and nanoparticles of less than 10 nm in size were identified by transmission electron microscopy (TEM), but the formation of metastable copper hydride could not be conclusively excluded.

Chemical generation of volatile species of palladium have been generated in the presence of diethyldithiocarbamate (DDTC) as a modifier for the first time [19]. A comprehensive study of the generation process, its efficiency and mechanistic aspects has been conducted, including identification of generated volatile species by TEM and DART-MS, again in co-operation with Dr. O. Benada and also with National Research Council Canada (Dr. R. Sturgeon and Dr. Z. Mester). Based on those results a parallel but dual-route mechanism of generation was suggested, one attributed to generation of volatile DDTC chelate of Pd and a second to nanoparticle formation. The conclusions given in this paper are supposed to contribute to clarify the general mechanism of volatile species generation of transition metals. This research was managed by our team with a total contribution of 80%. Only TEM measurements were conducted in collaboration with Institute of Microbiology of the CAS (Dr. O. Benada). Two

colleagues from NRC (Canada) provided access to their facilities (DART-MS), which are not available at our lab, and contributed with a proposal of the mechanism of generation.

Since the developed analytical methods that relied on chemical way of generation of volatile species [14,19] were limited by rather low efficiencies and reproducibility, still unclear mechanism of generation and identity of volatile species (nanoparticles, “strange” hydrides, chelates?), we moved over to a photochemical vapor generation (PVG). This relatively new technique offers to generate well defined volatile species that, in the case of transition metals, are usually metal carbonyls (like the well-known nickel tetracarbonyl). In this field, we gained important knowledge with PVG of Se when our group focused on determination of PVG efficiency by means of  $^{75}\text{Se}$  radioactive indicator [13] (35% contribution). Subsequently, we achieved highly efficient PVG of molybdenum as the first ever [28], which was accomplished in a flow-through photoreactor using formic acid as a reaction medium. The efficiency could be further enhanced by the presence of  $\text{mg L}^{-1} \text{Fe}^{3+}$  ions. We verified the accuracy and feasibility by the analysis of real samples and certified reference materials and even proposed the mechanism of generation which is usually omitted in the current literature. This “breakthrough” work opened a new route of research to other analytes from the 6<sup>th</sup> group of the periodic table. It was quickly followed up by a paper on PVG of tungsten [40]. However, to achieve PVG of W was not as “easy and straightforward” as for PVG of Mo. The addition of  $\text{Cd}^{2+}$  ions as a sensitizer at hundreds of  $\text{mg L}^{-1}$  was found crucial to initiate the photochemical reaction, nevertheless the mechanism of its action remained unanswered despite a significant investigative effort. After optimization we verified the accuracy and feasibility of this methodology by the analysis of water samples and certified reference material. The achieved limit of detection below  $1 \text{ ng L}^{-1}$  with ICP-MS as the detector was found amazing. Our group made a fundamental contribution to those two papers on PVG of Mo [28] and W [40]. The foreign partner represented by R. E. Sturgeon from National Research Council (Canada) was invited as a world leading specialist and pioneer in the field of photochemical vapor generation. He contributed (20%) remotely with a proposal of the mechanism of generation and planning special experiments that helped to strengthen the manuscript.

#### **(vi) Application of unique features of advanced methods of As speciation analysis based on hydride generation**

The research activities concerning application of advanced hydride generation based methods in speciation analysis of arsenic are a continuation of research from previous years. Since the hydride generation based methods are to a great extent unique and complementary to more common HPLC-ICP-MS based arsenic speciation analysis, our capabilities opened doors to several international cooperations. The research was aimed at achieving the best possible limits of detection, which would allow analysis in corresponding to low level of exposure, as well as method development for fast and practical analyses of As species. Fundamental studies aimed at deepening the understanding of the processes of hydride generation and assuring accurate analysis.

We continued in the development of the methodology based on selective hydride generation interfaced to a cryotrap (CT) for the speciation analysis of As metabolites in biological and other matrices. In this vein we designed a new miniaturized CT - Ushaped quartz capillary. It is capable to collect all relevant arsenic species with 100% trapping/volatilization efficiency and it has a potential to become a routinely employed device [4]. This research was exclusively done by our team.

The development of methodology has been to a great extent tailored to immediate use in toxicological research. Foremost, it found immediate application in cooperating group of Prof. M. Stýblo at Department of Nutrition, University of North Carolina at Chapel Hill (UNC), USA, a leading laboratory in the field of toxicology of chronic effects of arsenic exposure. A method

for analysis of toxicologically relevant As species in whole blood and blood plasma was developed at our laboratory and then validated for a routine use at UNC by our team member [18]. The methodology was based on selective hydride generation interfaced to a CT with ultrasensitive ICPMS detection. Presently, this is the sole method capable of this analysis at natural levels, since the concentrations of arsenic in blood are much lower than in e.g. urine (total As content below  $\mu\text{g L}^{-1}$ ); previously only speciation from blood with elevated As content from chronic exposure or therapeutical treatment were published. The method was developed by our team, then transferred to UNC for routine application. UNC personnel training and final method validation using unique blood samples was performed under supervision of TEA team member during his stay at UNC.

Unparalleled sensitivity of HG-CT-ICP-MS based speciation analysis of arsenic also reflected in cooperation with National Research Council Canada, a leading producer of certified reference materials. We participated in certification campaign of water reference materials containing less than  $1 \mu\text{g L}^{-1}$  total As. We provided data for of As species content in reference materials. Since we were a sole laboratory capable of this analysis, our data are base for information values in the certificates. Certification of seawater reference materials NASS-7 and CASS-6 is described in Ref. [26], also participation in certification of river water SLRS-6 and drinking water AQUA-1 reference materials were acknowledged in the corresponding certificates.

We were also interested in the development and inter-validation of a method of selective hydride generation with ICP-MS after microwave-assisted extraction with the main focus on the samples of marine origin [31]. The method of selective hydride generation enables to measure toxic inorganic arsenic content without a prior step of species separation by chromatography and is a feasible option for high-throughput and large-scale screening of inorganic arsenic content in relevant seafood samples. In this work, we also employed a reference method based on liquid chromatography, hydride generation and AFS with the FIGS atomizer, which enabled to provide new data on inorganic arsenic and other As species in new certified reference materials released by National Research Council Canada. Despite a great co-operation with foreign partners (University of Aberdeen, UK and Matís, Iceland), who participated in a comparative study, this research was managed and organized by our lab.

A very practical outcome was also achieved in the field of speciation analysis of arsenic in baby food [17]. A setup of a hydride generator with the CT and AAS was modified to directly introduce a sample slurry of baby food. Several procedures for sample preparation were tested but the best results were obtained with  $3 \text{ mol L}^{-1}$  HCl under heating and ultrasound assisted agitation. Limits of detection for three As species are superior to those typically reported for standard methodology HPLC-ICPMS, although the investment and running costs are substantially lower. This research was conducted by a Ph.D. student from Brazil within his scientific international visit at our lab but the research was managed by our team. Some comparative measurements were carried out at partner organization as well.

We developed a postcolumn hydride generator designed for determination of toxicologically relevant arsenic species. We have achieved equal generation efficiency of arsanes from arsenite, arsenate, methylarsonate and dimethylarsinate, which has not been reported in literature yet. Two benefits thus emerge: there is just one standard required (e.g. dimethylarsinate) to quantify all four species and time for calibration is substantially shortened because dimethylarsinate typically elutes from the chromatography column at short retention times [7]. This research was exclusively done by our team.

We also investigated in detail a serious molecular rearrangement (demethylation) of methylarsonate and dimethylarsinate during hydride generation from HCl which is the most common generation medium [11]. This paper refutes the general assumption that only corresponding arsanes are volatile products from methylated As species. This behavior can

jeopardize accuracy of As speciation analysis based on generation of substituted hydrides which is occasionally used for toxicological studies. We identified the hydrolytic products of NaBH<sub>4</sub> responsible for demethylation. This research was exclusively done by our team.

In a following paper [20], we tried to make use of this behavior, namely, that under certain conditions of HG the bond between As and C can be broken. We investigated HG activity of complex As species, i.e. arsenosugars, with a focus on reaction mechanism. The knowledge of the mechanism of HG from complex organo-arsenic species is important because HG is a common derivatization step in arsenic speciation analysis scenarios. The highest yield of volatile hydrides from arsenosugars was obtained from sulfuric acid medium. With the knowledge of the mechanism we have also improved the design of the generator to enhance the generation efficiency. This research was managed by our team. Prof. Mikšík from the Institute of Physiology of the CAS participated in identification of arsenosugar species in the seaweed extracts as the instrumentation of ESI-MS is not available at TEA laboratory.

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**Please note that the references are listed in the same order (according to the year of publication) as in the attached report with bibliometric evaluation.**

## Research activity and characterisation of the main scientific results

### Current Topics Electromigration methods for pre-concentration and separation of bioparticles

In the history of FPS department, there has been a wealth of studies of separation of bioparticles by electromigration methods as regards both the types of bioparticles and the methods employed. Within 2015–2019, several studies on this topic have appeared, including determination of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteria in blood by capillary zone electrophoresis (M. Horká et al.: *Anal. Chim. Acta* 2015, 868, 67; <http://dx.doi.org/10.1016/j.aca.2015.02.001> ) or capillary electrophoresis with preparative isoelectric focusing preconcentration for sensitive determination of amphotericin B in human blood serum (J. Šalplachta et al.: *Anal. Chim. Acta* 2019, 1053, 162; <http://doi.org/10.1016/j.aca.2018.12.010> ). Some of the studies within this topic have also involved characterization of the separated species with off-line matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, including utilization of red nonionogenic tenside labeling, isoelectric focusing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the identification of uropathogens in the presence of a high level of albumin (M. Horká et al.: *ACS Infect. Dis.* 2019, 5, 1348; <http://dx.doi.org/10.1021/acsinfecdis.9b00045> ), electrophoretic techniques for purification, separation and detection of Kayvirus with subsequent control by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and microbiological methods (M. Horká et al.: *J. Chromatogr. A* 2018, 1570, 155; <http://dx.doi.org/10.1016/j.chroma.2018.07.078> ), identification of bacterial uropathogens by preparative isoelectric focusing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (J. Šalplachta et al.: *J. Chromatogr. A* 2018, 1532, 232; <http://dx.doi.org/10.1016/j.chroma.2017.11.072> ), or preparative isoelectric focusing of microorganisms in cellulose-based separation medium and subsequent analysis by CIEF and MALDI-TOF MS (M. Horká et al.: *Anal. Chim. Acta* 2017, 990, 185; <http://dx.doi.org/10.1016/j.aca.2017.08.046> ). The latter three articles have not been included in the 20 papers serving as a basis of the FPS department evaluation. All electromigration separations and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry characterizations have been carried out at the FPS department.

### Isoelectric points of pI markers from capillary isoelectric focusing

Isoelectric focusing is a very well established and possibly the most traditional continuously developed topic in the FPS department, and it has been elaborated in both analytical and preparative scales. Also, the formats of the separation space have been different, including capillaries, gels, cellulose-based separation media in channels of various profiles, and divergent flow arrangement in nonwoven fabrics. Within 2015–2019, possibly the most important contribution dealt with characterization of isoelectric points of low-molecular-mass nitrophenol-based compounds suitable for the effective tracking of pH gradient in isoelectric focusing (F. Duša et al.: *Anal. Chim. Acta* 2019, 1076, 144; <http://dx.doi.org/10.1016/j.aca.2019.05.010> ). Other applications of isoelectric focusing in the time period under evaluation included, e.g., a study of capillary isoelectric focusing as a useful tool for detection and quantification of lactic acid bacteria in milk (F. Růžička et al.: *Food Anal. Methods* 2016, 9, 3251; <http://dx.doi.org/10.1007/s12161-016-0522-6> ), a study of preparative isoelectric focusing in a cellulose-based separation medium (J. Šalplachta et al.: *J. Separ. Sci.* 2017, 40, 2498; <http://dx.doi.org/10.1002/jssc.201700036> ), or a contribution on preparative and capillary

isoelectric focusing for detection and identification of *Aspergillus conidia* in complex sample matrices (M. Horká et al.: *J. Separ. Sci.* 2019, 41, 4203; <http://dx.doi.org/10.1002/jssc.201800547> ). The latter three articles have not been included in the 20 papers serving as a basis of the FPS department evaluation. All isoelectric focusing experiments have been carried out at the FPS department.

### **Synthesis and testing of monolithic silica capillary columns**

This topic has been well established in the FPS department portfolio, with our early pertinent contributions dating back to 2010 and 2012. Within 2015–2019, there have been several follow-up contributions. The first of these involved phosphatidylcholine covalently linked to a methacrylate-based monolith through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide coupling as a biomimetic stationary phase for capillary liquid chromatography (D. Moravcová et al.: *J. Chromatogr. A* 2015, 1402, 27; <http://dx.doi.org/10.1016/j.chroma.2015.05.004> ). In this work, characterization of bulk materials and columns by FTIR spectroscopy has been performed by cooperating institutions while the synthesis, functionalization and chromatographic testing of columns was done at FPS. Another contribution involved hybrid silica–organic monolithic columns, namely, bridged polysilsesquioxane-based wide-bore monolithic capillary columns for hydrophilic interaction chromatography (J. Šesták et al.: *J. Chromatogr. A* 2017, 1479, 204; <http://dx.doi.org/10.1016/j.chroma.2016.11.062> ). This contribution was accomplished in close cooperation with the BAI department of the Institute, with the amount of work distributed equally between the BAI and FPS departments. Yet another contribution involved immobilization of a phosphonium ionic liquid on a silica monolith for hydrophilic interaction chromatography (D. Moravcová et al.: *J. Chromatogr. A* 2018, 1552, 53; <http://dx.doi.org/10.1016/j.chroma.2018.04.012> ). This study resulted from a close cooperation between the FPS department and the research group of Dr. Susanne K. Wiedmer at the University of Helsinki, Finland. The contribution of the colleagues from the University of Helsinki involved the synthesis and NMR characterization of the trioctyl(3/4-vinylbenzyl)phosphonium chloride ionic liquid whereas the column preparation, functionalization with the ionic liquid and chromatographic characterization were done at the FPS department.

### **Instrument developments of capillary liquid chromatography**

Development of miniaturized instruments for liquid chromatography has been a very traditional topic at the FPS department. Within 2015–2019, the first paper on this topic has been a well-received review of instrument platforms for nano liquid chromatography (J. Šesták et al.: *J. Chromatogr. A* 2015, 1421, 2; <http://dx.doi.org/10.1016/j.chroma.2015.07.090> ). Another contribution from this field has been concerned with nanolitre-scale cell based on L-shaped silica capillary and optical fibre for absorption photometric detection in capillary liquid chromatography (J. Šesták et al.: *Anal. Chim. Acta* 2019, 1073, 99; <http://dx.doi.org/10.1016/j.aca.2019.04.072> ). These results have been achieved at the FPS department without external collaboration.

### **Pressurized hot water extraction of plant materials**

The topic of pressurized hot water extraction of plant materials has been started in the former Department of Separations with Compressed Fluids. Within 2015–2019, two papers from the FPS department have appeared on this topic, with the first paper being focused on pressurized hot water extraction of high-value proteins from *Sambucus nigra* L. (black elder) branches (J. Šalplachta, B. Hohnová: *Ind. Crop. Prod.* 2017, 108, 312;

<http://dx.doi.org/10.1016/j.indcrop.2017.06.053> ). Another paper was concerned with a more conventional topic of pressurized hot water extraction followed by high-performance liquid chromatography for determination of polyphenols in *Sambucus nigra* L. branches in dependence on vegetative period of the plant (B. Hohnová et al.: *J. Food Nutrition Res.* 2017, 56, 299). This latter paper has not been included in the 20 papers serving as a basis of the FPS department evaluation. All pressurized hot water extraction investigations have been carried out at the FPS department without external cooperation.

### **Novel formats of fused silica capillaries from etching with supercritical water**

At the temperatures and pressures near its critical point (647 K, 22.06 MPa), water is known to dissolve silicon dioxide in both crystalline (quartz) and amorphous (fused silica) forms. Therefore, we started to use in-situ generated near- and supercritical water to modify the inner surfaces and diameters of fused silica capillaries for applications in analytical separations. The first paper on this topic has appeared in 2013, and, to our knowledge, the FPS department is still the only workplace where this ability of near- and supercritical water has been used for the purpose. Within 2015–2019, several new formats of supercritical water-treated fused silica capillaries were developed and applied in separations of microorganisms and bioparticles by electromigration methods. These efforts included online concentration of bacteria from tens of microliter sample volumes in roughened fused silica capillary with subsequent analysis by capillary electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (M. Horká et al.: *ACS Infect. Dis.* 2020, 6, 355; <http://dx.doi.org/10.1021/acsinfecdis.9b00200> ), or preconcentration and separation of bacteria by volume coupling electrophoresis on supercritical water-etched fused silica capillary with two segments of different internal diameters and inner surface roughnesses (M. Horká et al.: *Anal. Bioanal. Chem.* 2018, 410, 167; <http://dx.doi.org/10.1007/s00216-017-0706-y> ). Another unique methodology involved the formation of inner surface roughness gradient within fused silica capillary (M. Horká et al.: *J. Separ. Sci.* 2016, 39, 3827; <http://dx.doi.org/10.1002/jssc.201600660> ). The latter two articles have not been included in the 20 papers serving as a basis of the FPS department evaluation. All experiments with supercritical water as well as all electromigration separations of bioparticles have been carried out at the FPS department without external collaboration.

### **Interactions of amphiphilic substances with biomembranes**

This research topic has been brought to the FPS department by Dr. Filip Duša on his return from a postdoctoral stay in the group of Dr. Susanne K. Wiedmer at the University of Helsinki. Therefore, there is a strong coauthorship by the colleagues from Finland on the papers on this topic. The first of the selected studies on this topic concerned nanoplasmonic sensing and capillary electrophoresis for fast screening of interactions between phosphatidylcholine biomembranes and surfactants (F. Duša et al.: *Langmuir* 2018, 34, 5889; <http://dx.doi.org/10.1021/acs.langmuir.8b01074> ). Another study dealt with determination of the main phase transition temperature of phospholipids by nanoplasmonic sensing (W. Chen et al.: *Sci. Rep.* 2018 (8), 14815; <http://dx.doi.org/10.1038/s41598-018-33107-5> ). The third selected study was titled Immobilization of natural lipid biomembranes and their interactions with choline carboxylates. A nanoplasmonic sensing study (F. Duša et al.: *Biochim. Biophys. Acta – Biomembranes* 2020, 1862, 183115; <http://dx.doi.org/10.1016/j.bbamem.2019.183115> ). Other contributions from this field that have not been included in the 20 papers serving as a basis of the FPS department evaluation focused on distribution of local anesthetics between aqueous and liposome phases (S. K. Ruokonen et al.: *J. Chromatogr. A* 2017, 1479, 194; <http://dx.doi.org/10.1016/j.chroma.2016.12.005> ) and on calcium dependent reversible aggregation of *Escherichia coli* biomimicking vesicles enables formation of supported vesicle

layers on silicon dioxide (F. Duša et al.: *Front. Mater.* 2019, 6, 23; <http://dx.doi.org/10.3389/fmats.2019.00023> ).

### **Electrokinetic injection**

Within 2015–2019, this (sub)topic has been worked on during the postdoctoral stay of Dr. Jozef Šesták with Dr. Wolfgang Thormann at the University of Bern, Switzerland. The first paper on the topic was titled Insights into head-column field-amplified sample stacking: Part I. Detailed study of electrokinetic injection of a weak base across a short water plug (J. Šesták, W. Thormann: *J. Chromatogr. A* 2017, 1502, 51; <http://dx.doi.org/10.1016/j.chroma.2017.04.041> ), and the subsequent contribution has been titled Insights into head-column field-amplified sample stacking: Part II. Study of the behavior of the electrophoretic system after electrokinetic injection of cationic compounds across a short water plug (J.

Šesták, W. Thormann: *J. Chromatogr. A* 2017, 1512, 124; <http://dx.doi.org/10.1016/j.chroma.2017.06.073> ). The third contribution, dealing with fundamental aspects of field-amplified electrokinetic injection of cations for enantioselective capillary electrophoresis with sulfated cyclodextrins as selectors (J. Šesták et al.: *J. Chromatogr. A* 2018, 1558, 85; <http://dx.doi.org/10.1016/j.chroma.2018.05.022> ), has not been included in the 20 papers serving as a basis of the FPS department evaluation.

### **Heart proteomics**

During her research stay in the group of Dr. Jennifer Van Eyk at The Cedars-Sinai Medical Center (Los Angeles, CA, USA), Dr. Miroslava Šťastná has taken part in several studies of heart proteomics. One of these articles was a review titled Exploring ribosome composition and newly synthesized proteins through proteomics and potential biomedical applications (M. Šťastná et al.: *Expert Rev. Proteomics* 2017, 14, 529; <http://dx.doi.org/10.1080/14789450.2017.1333424> ). Another review was titled Posttranslational modifications of lysine and evolving role in heart pathologies - Recent developments (M. Šťastná, J. V. Van Eyk: *Proteomics* 2015, 15, 1164; <http://dx.doi.org/10.1002/pmic.201400312> ). The third contribution concerned dynamic proteomic and miRNA analysis of polysomes from isolated mouse heart after Langendorff perfusion (M. Šťastná et al.: *J. Vis. Exp.* 2018, 138, e58079; <http://dx.doi.org/10.3791/58079> ).

### **Thermodynamic measurements and models of systems with supercritical fluids**

In the evaluated period 2015–2019, the role of this topic in the FPS department portfolio has been significantly reduced for two major reasons. One reason was the lack of dedicated young generation to take up this relatively difficult topic. The other reason was that, given the methodical background and in-house designed instruments available in FPS, the accessible classes of prospective analytes (solutes) have largely been exploited before 2015. This applies to both the solubilities of nonpolar organic solids in pressurized hot water and the partition coefficients of low-volatility organics between ionic liquids and supercritical carbon dioxide. Therefore, only two theoretical papers on thermodynamic subjects have been published. One of these is a correlation of limiting partition coefficients of organics between water and CO<sub>2</sub> employing only pure component properties (M. Roth: *Environ. Sci. Technol.* 2016, 50, 12857; <http://dx.doi.org/10.1021/acs.est.6b03210> ). The other paper emphasizes the important role of cohesive energy density / internal pressure ratio as a basis of important differences in solvent tunability between water and other near- and supercritical fluids in common use (M. Roth: *Molecules* 2019, 24, 961; <http://dx.doi.org/10.3390/molecules24050961> ). This latter paper has not been included in the 20 papers serving as a basis of the FPS department evaluation.

## Research activity and characterisation of the main scientific results

Research activity of Department of Environmental Analytical Chemistry in the evaluated period resulted in 46 publications in peer-reviewed journals with impact factor and 3 publications in journals without impact factor. Other results of ENV team included 2 chapters in books, 45 contributions in conference proceedings, 1 patent and 4 applied results.

Our research was largely funded by grants from the Grant Agency of the Czech Republic (GA CR). During the evaluated period the members of ENV were principal investigators of two projects:

- “Essential oils as tool for saving and increasing of culture heritage on paper“, Ministry of Culture of the Czech Republic, NAKI 63, DF11P01OVV28, 2011-2015
- „New approach for analysis of water-soluble organic compounds in fine fraction of atmospheric aerosol“, GA CR, P503/14/25558S, 2014-2016

and the co-investigators of three other projects:

- „Centre for studies on toxicity of nanoparticles“, Centre of Excellence, GA CR, P503/12/G147, 2012-2018
- „Mechanisms of toxicity of biofuel particulate emissions“, GA CR, P503/13/1438S, 2013-2015
- „Photocatalytic technology for purifying air in the interior and exterior of buildings: Overall environmental impact“, GA CR, P104/19/12109S, 2019-2021

Moreover, our research was partly funded by two one-year projects in the frame of Regional co-operation of districts and institutes of the Czech Academy of Sciences:

- „Comparison of aerosol particle emissions from different types of small combustion devices“, R200311701, 2017
- „Combustion of solid fuels in old and modern boilers used in the Moravian-Silesian region for domestic heating: characterization of emissions and calculation of risk of lung cancer from emission factors of carcinogenic polycyclic aromatic hydrocarbons“, R200311921, 2019

Research activity of the Department of Environmental Analytical Chemistry was mainly focused on the study of chemical composition and health effects of airborne fine (PM<sub>2.5</sub>; i.e., particulate matter (PM) with a particle diameter of less than 2.5 μm), and submicron (PM<sub>1</sub>; PM with a particle diameter of less than 1 μm) particles including ultrafine particles (UFP) and nanoparticles (NPs), i.e., PM with a particle diameter of less than 100 nm. Fine and especially submicron particles have adverse effects on human health because these small-sized particles are able to penetrate deep inside the alveolar regions of human lungs and UFP/NPs are even able to translocate from lungs to other organs and tissues. Effect of NPs on human health is subject of interest of many in vitro studies, but studies with realistic exposure routes and doses assessing the effects of NP in model organisms in vivo are still rare. Respiratory tract during inhalation is the main route by which NPs enter the human body. The inhalation of NPs hence represents the most dangerous threat for industrial workers producing or using nanomaterials. To mimic this form of exposure, we modelled long-term inhalation of NPs in mice.

In the frame of the project „Centre for studies on toxicity of nanoparticles“ (GA CR, P503/12/G147) we focused on a long-term inhalation experiments on laboratory mice, during which the mice were exposed to metal oxide NPs, and on toxicological studies on airborne ultrafine- and nano-particles and organic compounds bound to them. In in vivo experiments we studied transport of inhaled NPs composed of metal oxide (MnO, Mn<sub>2</sub>O<sub>3</sub>, CdO, PbO, TiO<sub>2</sub>, ZnO and CuO) and their allocations in mice organs. The studied NPs were selected as products of technological processes and because of the presence of relevant metals in ambient aerosol. Adult female mice were exposed to engineered metal oxide NPs (size range of 7 – 40 nm, number concentration of NPs up to ca 2×10<sup>6</sup> particles/cm<sup>3</sup>) by inhalation in a whole body inhalation chamber (composed of four exposure cages) continuously (for 24 h/day, 7

days/week) under controlled experimental conditions (illumination, temperature, relative humidity) for long-term period (6 - 11 weeks). Nanoparticles were synthesized in situ via aerosol route in a hot wall tube flow reactor using granules or metal wire (CdO, PbO, ZnO) or organic precursor (MnO.Mn<sub>2</sub>O<sub>3</sub>, TiO<sub>2</sub>, CuO). At the end of exposure, mice were sacrificed and mice organs were collected for chemical, biochemical, histological, histochemical, immunological, genotoxic and electron microscopic analyses. Five organs (lung, liver, kidney, spleen and brain) were collected and analysed from both mice exposed to NPs and the control group of mice without exposure to nanoparticles. During the evaluated period, we published 6 papers with the results of inhalation experiments on mice. Comprehensive approach to inhalation exposures of mice to metal oxide NPs and subsequent analysis of mice organs required the cooperation of experts with different specializations. Hence, toxicological analyses of mice organs were performed by collaborating experts from Masaryk University Brno, Institute of Animal Physiology and Genetics of the CAS Brno, Institute of Experimental Medicine of the CAS Prague, University of Veterinary and Pharmaceutical Sciences Brno and Veterinary Research Institute Brno. Therefore, 6 mentioned publications are joint works of ENV and above mentioned cooperating institutions. ENV in these exposure studies was responsible for the preparation of defined engineered NPs, characterisation of generated NPs, performing of inhalation in vivo experiments in a whole-body inhalation chamber with controlled exposure to NPs and analysis of metals of inhaled NPs in target mice organs using atomic absorption spectrometry.

Obtained results indicate that the inhaled both cadmium oxide (CdO) and lead oxide (PbO) nanoparticles not only accumulated in lungs (primary organ) but they were also transported by blood to other (i.e., secondary) organs (i.e., liver, kidney, spleen and brain) causing serious damage at tissue as well as cellular level. CdO (Dumková et al., *Int. J. Mol. Sci.* 2016, 17, 874; Lebedová et al., *Environ. Sci. Pollut. Res.* 2016, 23, 24047-24060) and PbO (Dumková et al., *Part. Fibre Toxicol.* 2017, 14, 55; Lebedová et al., *Nanotoxicology* 2018, 12, 290-304 \*) NPs were found in all studied organs. Accumulation of Cd and Pb in the lung, liver and kidney occurred already after 24 h and in the brain and spleen after 72 h of exposure. Pb and Cd concentration in all tissues as well as intensity of adverse effects gradually increased with dose and exposure time, indicating increasing potential health risk with longer exposures.

Zinc and copper are present in active sites of enzymes and as essential elements play an important role in cellular metabolism, but ZnO or CdO NPs, that enter the organism, have numerous toxic health effects. Paper dealing with the effect of ZnO NPs inhalation was focused only on lungs. The zinc content in the lungs of mice exposed to ZnO NPs for 3 days was surprisingly higher than those in mice exposed for 3 months (Rossner et al., *Toxicol. Sci.* 2019, 168, 190-200). A stark difference in the response of the organism to acute (3 days) and subchronic (3 months) exposure to \* 12 publications selected for a detailed evaluation during the first phase is indicated in bold ZnO NP was found. Long-term exposure was associated with the induction of protective mechanisms in the lungs, whereas short-term inhalation failed to activate such processes. As in the case of ZnO NPs, the publication studying the influence of CuO NPs also focused only on a single organ, namely spleen (Holář et al., *Nanotoxicology* 2019, 13, 952-963). The copper content in the spleen of mice exposed to NPs did not change significantly during a long-term (3 months) inhalation of CuO NPs and, furthermore, the copper content in the spleen of exposed and control mice was similar. However, three months inhalation of CuO NPs differentially affected the cells of the innate and adaptive immune system in the spleen.

Other results obtained during the inhalation of other metal oxide NPs are in preparation for publication.

We also participated in the study of the effect of the nanoparticles on plants. The environmental fate of airborne nanoparticles and their toxicity to plants is not yet fully understood. In cooperation with Global Change Research Institute of the CAS Brno we exposed barley plants (Večeřová et al., *Environ. Poll.* 2016, 218, 207-218) or spruce seedlings

(Večeřová et al., *Environ. Poll.* 2019, 253, 646-654) to CdO nanoparticles for 3 weeks. In addition to NPs, spruce seedlings were also exposed to other stress conditions (i.e., higher temperature and vapour pressure deficit, VPD). Results showed direct absorption of CdO NPs into leaves or needles from the air via penetration through stomata, however, higher temperature and VPD reduced NPs penetration into needles due to stomatal closure. CdO NPs influenced physiological and metabolic processes in studied plants, especially total content of primary metabolites. This impact strengthened with increasing time of exposure. ENV in exposure studies of plants prepared defined engineered NPs, characterised generated NPs, performed plants exposure in an inhalation chamber and analysed Cd in leaves, needles and roots using atomic absorption spectrometry.

Other two papers dealt with the evaluation of the health effects on workers exposed to nanoparticles (Lischková et al., *Monatsh. Chem.* 2019, 150, 511-523; Hubacek et al., *Ind. Health* 2019, 57, 741-744).

Study of atmospheric aerosols in ambient air was concentrated on the characterization of aerosols and chemical composition of aerosols in the size fraction PM<sub>1</sub> and PM<sub>2.5</sub> in polluted regions. Chemical analyses were largely aimed to the determination of toxic compounds (i.e., polycyclic aromatic hydrocarbons; PAHs) and organic molecular markers of significant anthropogenic sources of aerosol. Organic molecular markers are organic compounds that allow the specific or selective identification of emission sources, such as biomass burning (BB), coal combustion (CC), traffic, cooking, etc. Analysis of organic markers in aerosols generally comprised the following compounds: monosaccharide anhydrides (MAs), resin acids, hopanes, steranes, n-alkanes, acyclic isoprenoids, methoxyphenols and saccharides. Some compounds (MAs, hopanes, PAHs, ...) were used to calculate diagnostic ratios. We identified the emission sources of aerosols and estimated health risks of aerosols. The studies contributed to discussion about the importance of local home heating to air quality in highly urbanised areas in the Czech republic or in Europe in general. In the frame of the grant project „Centre for studies on toxicity of nanoparticles“ (GA CR, P503/12/G147) we studied atmospheric aerosols at different types of locations ranging from urban (i.e., Ostrava, Mladá Boleslav, Čelákovice), rural (KladnoŠvermov) to background (Košetice) site. The results from Ostrava and Mladá Boleslav were published, while results from other locations are still in preparation for publication. Results of the analysis of aerosols were published in five papers.

In paper by Mikuška et al. (*Atmos. Environ.* 2015, 105, 97-108) we characterized the organic compounds in PM<sub>2.5</sub> aerosols collected in winter 2012 in OstravaRadvanice. Ostrava in the Moravian-Silesian region (Czech Republic) is a European air pollution hot spot for airborne particulate matter (PM), PAHs, and UFPs, that is why, the analysis of air pollution in Ostrava is subject of interest of many studies. The campaign was carried out under two different meteorological scenarios. During a smog episode, high concentrations of PM<sub>2.5</sub> aerosols and bound organic compounds were found, while in period after the smog episode much lower concentrations of aerosols were observed. The high concentration of PM<sub>2.5</sub> aerosols during the smog episode was attributed to a combination of reduced vertical dispersion due to temperature inversion and the emissions originated from local and regional combustion of wood and coal in the frame of residential heating. Furthermore, back-trajectory analysis for Ostrava sampling site indicated that transboundary transport of polluted air from the Silesian Voivodeship in Poland might contribute to high aerosol concentration in the studied Ostrava site during the smog episode. The combustion of coniferous wood and coal in residential heating and traffic were identified as the biggest emission sources of organic compounds associated with the PM<sub>2.5</sub> aerosols collected during the winter campaign in Ostrava-Radvanice. The industrial production of coke and iron was another important contributor to the concentrations of BaP and other carcinogenic PAHs. Different emission sources of aerosols were proved during smog and post-smog period, which indicates that the level of air pollution in Ostrava was determined to a great deal by the overall meteorological situation at sampling site.

Another publication (Křůmal et al., *Atmos. Pollut. Res.* 2017, 8, 930-939) characterized the content of organic compounds in PM<sub>1</sub> aerosols collected in winter 2013 in Mladá Boleslav. Mladá Boleslav represents relatively small town with large automobile factory near town centre. Combustion of solid fuels (coal and wood) for household heating and the heating plant located inside the automobile factory was identified as the dominant emission sources of aerosols sampled in the studied locality during the campaign. The composition of aerosols was affected by local emissions and also by regional transport of polluted air from small villages nearby Mladá Boleslav. The following three publications were the result of collaboration ENV with colleagues from other institutions who analysed aerosols simultaneously in joint campaigns in Mladá Boleslav (2013) and again in Ostrava (2014). Our contribution to these publications included the concentrations of selected molecular markers (levoglucosan for BB, homohopanes for CC and traffic) in PM<sub>1</sub> aerosol, enabling unambiguous identification of emission sources of aerosols in studied areas. The study from the campaign in Mladá Boleslav in winter 2013 (Hovorka et al., *Atmos. Environ.* 2015, 113, 98-107) applied Positive Matrix Factorization (PMF) to elemental composition of aerosol particles and organic tracers of biomass and coal burning to identify sources of fine and coarse PM in a residential district of Mladá Boleslav. BB, CC, traffic and industry were identified as sources of the fine PM while combined biomass and coal combustion, road dust, and abrasion of car brakes and tyres were revealed sources of the coarse PM. A paper dealing with the results from campaign in Ostrava in winter 2014 (Leoni et al., *Environ. Pollut.* 2018, 234, 145154) identified the major sources of fine atmospheric particles in Ostrava down to nanoparticles using PMF. The major sources of fine PM during the winter were secondary inorganic aerosols (SIA), CC and BB. Further study from campaign in Ostrava in winter 2014 (Kozáková et al., *Environ. Sci. Pollut. Res.*, 2019, 26, 16751692) compared an air quality between an urban site (Ostrava - Radvanice) and a suburban site (Plesná) to distinguish the sources of the fine and coarse PM and determine their local and/or regional sources using the PMF.

Two other publications dealt with aerosols in Brno. The first one (Křůmal et al., *Atmos. Pollut. Res.* 2015, 6, 917-927) compared the composition of urban PM<sub>1</sub> aerosols in Brno (large city) and Šlapanice (small town) in winter and summer seasons of 2009 and 2010 with focus on MAs, monocarboxylic acids and organic (OC) and elemental (EC) carbon. The contribution of anthropogenic emissions and biogenic emissions to contents of OC and EC in PM<sub>1</sub> aerosols was evaluated too.

The second paper (Mikuška et al., *Atmos. Pollut. Res.* 2017, 8, 576-586) was the outcome of the thesis of N. Kubátková, student at Brno University of Technology. Within the study, we optimized the extraction procedure and derivatization process to be applicable simultaneously for all the studied particulate organic compounds. Developed method was applied to the analysis of PM<sub>2.5</sub> aerosols collected in Brno in different seasons in 2010 and 2011. Wood burning products predominated in winter and autumn, while the compounds produced by biological sources or via soil resuspension had the highest concentrations during spring and summer.

Next to ambient aerosols, within a project „Mechanisms of toxicity of biofuel particulate emissions“ (GA CR P503/13/1438S, 2013-2015) we compared the toxicity and composition of major particulate components in the emissions from vehicle engines powered by classic fuels and biofuels under various experimental conditions (test cycles and engine technology). Results of the project were published in three papers. Paper by Vojtíšek-Lom et al. (*Fuel* 2017, 197, 407-421) investigated the performance of a mix of two emerging biofuels, butanol and hydrotreated vegetable oil (HVO), in an Iveco Tector diesel engine. We found that, compared to diesel fuel, blends of 30% of either n-butanol or isobutanol into HVO resulted in a 70-80% decrease in the emissions of EC and carcinogenic PAHs and a moderate decrease in the emissions of nitrogen oxides, while the emissions of total particle mass have decreased by more than one half. Moreover, all studied biofuels exhibited, relative to diesel fuel, a decrease in the concentration of PAHs and n-alkanes in emissions, while the concentrations of hopanes and steranes originated from lubricating oil were comparable across all fuels. In another paper

(Coufalík et al., Environ. Sci. Pollut. Res. 2019, 26, 29012-29019) we investigated the influence of bio-components in conventional fuels on metal contents in emissions from diesel and spark-ignition engines. We determined the total contents of Ba, Ce, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn in emissions, fuels, and lubricating oils. We found that metal contents in emissions were not influenced only by metal concentrations in fuels, but also by metals originating from engine wear and lubricating oils and by engine load and the resulting mass of particulate matter. The use of biodiesel (especially methylesters of rapeseed oil) reduces the total mass of particulate and metal emissions from diesel engines. Both previous papers were the results of ENV cooperation with other partners of the grant project. ENV contribution to these publications included engine emissions sampling and the determination of the content of organic compounds (Vojtíšek et al., 2017) or metals (Coufalík et al., 2019). In addition, we also took part in writing the paper (Vojtíšek et al., 2017) while the second paper (Coufalík et al., 2019) was written by ENV team. Last study from this project (Coufalík et al., Atmos. Environ. 2016, 140, 469-475) determined bioaccessible fraction of metals in atmospheric aerosol according to the extraction yields of six simulated lung fluids (SLFs). The chemical composition and surface tension of the SLFs were found as the main parameters influencing extraction yields. The bioaccessible portions of metals determined by extraction in Gamble's solution and the newly proposed "Simulated Alveoli Fluid" (SAF) were, for most of the studied metals, substantially lower than those determined by extraction in water or artificial lysosomal fluid. We concluded that the bioaccessibility of metals in atmospheric aerosol should be assessed by SLFs with low surface tension (i.e., SLFs with dipalmitoyl phosphatidyl choline), which simulated better the behaviour and composition of native lung surfactant, despite the lower extraction efficiency. The bioaccessibility of metals in aerosol assessed by means of the extraction in water or artificial lysosomal fluid can be overestimated.

During the co-operation of Department with VSB-Technical University of Ostrava (VSB-TUO), done in the frame of Regional cooperation of districts and institutes of the CAS, we compared the emission factors of gaseous and particulate organic compounds from the combustion of different fuels (coal, wood, pellets, briquettes) in two old-type and two modern-type boilers during the reduced output operation used for household heating (Křůmal et al., Chemosphere 2019, 229, 51-59). Emission factors of organic markers depended on the fuel and boiler used. The emission factors of CO and particulate organic compounds including PAHs and organic markers were much higher in old-type boilers, especially with the combustion of brown and hard coal. The modern-type (especially automatic) boilers were the most environmentally friendly. Characteristic ratios of selected organic compounds/markers applied for source identification were calculated and compared with literature. ENV team was responsible in this study for the sampling of relevant emissions, analysis of organic compounds in emissions of boilers, and we wrote the paper.

Analyses of aerosols mentioned in previous publications were based on sampling aerosols on filter and subsequent analysis of filters in a laboratory filter using GC-MS or ICP-MS. Sampling of aerosols on filters allows the analysis of trace concentrations of aerosol components and the simultaneous determination of several different types of compounds from each filter. However, this approach is time consuming and may subject to various sampling artifacts. In addition, the time resolution of filter sampling technique is mostly low, whereas the chemical composition of aerosols varies significantly with time and place. To detect fast changes in aerosol composition, there is a need for a continuous sampling technique that could be combined with a near realtime method for the detection of particulate components.

To accomplish this requirement, we have recently developed a continuous aerosol collector, an Aerosol Counterflow Two-Jets Unit (ACTJU), based on a Venturi scrubber. The ACTJU captured aerosol particles by water droplets within the collision of two jets of fine water droplets formed by spraying of deionized water at room temperature by high-velocity air in two opposed Venturi nozzles (Mikuška and Večeřa, Anal. Chem. 2005, 77, 5534-5541). The effluent, the deionized water with collected aerosols, is continuously withdrawn from the ACTJU for on-line

analysis of watersoluble components of collected aerosols. The ACTJU enabled quantitative collection of particles larger than 300 nm in diameter, while particles smaller than 300 nm were collected with lesser efficiency. To collect quantitatively also these small-sized particles, we developed a new water-based Condensation Growth Unit (CGU) in the frame of the project „New approach for analysis of water-soluble organic compounds in fine fraction of atmospheric aerosol“ (GA CR, P503/14/25558S, 2014-2016). Condensation of water vapour in the CGU enlarges nanometer sized particles to larger sizes in the supermicrometer range and the formed droplets are then collected into water in the ACTJU collector. The aerosol sampler, formed by placing the CGU upstream of the ACTJU collector, accomplished the quantitative collection of fine and ultrafine aerosol particles down to a few nanometers in diameter (Mikuška et al., *Anal. Chim. Acta* 2018, 1020, 123-133). The continuous collection of aerosols into the water in the CGU-ACTJU sampler, coupled with on-line detection devices, provided the timeresolved in-situ automated measurement of changes in the concentration of watersoluble aerosol components. Coupling of the CGU-ACTJU sampler with flow method and direct chemiluminescent detection of particulate nitrite or nitrate enabled time resolution of up to 1 s. On-line ion chromatography determination due to the preconcentration step allowed the measurement of anions (fluoride, chloride, nitrite, nitrate, sulphate and phosphate) and dicarboxylic acids (Čapka et al., *Atmos. Environ.* 2020, 222, 117178) in atmospheric aerosol with a time resolution of 1 h. Alternatively, when the aerosol components cannot be analysed on-line, the ACTJU concentrate was collected using a new developed fraction collector (Čapka and Mikuška, *Chem. Listy* 2018, 112, 196-200) according to a defined time schedule into glass vials for later off-line analysis in the laboratory. In this way, we determined levoglucosan and dehydroabietic acid in atmospheric aerosol with a time resolution of 2 h involving their off-line preconcentration and determination by liquid chromatography – mass spectrometry in laboratory (Coufalík et al., *Chemosphere* 2018, 210, 279-286). Joint publication formed as a result of our collaboration with Dr. Changjin Hu (Laboratory of Atmospheric Physico-Chemistry, Anhui Institute of Optics and Fine Mechanics, Chinese Academy of Sciences, Hefei) dealt with the study of iodine oxide particles (IOPs) formed from O<sub>3</sub>-initiated photooxidation of diiodomethane (Wei et al., *RSC Advances* 2017, 7, 56779-56787). The ENV contribution consisted in the application of annular diffusion denuder to remove the residual O<sub>3</sub> and CH<sub>2</sub>I<sub>2</sub> at the exit of the flow reactor to end the gaseous reaction of CH<sub>2</sub>I<sub>2</sub> with ozone.

Other part of our activity was devoted to the determination of trace metals in environmental samples. The toxicity and bioaccumulation of heavy metals, especially the mercury and its species, represent a significant health risk to the population.

Diffusive gradient in thin films (DGT) technique was applied to assess cadmium and copper uptake by radish (Dočekalová et al., *Talanta* 2015, 134, 153-157) or to characterize the content of traffic-related metals in the urban PM accumulated on textile fiber filters in air-conditioning system (Dufka and Dočekal, *J. Anal. Methods Chem.* 2018, 2018, 9698710). The DGT technique was shown as a useful tool for estimation of plant uptake or for measurement of bioaccessibility of the water-soluble, mobile fraction of transition metals in soils or PM.

P. Coufalík in the course of cooperation with Masaryk University Brno dealt in detail with the fate and speciation of mercury in various samples (mainly from James Ross Island, Antarctica) with respect to an origin of the contamination (Coufalík et al., *Antarct. Sci.* 2015, 27, 280-289; Coufalík et al., *Bull. Environ. Contam. Toxicol.* 2018, 101, 549-553; Zvěřina et al., *Environ. Monit. Assess.* 2018, 190, 13). Moreover, the biogeochemical cycle of mercury in the environment and mercury biomethylation in the ecosystem were studied (Coufalík et al., *Microchem. J.* 2018, 140, 8-13; Coufalík et al., *Pol. Polar Res.* 2016, 37, 477-491; Zvěřina et al., *Environ. Sci. Pollut. Res.* 2017, 24, 1424-1431). Mercury and its species were also analysed in anthropogenically contaminated soils from the Czech Republic and ability of present microorganisms to accumulate and transform Hg in soil was investigated (Száková et al., *J. Environ. Sci. Health A* 2016, 51, 364-370). In another paper, coal from the Faroe Islands was characterized for the content of minerals and trace elements including mercury

(Kuboušková et al., *Int. J. Coal Geol.* 2016, 165, 157-172). Other papers described a new analytical procedure for the direct determination of HgS in environmental samples by means of thermal desorption coupled with atomic absorption spectrometry (Coufalík et al., *Spectrochim. Acta B* 2016, 118, 1-5) or a new method for the simultaneous determination of cadmium and iron in foodstuffs (Zvěřina et al., *Food Chem.* 2019, 298, 1-5, 125084). The bioaccumulation of cadmium and zinc was studied by cultivating selected species of higher plants on contaminated soils (Vašinová Galiová et al., *Environ. Monit. Assess.* 2019, 191, 181). The contribution of P. Coufalík to these publications consisted of the collection of the Antarctic samples of lichens, algae and cyanobacteria, collection of volcanic rocks, determination of mercury and its species in all studied samples, determination of cadmium and zinc in samples of plants, and above all, in the writing of publications.

ENV team also dealt with the determination of gaseous compounds, such as ammonia and biogenic and anthropogenic volatile organic compounds (VOCs). Biogenic volatile organic compounds (BVOCs) play an important role in atmospheric chemistry, because they are involved in chemical reactions that produce tropospheric ozone and atmospheric aerosols. ENV team collaborated with Global Change Research Institute of the CAS Brno at the study of monoterpene (MT) emissions from Norway spruce, the most representative forest species in central and northern Europe. The collaboration resulted in two joint papers. The first publication by Křůmal et al. (*Talanta* 2016, 153, 260-267) compared analytical parameters of three methods during the measurement of monoterpenes. The Cylindrical Wet Effluent Diffusion Denuder - GC (CWEDD-GC), Tenax tubes - GC and Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOF), were compared first in laboratory and then all methods were applied to the determination of MTs in real conditions in a Norway spruce forest. The CWEDD-GC was the only method that allowed sampling of monoterpenes with short time intervals of 2 – 5 min and, moreover, it enabled together with Tenax tubes-GC the determination of concentrations of individual monoterpenes due to subsequent analysis of samples by GC while the PTR-TOF measured only the sum of all monoterpenes. In the second paper (Juráň et al., *Agr. Forest Meteorol.* 2017, 232, 500-513), PTR-TOF-MS and CWEDD-GC were applied to the measurement and subsequent modelling of concentrations and fluxes of biogenic and anthropogenic VOCs above a Norway spruce forest during a field campaign focusing on isoprene and monoterpenes as the most important representatives of BVOCs. Obtained results contribute to better understanding emissions of BVOCs in central Europe. ENV contribution in the papers consisted in the measurement of MTs using the CWEDDGC and writing the papers.

Next to BVOCs we published paper dealing with the measurement of emission and dry deposition of ammonia in the vicinity of naturally ventilated animal building (Zapletal and Mikuška, *Atmosfera* 2019, 32, 337-350). ENV contribution consisted in NH<sub>3</sub> measurement based on a combination of continuous collection of NH<sub>3</sub> into water in the cylindrical wet effluent diffusion denuder and fluorimetric detection of collected NH<sub>3</sub> and participation at writing of paper.

Within the grant project „Photocatalytic technology for purifying air in the interior and exterior of buildings: Overall environmental impact“ (GA CR, P104/19/12109S, 2019-2021) we cooperate with J. Heyrovsky Institute of Physical Chemistry of the CAS in Prague at the development of quantitative methodology for the determination of the efficiency of the photocatalytic technology for the removal of NO<sub>x</sub> from the air. So far, we have not published any papers.

We completed the project “Essential oils as tool for saving and increasing of culture heritage on paper“ (MC CR, DF11P01OVV28, 2011-2015) focused on the use of essential oils (EOs) to protect damaged books and other archives on paper basis that are affected by microbial activities. We determined the antimicrobial properties of 15 essential oils for 17 microorganisms (bacteria and fungi). The most effective EOs were *Lavandula angustifolia*,

*Cymbopogon nardus*, *Citrus aurantifolia*, *Juniperus communis*, *Myrtus communis* and *Cinnamomum zeylanicum* (Křůmal et al., Chem. Pap. 2015, 69, 1084-1092). We also proposed a disinfectant for the treatment of cellulose and/or ligno-cellulose materials against attack of fungi and for elimination of fungi in these materials and designed way of its application and method of protection of materials. The disinfectant is particularly suitable for indoor use in libraries and depositories (Křůmal and Večeřa, 2017, Czech patent, No. 306676).

Three other publications are the result of collaboration of Department with Brno universities. The co-operation with the University of Defence Brno resulted in the development of a portable device for fast analysis of explosives in the environment (Čapka et al., J. Chromatogr. A 2015, 1388, 167-173). This novel portable device is able to determine selectively most of nitramine- and nitroester-based explosives as well as inorganic nitrates at trace concentration in water or soil extracts in less than 8 minutes. Further cooperation with University of Defence Brno and with Mendel University Brno led to another paper that presented a method for the automatic identification of outliers in environmental time series based on nonparametric statistical techniques (Čampulová et al., J. Chemometr. 2018, 32, e2997). In cooperation with Brno University of Technology we developed a method for routine determining the ecotoxicity of water-soluble compounds for laboratory practices using UV-VIS spectrophotometry (Čapka et al., Fresen. Environ. Bull. 2015, 24, 4758-4762).

Besides the 46 publications in peer-reviewed journals with impact factor, we also published three papers in journals without impact factor. The first one dealt with the statistical analysis of the effect of inhaled manganese oxides ( $MnO.Mn_2O_3$ ) NPs on weight of internal organs of laboratory mice induced by a long-term inhalation of NPs (Zeman et al., J. Phys. Conf. Ser. 2015, 617, 1-6). The second one investigated the contents of selected heavy metals in the *Usnea antarctica* lichens from James Ross Island for monitoring of increasing pollutant burden of the Antarctic environment (Zvěřina et al., J. Czech Polar Reports 2016, 6, 238-239). The third study, done in collaboration with University of Defence in Brno, characterized the UFPs in military rooms used by soldiers of the Czech Armed Forces during their working hours and assessed the risk of UFPs on the health of soldiers (Kellnerová et al., Inżynieria Mineralna – czasopismo polskiego towarzystwa przeróbki kopalin 2019, 1, 163-167).

We published four applied results:

- Methodology of application of essential oils and their components to achieve fungistatic effect in depositories paper artifacts endangered by microbial attack due to high relative humidity. Milichovský M., Večeřa Z., Neuvirt J.; MK-S 530/2011 OVV, 2016.
- Disinfectant for treatment of cellulose and/or ligno-cellulose materials against attack of fungi and for elimination of fungi in these materials, its application and methods of protection of materials. Křůmal K. and Večeřa Z.; Czech patent, No. 306676, 2017.
- A multi-application device for sampling and dosing of liquid samples. Čapka L., Mikuška P., Šesták J.; Model number: 30849, 2017.
- Portable analyzer for explosive detection. Čapka L., Večeřa Z., Mikuška P., Šesták J.; Model number: 30954, 2017.

In addition to above mentioned research, the Department also performed various analyses for companies in the form of paid services. The extent of these analyses was insignificant in comparison with the main research. Mostly we analysed combustion products in experimental buildings for the Ministry of Defence. We also co-operated with the company VF Černá Hora, a.s., in the development of device for sampling methane and carbon dioxide from the air and determination of  $^3H$  a  $^{14}C$  in air around nuclear power plants.

## Research activity and characterisation of the main scientific results

### Electromembrane extraction

Fundamental operational principle and instrumental set-up of electromembrane extraction (EME) suggest that electrolysis may play an important role in the EME process. In a series of three consecutive publications [1-3], the fundamental principles of electrolysis in EME were theoretically investigated, effects of electrolysis on EME performance were quantitatively described, and practical hints for a possible remedy of this unwanted EME feature were suggested. The omnipresent electrolysis of water during EME significantly affects pH of operational solutions and particularly of acceptor solutions, which are usually in  $\mu\text{L}$  volumes [1]. The decrease or increase of pH values in operational solutions changes the ionization state of target analytes and matrix components and usually deteriorates the EME performance [2]. As a suitable remedy, buffered operational solutions, or solutions containing high concentrations of weak acids or bases should be used instead of traditionally employed low concentrations of strong acids/bases. Application of optimized operational solutions efficiently eliminates the electrolysis, ensures considerably improved EME performance, and operation of the EME system at higher extraction currents [2,3]. The effects of electrolytically-induced local pH changes in a narrow layer close to the phase interface were investigated and the importance of application of buffered solutions was highlighted [4].

Micro-electromembrane extraction ( $\mu\text{-EME}$ ) through free liquid membrane (FLM) is performed in a narrow, transparent capillary tubing and requires only  $\mu\text{L}$  volumes of all operational solutions. As all phases are used in form of free liquids, the tubing can be filled with virtually unlimited number of immiscible phases. The phases are consecutively filled one after another into the tubing and form extraction systems with multiple phases. The multiple-phase arrangement can be used for tailor-made microextractions, e.g. for the simultaneous  $\mu\text{-EME}$  of cations and anions [5] and salt removal from saline samples [6]. As the number of the plugs, their composition, volume and sequence in the tubing can be chosen specifically for each particular application, high variability of the extraction process can easily be achieved. A five-phase micro-extraction system, which consists of aqueous donor solution (central phase) sandwiched between two organic FLMs and two aqueous acceptor solutions (peripheral phases), was employed for the simultaneous  $\mu\text{-EME}$  of various anions and cations. Simultaneous  $\mu\text{EME}$  of ibuprofen and procaine was demonstrated as a proof of principle of the proposed multiple-phase microextraction from urine sample [5]. Composition of each phase was optimized in order to obtain rapid migration of target analytes in the opposite direction and the two acceptor solutions were analyzed. In an even more advanced arrangement, selectivity of the organic phases was fine-tuned and the transfer of small inorganic cations and anions from the central phase was achieved. By using this principle, a saline sample was used as the central phase in the five-phase  $\mu\text{-EME}$  arrangement,  $\text{Na}^+$  and  $\text{Cl}^-$  migrated through two tailored FLMs to waste solutions and the sample (i.e. the central aqueous phase) was rapidly desalted. This approach was able to achieve up to 99.3% salt removal from samples with physiological salt concentrations in less than 12 min and to retain 95 – 98% of biochemical macromolecules for subsequent CE, or MS analysis in less than a drop of a sample [6]. This is an important finding for the development of miniaturized desalting procedures of, e.g. biological samples, which are usually available in limited volumes.

Selectivity of sample pretreatment techniques is of major importance for efficient preconcentration of target analytes and elimination of matrix components. Selectivity of EME was fine-tuned by modification of operational solution composition. The most convenient approach is the direct modification of the phase interface between the donor and the acceptor solution and a significant selectivity improvement has been demonstrated by preparing the liquid membrane from a highly retaining organic solvent and a highly selective modifier. Nitrobenzene was doped with a purpose-synthesized macrocyclic compound (bambus[6]uril) and the mixture formed a highly selective phase interface for particular inorganic anions. The

transfer of major matrix ions (chloride, sulfate and nitrate) was up to five orders of magnitude lower than the transfer of target analytes (bromide, iodide and perchlorate) and the developed system was applied for highly selective EMEs of trace concentrations of target anions from environmental samples [7]. The liquid membrane functionality can be altered also by using ionic modifiers. Cationic carrier, bis(2-ethylhexyl)phosphate, was selected as a suitable modifier for the extraction of selected heavy metal cations from saline samples. Both, the individual effect of each major matrix cation (sodium, potassium, calcium, magnesium) and their synergistic effect on EME of the trace heavy metal cations were evaluated. In both cases, a decrease in extraction efficiency was observed when the matrix cations were present in the sample, which was more significant for calcium and magnesium [8]. Another possibility for selective extractions is the application of the formerly developed multiple-phase  $\mu$ -EME concept. Composition of the aqueous and organic phases can be optimized and selected groups of analytes (based on their physicochemical properties) can be transferred to a specific phase and can be separated into several fractions. Basic drugs with different acid-base strengths were fractionalized into two separate aqueous phases with different pH and composition based on their  $pK_a$  values [9]. Fundamental principles of EME were comprehensively described and understood by the application of  $\mu$ -EME through FLM. Three adjacent plugs of aqueous donor solution (containing model analytes), organic FLM, and aqueous acceptor solution were formed for each  $\mu$ -EME. After  $\mu$ -EME, the three solutions were recovered from the tubing, concentrations of the analytes in each solution were determined by CE-UV, and time-dependent distribution of the analytes among the three phases was established. An excellent fit was achieved between the theoretical model for  $\mu$ -EME of charged analytes and the experimentally determined distribution of model analytes among the three immiscible phases and confirmed, for the first time, transfer of analytes in their charged forms [10]. In addition, selective fractionation of transferred analytes into FLM and acceptor solution was achieved by fine-tuning the composition of the latter. Preconcentration ability of the  $\mu$ -EME through FLM was increased by the application of a dynamic set-up with a stirred donor solution. A two-phase  $\mu$ -EME arrangement (i.e. the FLM acts as the phase interface for the elimination of the matrix components as well as the acceptor solution) was suggested for the ease of operation and accelerated the EME transfer. The novel twophase  $\mu$ -EME method was applied for the efficient extraction of model acidic drugs from human urine, human serum and wastewater samples [11].

### **Automation of microextraction/analytical methods**

Automated analysis of complex samples requires automation of the analytical as well as of the sample pretreatment process. This is usually a challenging task in the analysis of biological samples, which are available in limited volumes, since the sample pretreatment process has to be performed in a miniaturized format and its direct coupling to standard analytical methods might not be easily adapted.

On the other hand, volumes of operational solutions in LPME and CE are in  $\mu$ L range and are thus perfectly compatible. Two major directions were investigated in the evaluated period aiming at

(semi)automation of (i) SLM extractions or (ii)  $\mu$ -EMEs with an analytical system.

In the research on semi-automation of SLM extractions, commercial CE instruments were employed due to the ease of operation and comfort of the operators. A specifically designed microextraction units compatible with the commercial CE instrumentation and autosampler carousels were designed and their fabrication from off-the-shelf cheap and disposable consumables was suggested. These units ensured convenient replacement and possibility to dispose them of after a single use. This was the major prerequisite for a rapid and reliable analysis of complex samples, such as human body fluids, environmental and food samples.

CE is usually characterized by relatively low sensitivity, which is particularly pronounced with standard

UV-Vis detection due to the short optical pathlength through the narrow-bore separation capillary. Sensitivity enhancement in CE can be achieved by various on-capillary preconcentration modes. Nevertheless, on-capillary preconcentration are not possible for complex samples, such as human body fluids, which usually require an additional sample pretreatment to remove matrix interferences. In-line coupling of extractions through supported liquid membrane (SLM) to large electrokinetic injection and transient isotachopheresis-capillary zone electrophoresis (tITP-CZE) was described as a convenient method for direct and sensitive analysis of human body fluids [12]. A significant improvement was achieved for model basic drugs and the analytical sensitivity (i.e. enrichment factors) increased up to 340 times compared to standard hydrodynamic injection and CZE of the drugs. SLM extractions use polymeric supports, which are impregnated with a suitable water-immiscible solvent just before the extraction. The SLM then separates the complex sample from a clean acceptor solution and acts as a semipermeable phase interface that enables selective transfer of target analytes from the donor solution to the acceptor solution. At the same time, SLM retains matrix interferences and disables their transfer to the acceptor solution. One major disadvantage of using SLMs in automated systems is their need for impregnation before the extraction process. Polymer inclusion membranes (PIMs) on the other hand are dry and non-porous membranes that can be incorporated into a microextraction device ahead of the extraction process and stored without noticeable deterioration in extraction performance. A preassembled microextraction unit with PIM was designed and used for in-line extraction and CZE determination of formate in toxicological studies [13]. Formate (the major metabolite in methanol poisoning) was determined in undiluted human serum and whole blood by CZE with simultaneous C<sup>4</sup>D and UV-Vis detection. A complete automation of the entire analytical procedure, i.e., of formate extraction, injection, CZE separation and quantification, was achieved. Sensitivity of the method was sufficiently low to clearly distinguish between endogenous and toxic levels of formate in healthy and methanol intoxicated individuals. In medical treatment, co-administration of basic and acidic drugs might be applied for a more efficient synergistic effect of the two different pharmaceuticals. Nevertheless, conventional sample treatment techniques are suitable for extractions of compounds with similar physicochemical properties only and simultaneous extraction of basic and acidic drugs is usually not possible. A targeted SLM extraction method was developed that enables automated simultaneous extraction of basic and acidic drugs in-line coupled to CE and was applied to the direct analysis of both groups of compounds in undiluted urine samples [14]. A CE method was optimized in order to achieve the simultaneous determination of the two groups of analytes in a single CE run and the automated method was applied for a pharmacokinetic study of drugs in urine sample with sample throughput of more than 6 samples per hour. Novel and more efficient phase interfaces for in-line coupling of SLM extraction to CE were also investigated. Consumption of organic solvents and extraction time were reduced and extraction efficiency has improved for novel materials based on thin polypropylene [15] and nanofibrous polyamide 6 [16] membranes, with no obvious limitations for the direct analysis of complex body fluid samples.

SLM extractions enable efficient semi-automation of the entire analytical workflow. Nevertheless, their full automation is challenging due to the need for a regular impregnation/replacement of the membrane, which is done manually. From that point of view, application of automatically renewable phase interfaces presents an interesting alternative for the development of a fully automated sample pretreatment and analytical system.  $\mu$ -EME through FLM was examined for this purpose in a proof-of-concept study and the manual handling of the three liquid phases was done in a semi-automated fashion using a bidirectional syringe pump [17]. A three-phase  $\mu$ -EME system was formed by consecutive withdrawal of microliter volumes of a donor solution, a FLM and an acceptor solution into a disposable microextraction unit. Extraction performance was enhanced by the application of electric field, which significantly accelerated the extraction process and improved extraction efficiencies and exhaustive extractions of model analytes were possible in 10 min from raw human urine and

serum samples. The concept of  $\mu$ EME through FLM was later completely automated. The miniaturized extraction system consisted of a computer-controlled microsyringe pump and a multiposition rotary valve for handling aqueous and organic solutions at a low microliter volume and acted as a front-end to on-line liquid chromatographic separation. The organic membrane was automatically renewed and disposed of in every analytical cycle, thus minimizing analyte carry-over effects while avoiding analyst intervention. The applicability of the automated microfluidic device was demonstrated by the liquid chromatographic determination of nonsteroidal anti-inflammatory drugs in  $\mu$ -EME processed complex samples, such as urine and influent wastewater [18]. The developed system represents an autonomous device operated in a fully unattended regime and holds a great promise for running unmanned analyses including clean-up, extraction, analysis and quantification of high matrix samples.

### Theory and methodology of CE

Electrolyte systems for moving boundary ITP (where leader and terminator can be also present in the terminating and leading zone, respectively) were investigated with the aim of extending their application range also to acidic and alkaline medium. It was found that in such systems, like in ITP in classical format, the solvent ion can be employed as terminator when its effective mobility is sufficiently decreased by a counter-constituent of suitable  $pK_a$ . This concept proved to be applicable both for cationic ITP in the acidic region and anionic ITP in the alkaline region. In the former case,  $H^+$  serves as essential terminator and application of the general theory of moving-boundary ITP allowed definition of stacking criteria and obtaining explicit equations for the zone-related boundary mobilities, concentrations and particularly for the terminating zone mobility that provides a generalized definition of the  $H^+$  effective mobility [19]. A similar approach was applied to anionic ITP systems in the alkaline region where the  $pK_a$  of the counterionic base is selected to keep the mobility of the  $OH^-$  zone low enough to serve as an essential terminator. A new generalized expression for the mobility of the  $OH^-$  zone was derived that enabled prediction and tuning of its value to meet practical requirements [20].

It was shown that the general concept of resolution used in many separation techniques is applicable also to the method of electrophoretic focusing on inverse EMD gradient [21]. In this new separation principle, the separation of analytes along the electromigrating EMD profile proceeds so that each analyte is focused and concentrated within the profile at a particular position given by its  $pK_a$  and ionic mobility. A fundamental equation was derived, expressing resolution of a pair of analytes as a function of the difference in their effective mobilities, of constants characterizing the diffusional and focusing properties of the analytes and the system, and of passed electric charge and electric current, both related to the time point of detection. The derived equations show that resolution is proportional to the square root of the product of passed electric charge and current; for constant current operation, this proportionality simplifies to the square root of time. The presented theory offers an easy solution for the enhancement of the separation performance of the method.

Chiral ITP of methadone using inverse cationic configurations with  $H^+$  as leading component and multiple isomer sulphated- $\beta$ -cyclodextrin (S- $\beta$ -CD) as leading electrolyte additive, has been studied utilizing dynamic computer simulation and a calculation model based on steady-state values of the ITP zones [22]. It has been shown for the first time that inverse cationic chiral ITP configurations in presence of S- $\beta$ -CD can be employed to form ITP zones of enantiomers of a weak base. It was found that by varying the concentration of S- $\beta$ -CD in the leader, either both methadone enantiomers, only S-methadone which has a weaker complexation or neither of the two enantiomers formed ITP zones. Experiments that confirmed theoretical findings showed potential application to micropreparative purification of S-methadone by ITP.

Fundamental studies on electrophoretic effects induced by a phase of mixed micelles, that migrates surrounded with background electrolyte, were undertaken using CE with capacitively coupled contactless conductivity ( $C^4D$ ) or UV-Vis detector. It was established that mixed

micelles under electrophoresis are subject of evolution in terms of mobility, peak area and presence of sub-zones enforced by the composition of micellar phase, segment length and applied voltage. Established features allowed us to explain the electrophoretic behaviour of nanoparticles in the CE system and it was postulated that a pseudomicellar state of nanoparticles can be useful term in analyzing the migration phenomena of nanoparticles within micellar environment [23,24]. A new portable CE instrument was developed, which enables the concurrent separation of anions and cations in parallel channels. Each channel has a separate buffer container to allow independent optimization of separation conditions. The system employs a miniature automated syringe pump, which can apply both positive and negative pressures and the separations are performed in a thermostatted compartment for improved reproducibility in field conditions. The instrument has a compact design, with all components arranged in a briefcase with portable dimensions and a weight of less than 15 kg [25]. A simple sample injection procedure compatible with commercial CE instruments was developed, which enables handling sample volumes as little as 250 nL for the analyses of samples with limited volumes. Single-use micro-sampling inserts were prepared by thermal modification of polypropylene micropipette tips and the inserts were accommodated in standard CE vials in CE autosampler carousel. Injections from sub- $\mu\text{L}$  samples were carried out in conventional as well as in short-end injection mode, were compatible with standard fused silica capillaries (25 – 100  $\mu\text{m}$  i.d.), with various background electrolyte solutions, and various detection modes (UV-Vis,  $\text{C}^{4\text{D}}$ , ESI-MS). Excellent repeatability of replicate injections from 250 nL to 3  $\mu\text{L}$  was achieved for model analytes, which was comparable with that for replicate injections from standard CE vials [26]. A new CE- $\text{C}^{4\text{D}}$  method was developed for the rapid (in less than 4 min) determination of meldonium in urine. Direct injection was possible after 1:9 dilution of urine with deionized water. The analytical performance data proved good applicability of the method to the determination of various meldonium concentrations in urine and good long-term performance of the analytical system. The method might be particularly useful in analyses of large batches of samples for the testing of meldonium-positive vs. meldonium-negative urine [27]. A new detection system based on the application of novel laser emitting diodes (LEDs) was developed for analytical separations performed in fused silica capillaries. The system was suitable for portable operation and offered a number of advantages over traditional light sources including robustness, small size and low-cost. The main devise of the presented system was extension of the operation range to deep-UV range, which is traditionally difficult with standard LEDs and other alternative light sources [28,29].

### **Electrolyte systems for the CE-MS combination**

The possibilities of reaching higher sensitivity in ITP analyses of complex samples with ESI-MS detection were investigated on the example of analysis of diclofenac and ibuprofen in environmental waters [30]. Optimization of the MS detector variables have shown that fragmentor voltage and working in the SIM mode with collection of data for multiple fragments increase the method specificity and approx. double its sensitivity. Combination with an off-line SPE preconcentration step resulted in a very high sensitivity of the described methodology with a LOD below  $2 \times 10^{-12}$  M, corresponding to analyte levels of 0.6 pg/mL of diclofenac and 0.4 pg/mL of ibuprofen. The results demonstrated that ITP-MS has the potential to reach sensitivities comparable to HPLC-MS.

Research of electrolyte systems for ITP with ESI-MS detection, that represents a potentially more sensitive alternative to other CE-based techniques, was focused on moving-boundary ITP systems in acidic and alkaline medium. Theoretical results regarding the use of solvent ions as essential terminator were employed for successful setup of very simple systems composed of ESI-compatible volatile components. Application to acidic cationic moving-boundary ITP was demonstrated with a system consisting of 10 mM ammonium acetate as the leader and 10 mM acetic acid with 2 mM ammonium as the terminator, used for the analysis of 2-hydroxyatrazine and 2-hydroxyterbutylazine in waters. Despite using direct sample

injection without any preconcentration step, the method showed much better sensitivity than so far published electrophoretic methods, with LODs below 0.1 ng/mL. The applicability of the method was confirmed on analyses of untreated and spiked drinking and river water [19]. The properties of alkaline anionic moving-boundary ITP systems composed of acetic acid and selected weak bases (ammonium, triethylamine and ethanolamine) were shown by stacking diagrams, simulation and model experiments. Experimental results confirmed that this methodology is well suitable for sensitive and selective analyses of very weak acidic analytes (e.g. sulfonamides or chlorophenols). A practical example of analysis of sulfonamides in waters demonstrated the potential to reach high sensitivity with LOQs below  $10^{-9}$  M, without any sample pretreatment or preconcentration [20].

The first practical implementation of electrophoretic focusing on inverse EMD gradient, was described in combination with ESI-MS detection [31]. A simple electrolyte system consisting of two volatile components (maleic acid and 2,6-lutidine) was shown to be surprisingly robust and allowed for sensitive analyses of weak acids in the  $pK_a$  range 6-9. Application to analysis of sulfonamides in waters demonstrated the potential of the method for fast analyses of trace ionic analytes and reached LODs around  $3 \times 10^{-9}$  M (0.8 ng/mL). In a further study [21], resolution enhancement was achieved by the application of negative pressure at the inlet vial to partially compensate the fast transport of the profile towards the detector by combined EOF and ESI suction. Experiments have shown a considerable improvement of separation performance which allowed for effective analysis of multicomponent samples. This has been demonstrated by simultaneous analyses of four sulfonamides and three dichlorophenols in waters with very low LODs around the  $10^{-9}$  M level without any sample pretreatment.

## Reviews and book chapters

Senior scientists of the team are well established researchers in their areas and are regularly invited to publish review articles. All review articles published in the 2015 – 2019 period were invited contributions and were published in prestigious journals on analytical chemistry (Trends in Analytical Chemistry [32,33], Analytica Chimica Acta [34], Electrophoresis [35-43] and a book chapter for Elsevier [44]). The reviews and the book chapter have a direct impact on the formation of the view of the scientific community on particular topics of sample treatment [33,44] and separations based on electromigration [32,34-43], as shown by their good citation response. Moreover, summarizing latest developments in CE analyses of non-conventional body fluids [34] was an important step towards opening a completely new research direction at the department, namely, analysis of dried material spots.

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## Research activity and characterisation of the main scientific results

As the name of the Department suggests, the research team is involved in the development and applications of new bioanalytical instrumentation and protocols. Although capillary separations and mass spectrometry coupling were the main part of the work at the beginning of the evaluation period, the current research is much more diversified. Partly it is the result of the changes after the previous evaluation when several workers from the former Department of Glycomics and Proteomics joined the research team and partly by adding new research topics including the droplet microfluidics and applications of photon upconverting nanoparticles. Strong focus on miniaturization and microfluidic instrumentation and analyses of small sample amounts of biologically important molecules, including DNA, proteins, and glycans down to the single-cell level, represents the last part of the research. The following section briefly describes individual areas of the work represented by the publications selected for the evaluation. Note that the reference numbers reflect the publication order in the list of publications and not the text's appearance.

### **CE-ESI/MS – publications 1,5,9,15,16,22,26**

In 2015 we published results obtained during a two-year postdoctoral stay of G. Járvas in Brno from the group of Prof. A. Guttman in Vesprém, Hungary. In this work, we have used computer simulations and designed a microfabricated pneumatic electrospray nebulizer. The simulations showed the velocity fields of the gas flow at the exit port of the microfabricated electrospray nebulizers designed with different geometries. The results are essential for understanding the design parameters important for microfabrication of the ESI interface [26]. In the same year, we have also published an invited review article on the modeling of CE-ESI/MS interfaces in the prestigious journal *Mass Spectrometry Reviews* [1].

During the experimental studies on nanoelectrospray ionization at sub-nanoliter per minute flow rates, we have realized that at very low internal diameters, the electrospray tip can serve for both the separation and electrospray ionization. In the first paper [5], we have demonstrated that a high-resolution electrophoretic separation inside the electrospray capillary is possible. We have also provided both the theoretical and practical considerations for such an operation. In continuation of this work, we have developed a new method for the determination of drugs in blood plasma [9]. This work (Interface-free capillary electrophoresis-mass spectrometry system with nanospray ionization - Analysis of dexrazoxane in blood plasma) was done in collaboration with our colleagues from the Charles University who supplied the samples.

Analysis of complex glycan mixtures is an integral part of modern bioanalysis. For CE separations, the glycans are labeled with a charged, often fluorescent, group providing the necessary charge. One of our long term efforts in this field is the development of a tag with multiple positive charges for fast CE separation and high sensitivity MS detection. In the first published paper [15], we have described the behavior of oligopeptide labeled N-linked glycans and the role of multiply charged tags in the speed of analysis and ESI/MS sensitivity. It was clearly shown that the gas phase charge distribution does not correlate with that of the ions in solution and that permanent charged groups (e.g., quaternary ammonium) will be needed for practical analyses. When studying reactions of cationic tags for CE glycan analysis, we have found a formation side products making the quantitative analysis difficult. We have realized that this problem relates to the presence of N-acetyl glucosamine at the reducing end of the glycan and studied this previously unknown behavior in detail [16].

In routine work, the use of automated CE-MS instrumentation is essential to achieve reproducible results. With support from the CE group from Agilent (Waldbronn, Germany), we have developed a microfabricated liquid junction hybrid capillary electrophoresis-mass spectrometry interface for fully automated operation [22]. The interface, fabricated by laser machining from polyimide, provides excellent sensitivity and reproducibility without limitations on the size and type (coated, uncoated) of the separation capillary. While developed primarily for the CE-MS systems from Agilent, it can be used with practically any CE-MS system.

### **Microfluidics – publications 2,3,4,10,22,23**

Microfabrication and microfluidics represent an important part of our toolbox. It is often the best way of achieving the desired bioanalytical outcome in both the instrument development and methodology research. Although the institute does not have sufficient resources for the systematic development of high tech microfabrication technologies, exciting results can sometimes be obtained by combining expertise available in different departments. An example of such interesting research is the study of processes useful for etching of glass microchips with supercritical water [3]. In collaboration with the FPS department, we have demonstrated that hot/pressurized (supercritical) water can etch standard glass wafers with speed comparable to toxic HF-based etchants. Additional advantages include higher available aspect ratios and tuneability of the surface roughness. This is the first demonstration of using hot, pressurized water for etching microfluidic structures.

Another example of joining forces of two groups, this time with colleagues from the University in Copenhagen, Denmark, is the development of methods for rapid and straightforward preparation of thiol–ene emulsion-templated monoliths and their application as enzymatic microreactors [4]. The work describes the extension of the IMER technology previously developed in Brno into the cast microfluidic monolithic device designed in Copenhagen. This collaboration, partly done during the long term stay of our student in Copenhagen, continues in the form of mutual contacts (visits, lectures, defense examinations), editorial work, and joint grant application.

Droplet microfluidics is a new addition to our research portfolio. Initially, it started during the postdoctoral stay of E. Basova in our CEITEC (Masaryk University) part of the group when we focused on basic principles of Droplet microfluidics in (bio) chemical analysis [2]. More recently, we have applied the photon-upconversion nanoparticles under development in the Nanotechnology direction for barcoding with multiple barcode channels for droplet microfluidics [23]. Different ratios of photon upconverting nanoparticles were used to code analyte reagent concentrations in individual droplets for the first time. Since the coding depends on infrared excitation, it does not interfere with standard fluorescence detection used in droplet microfluidics.

Instrumentally similar to droplet microfluidics is also our work on single-cell analysis. An example of published output is the parallel single-cell analysis of active caspase-3/7 in apoptotic and non-apoptotic cells [10]. This work presents the design of an instrument for the monitoring of enzyme activity using chemiluminescence. Since the detection is based on single-photon, counting the enzymatic activity in individual cells can be monitored. Thus, instead of measuring the average values over many cells, standard in all current approaches, one can differentiate the chemistry of individual cells. This still ongoing research is performed with our colleagues from the Institute of Animal Physiology and Genetics (IAPG), who supply the cells in a different state of development.

### **Nanotechnologies – publications 7,8,18,21,23,24**

Nanotechnology is a vaguely defined field based on the physical dimensions of the studied objects. Generally, anything with at least one dimension below 100 nm is considered a

nanostructure. In our work, we are trying to utilize the unique properties related to nanoscale objects for chemical measurements. An example of such work is the utilization of the thin metal film as a resistivity chemical sensor [8]. This paper describes the change of resistance of a metal-film (50-200 nm) in response to the chemical binding of thiol-containing compounds. While the theoretical basis has been described in the 1950s, this phenomenon has not been explored in detail yet. We have microfabricated a set of sensors in the Wheatstone bridge arrangement and showed sensitivities in the subnanomolar range for thiolcontaining compounds. At the same time, non-thiolated analogs provided no signals even at molar concentrations. Since this work was not separately funded, we consider it as scouting research, which may lead to new research direction in the future. In continuation of the investigation of the preparation and application of thin metal films for bioanalysis and in collaboration with our partners from the Pardubice University, we have developed methods for the preparation of metal nanosheets and their application for shape-based coding [18]. In this work, conceived in Brno, we could prepare nanoplatelets with arbitrary shape weighing less than a nanogram. The particles were prepared from single or multiple metal layers providing both shape recognition by optical microscopy as well as magnetic and chemical properties for bioanalytical applications. The low weight of the particles in the ng-pg range allows their immobilization on the desired spot by only a few chemical bonds.

Another example of our efforts in nanotechnology applications is the use of inorganic nanofibers prepared by force spinning technology. In the paper resulting from the research, performed in collaboration with the company Pardam ( Roudnice n. L., CR), we describe the preparation of Zr and Ti containing inorganic nanofibers and their characterization for phosphopeptide enrichment in proteomics. Part of this joint collaborative research with the company Pardam has been patented.

The last direction in our nanotechnology-based research relates to the hiring of two new post doctorands after their return from abroad. In one case, A. Hlavacek is focusing on the preparation, modification, and applications of photon-upconversion nanoparticles. In some applications, fractionation of a crude mixture of nanoparticles may be critical for practical applications. We have published a new method for large-scale purification of photon-upconversion nanoparticles by gel electrophoresis for consecutive analog and digital bioassays [21]. The use of nanoparticles for SERS in salty solutions (e.g., body fluids) is often hampered by their precipitation. In our recent work, the nanoparticles were prepared with two types of ligands. One of the ligands defined the optimum distance from the analyte for the best SERS signal. The other one served as a regulator of a distance between the nanoparticles to prevent their precipitation. The amount of each of the ligands could be experimentally optimized [24].

#### **Omics related – publications 6,11,12,13,17,25**

One of the first results from the new members of the Department joining us from the dissolved former Department of glycomics and proteomic relates to the ongoing joint research with the Slovak Academy of Sciences. Two-dimensional gel electrophoresis, followed by digestion of selected protein spots and MALDI/MS or LC-MS analysis with database identification was used for the proteomic analysis of changes in the protein composition of MCF-7 human breast cancer cells induced by all-trans retinoic acid, 9-cis retinoic acid, and their combination [6]. Similar proteomic techniques were also used to study the consequences of the natural retinoid/retinoid X receptor ligands action in human breast cancer MDAMB-231 cell line [12]. In support of our proteomics related efforts, we have also studied the acetonitrileassisted enzymatic digestion, which can facilitate the protein sequence coverage for the bottom-up identification of proteins of cancer origin [17].

Glycan analysis (glycomics) is a significant counterpart of proteomics. Our research in this area is focused mainly on the development of CE-MS technology and synthesis of new, multiply-charged, cationic tags for derivatization of neutral glycans, as discussed in the CE-ESI/MS section. In a separate collaborative effort with our colleagues from the Institute of Biophysics, we have described the first successful system for the preparation of an immobilized lectin monolithic capillary column for selective capture and release of glycans coupled with electrochemical detection. The glycans released enzymatically from glycoproteins by the PNGase F were detected electrochemically after chemical modification with Os(VI) [25].

Our metabolomics related work is oriented mainly on the analysis of small ions in the exhaled breath condensate and biological fluids. This work is mainly instrumentally oriented and supported by colleagues from the local hospitals in the effort for the development of new point-of-care instrumentation. For example, a miniaturized CE-LIF instrument was constructed, and a new method based on capillary electrophoresis with laser-induced fluorescence detection was tested for sensitive determination of malondialdehyde in exhaled breath condensate and biological fluids [11]. In addition, a miniaturized membrane-based device, based on a semipermeable membrane and capillary action, was prepared. This disposable device is suitable for fast (seconds) blood plasma separation ideal for medical applications [13].

#### **DNA analysis – publications 14,20**

Most of the DNA analysis protocols involve DNA extraction from the biological specimen. Sometimes the amount of the DNA is tiny, and sample losses, typical for solid-phase extractions, may lead to unreliable analyses. We have been approached by our colleagues from the Roche Sequencing Solutions, Inc. to develop new protocols and instrumentation for electrophoresis based DNA extraction. Under the sponsored research program, we have first tested the preparative concentration of nucleic acid fragments by capillary isotachophoretic analyzer for the "lossless" DNA extraction [14]. The promising results with the DNA preparation from up to 30  $\mu$ l sample volumes have recently resulted in the development of a microfluidic device for preparative concentration based on epitachophoresis with over a thousand times higher loading capacity [20]. This technology is currently being patented, and the sponsored research continues.

#### **Highly interdisciplinary**

Most of our work can be considered to be interdisciplinary; however, this last example is quite different in both its start and results. A few years ago, one of our graduate students met our current collaborators from Mendel University in a pub. They didn't know each other, but discussed our research and exchanged contacts. During the subsequent visit at IACH, we started discussing the potential of using our recently prepared iron nanoparticles for the wood treatment. The initial experiments were very promising and led to the development of a new protocol for the preparation of highly concentrated iron nanoparticles from inexpensive and nontoxic chemicals. Since the wood capillaries are highly permeable for nanoparticles the iron penetrates deep into the wood and reacts with the tannin compounds. The resulting color can be fine-tuned in a wide range. Such a treatment can substitute for the widely used but more expensive and toxic ammonia treatment [19]. The process has been patented and licensed in the Czech Republic with international applications pending.