Characteristics of main research directions investigated at the institute and the achievements 2010−2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

The core activities of the Institute of Analytical Chemistry are research and development of new principles, methods and instrumentation in the field of analytical methods applicable for development of other scientific areas, especially biological and medical science, human health and environmental protection. Fundamental research is focused especially on separation and spectroscopic methods, systematic miniaturization and nanotechnology dealing with topics in proteomics, genomics, drug analysis, body fluid analysis and environmental monitoring. Top level is reached in research and development of electromigration analytical methods involving newly electromembrane extractions that is oriented both to fundamental research and to significant applications in medicine, biochemistry and environmental protection. Another progressive topic of research is the development of modern bioanalytical procedures based on separations, microfluidics, mass spectrometry and nanotechnologies aimed at the analysis of an individual cell. The research focused on development of analytical methods for trace element and speciation analysis based on atomic spectrometry continued very well, especially advanced approaches to generation, preconcentration and atomization of analytically useful volatile species. Development of electric field-based separation methods for biomolecules and bioparticles, application of supercritical water for modification of glass- and fused silica-based analytical separation devices, and development of chromatographic columns and miniaturization and automation of the separation methods continues successfully as well. Trace concentration of substances important in environmental protection were analyzed in aerosol particles, toxic metals were estimated in organs of experimental animals and in emission of automotive engines. Research is conducted in five scientific departments managed by renowned scientists who have achieved excellent scientific results. Based on results of previous evaluation, two of the departments were rearranged by combining with two other teams, which enabled to intensify research and utilize manpower in a better way. Department developing new bioanalytical instrumentation that reached the best level in previous evaluation continued constantly in its excellent scientific work. The team was augmented by several members of former Department of Proteomics and Glycomics in 2013. The department was restructured and based on the results of the previous evaluation most of the directions of former Department of Proteomics and Glycomics have already been closed. The new group members have been involved in new research directions towards targeted, cancer related analyses and development of new instrumentation for analyses of rare cells. Nowadays the Department of Bioanalytical Chemistry became the strongest team in the institute and is the most successful in obtaining financial support for grant projects, in international collaboration, recruitment of young perspective scientists as well as development of collaboration with renowned scientists from abroad. Publication of original papers is at high level, many of published papers are world class papers printed in international journals with high reputation. Last but not least, cooperation with companies resulted in patent applications, instrument donation and collaborative projects. The team members worked out several new bioanalytical technologies including microreactors for flow through and batch operation. Several unit operations have been performed using immobilized proteases, glycases or nanoparticles with specific affinities. New detection schemes and sensors based on luminescent quantum dots, chemiluminescence or resistance measurements including the underlaying instrumentation were proposed. Microseparation techniques for the analysis of DNA, proteins, peptides and glycans were developed using discrete capillary columns as well as microfluidic devices. This work also strongly related to the development of new mass spectrometry interfaces. A patent application for

coupling hydrodynamically closed isotachophoretic systems with electrospray ionization was awarded and licensed by the instrument manufacturer. Additional patent applications have been submitted for sample preconcentration and detection systems. Successful collaboration has been established with Agilent - one of the major instrument manufacturers. Research in electromigration methods, so far one of the main topic in the institute performed in the Department of Electromigration Methods, is oriented to development and improvement of progressive chemical methods for trace analysis and analysis of complex samples by capillary electrophoresis (CE). The composition of the team is constant. Research is focused on theory, methodology and new approaches to techniques utilizing electromigration stacking, combination of isotachophoresis (ITP) and capillary zone electrophoresis (CZE), focusing methods, combination of CE and mass spectrometry (MS), and methods based on electromembrane extraction (EME) and novel microextraction techniques. The integrated theory and methodology of the control and utilization of sample induced effects, of migration of analytes across liquid membranes, and of the effects of electromigration across electrophoretic moving boundaries substantially contributed to fundamental electrophoretic knowledge in separation science. In theory and methodology of CE, a new separation principle was described. It focuses weak non-amphoteric ionogenic substances and transfers them to the detector on an inverse electromigration-dispersion profile with pH decreasing towards the anode or cathode for focusing of anionic or cationic analytes, respectively. The important fundamental phenomena were investigated, brought about by omnipresent carbonates in the electrophoresis background electrolytes when alkaline medium is required and detection is performed by conductivity and indirect UV detection. The proposed theoretical approach allows prediction of behavior of individual systems and elimination of problems with qualitative and quantitative evaluation of results of analyses. A method of analysis of trace components in body fluids by on line ITP-CZE was developed allowing the determination of mefolinate, a metabolite of folic acid, in serum, blood and urine. A special form of ITP for sample concentration and elimination of electromigration dispersion was described, which involves isotachophoretic migration in multicomponent systems with moving sharp boundaries. This approach effectively compensates the limited amount of available system components compatible with ESI-MS detection. The developed general strategy for sensitive analyses by capillary ITP with ESI-MS detection is based on the newly formulated model of moving-boundary ITP where both the leading and terminating zones can be formed by various concentration ratios of the same substances, and on the newly defined concept of zone-related boundary mobility that allows describing the selectivity of a system by simple diagrams. Theory, together with recommended ESI-compatible substances of electrolyte system composition, allow advanced selectivity tuning for cationic and anionic ITP within a broad range of analyte mobilities by changing the composition of leading and/or terminating zones. The method offers very sensitive analyses by powerful stacking of the analytes on an ITP boundary. Its potential was demonstrated on a cationic analysis of the fungicide thiabendazole in orange juice and an anionic analysis of ibuprofen and diclofenac in waters with detection limits 0.1 nmol/L (20-30 ng/L). The developed new approaches to architecture of ITP systems include configurations that allow both accumulation of trace substances at a sharp boundary and their separation by generating more than one such boundary. New analytical methods based on EMEs across supported liquid membranes (SLMs) were developed, which brings substantial improvements in speed, selectivity, sensitivity, low costs, elimination of manual sample preparation when compared with contemporary methods for sample clean-up and preconcentration of heavy metal cations in environmental samples; determination of endogenous and therapeutic concentrations of lithium in body fluids; analysis of essential amino acids in body fluids; or trace determination of perchlorate (an inhibitor of iodide uptake to thyroid gland) in drinking and environmental water samples. A universal method, which enables examination of EME performance, was developed using CE with capacitively coupled contactless conductivity detection (CE-C4D). The method has proven to be a useful tool for the determination of SLM

selectivity and has evidenced for the first time that large proteins, such as human serum albumin, are efficiently retained on all examined SLMs and that transfer of other matrix components and analytes is strongly SLM dependent. Furthermore, the effects of selected operation parameters on efficacy and selectivity of EME were investigated and useful ways to their optimization and fine tuning were found by proper choice of the composition of electrolyte solutions and by addition of highly selective SLM modifiers. A completely new methodology was developed using EMEs across free liquid membranes (FLMs). FLMs are stable phase interfaces, their dimensions can be easily determined and manipulated and shapes of FLMs can also be altered. FLMs enable selective micro-extractions of analytes of interest from complex samples and the extraction process can be visualized in real time. These characteristics enable direct processing of raw biological samples, preconcentration of analytes and might help understand the fundamental principle of EMEs across liquid membranes. Various methods for down-scaled and rapid extractions of biological fluids were presented and combined with CE. These include methods for efficient pretreatment of complex biological samples by using micro-electrodialysis, direct coupling of disposable open-tubular ion-exchange precolumns to CE, and direct coupling of SLM extractions to CE. Moreover, the open-tubular precolumns and extraction devices with SLMs were designed for their direct use in commercial CE instruments. For example, a disposable sample pretreatment device with planar SLM was proposed for automated pretreatment and the analysis of formate in blood samples for rapid diagnosis of methanol poisoning. The pretreatment device required only microliter volumes of the blood sample and an organic solvent per extraction, and the overall analytical process including blood withdrawal, filling the SLM device, extraction of blood sample, injection into separation capillary and CE separation of formate from other anions took less than 4 min. The Department of Trace Element Analysis produces constantly results of high quality and develops wide international cooperation. In accordance with the adopted measures of the previous evaluation the department was supported by world-class instrumentation. The general target of research is to develop promising aspects of generation, preconcentration and atomization of volatile compounds for trace elemental and speciation analysis by atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), inductively coupled plasma mass spectrometry (ICPMS), and to employ methods of trace elemental and speciation analysis based on AAS, AFS and ICPMS for applications mainly in biomedical and environmental fields. The main research areas resolved in recent five years were: speciation analysis of toxicologically important metabolites of arsenic; trace elemental analysis in biological and environmental matrices; development of novel atomizers of volatile compounds; new approaches to preconcentration of volatile compounds by in-atomizer collection; generation of volatile compounds of transient metals. Methodologies based on hydride generation (HG), mainly interfaced to cryotrapping (CT) and to AAS and AFS as well as liquid chromatography with ICP-MS detection, for the speciation analysis of As metabolites in biological and other matrices were developed. The new methods were validated and implemented in practical studies. Among important results, the most significant is the development of a HG-CT method (with AAS, AFS or ICPMS detector) for the analysis of As species in homogenates of animal tissues. The method developed is unique since it makes possible a direct determination of trivalent As methylated species in tissues without extraction or digestion. This is practically the only method capable of analysis of unstable trivalent arsenicals in tissues. It helps to push the frontiers of arsenic toxicology. For quantitative analysis of unstable arsenic glutathione complexes, a HPLC method with gradient elution and postcolumn HG was also developed. It was applied for study of enzymatic arsenic methylation. A method based on HG-CT with ICPMS suited for As speciation analysis at ultratrace levels or in very small biological samples was developed. It was already implemented for an in-vitro study of mechanism of arsenic induced diabetes, and a population study of associations between arsenic species in exfoliated urothelial cells and prevalence of diabetes. This is, together with the methods with AFS detection mentioned below, the only method for arsenic speciation analysis at sub-ppt levels in the world. In order to develop

instrumentation and methodology of HG-CT-AFS, a new shielding unit of the atomizer was designed and a new approach to remove moisture from gases introduced to the atomizer. These measures resulted in an excellent performance of the HG-CT-AFS method. Detection limits reached were at least one order of magnitude better than those reported in the literature for the HG-CT-AFS approach and two orders of magnitude better than for our HG-CT-AAS method. It is suitable for analysis of water samples with a very low content of arsenic as well as for arsenic toxicological studies in biological microsamples. Other applications included study of processes in (sub)surface waters in natural As geochemical anomaly, finding a way to determine contaminant toxic arsenic species in N-methylglucamine antimonate drug, or development of a simple method for screening toxic arsenic species in rice and seafood. ICPMS was also proved superior in terms of sensitivity, accuracy, and temporal resolution over the method employing the potassium-binding fluorescent indicator for quantification of potassium concentration in cells. In the vein of development of novel atomizers of volatile compounds for AAS, investigation of dielectric barrier discharges (DBD) plasma sources started. The goals are complex: optimization of atomization conditions for individual analytes, subsequent validation of analytical applications as well as understanding the mechanisms of atomization. Following the study of ability of three plasma-based ambient pressure ion sources (including DBD) to desorb and ionize organic analytes for ambient mass spectrometry, Bi hydride as a model volatile compound was employed to explore perspectives of DBD atomizers. All features of DBD atomizers were compared with those of conventional quartz tube atomizers (QTA). Silanization of the inner DBD surface was essential improving sensitivity (3-times). DBD can be used for the routine determination of Bi, providing satisfactory repeatability and accuracy. This is the first report in the literature comparing performance of DBD atomizers with the conventional QTA. Generation of volatile compounds makes analyte preconcentration easy by collection in quartz atomizers for AAS. Using our quartz device for hydride collection, termed "trap-and-atomizer device", a procedure for determination of Sb in acetic acid leachates from pewter cups was developed, verified and applied. For preconcentration of Bi and Pb, optimization of experimental parameters followed by development and application of routine analytical methods were performed. Mechanisms of preconcentration and analyte atomization were studied and explained with the help of AAS and ICPMS detectors as well as of autoradiography and gamma radiometry. Also the capacity of the quartz surface was estimated and nature of bismuth interference during lead preconcentration was elucidated. Subsequently, a modular device, which makes possible to use a sapphire tube as the atomizer, was developed. Bi hydride was employed as a convenient model for volatile species. No significant difference between quartz and sapphire regarding atomization as well as in atomizer collection was found and virtually the same analytical characteristics were achieved. The use of sapphire tubes has a great potential for several applications including in-atomizer collection of volatile compounds of transient metals. Regarding generation of volatile compounds of transient metals, systems for generation of silver were optimized with the help of radiotracers. Transport losses were practically eliminated and detection limit as low as 1 ng/ml were reached. The further transient metal that was studied was gold. The first experimental evidence was provided that the product of its generation was in the form of nanoparticles. Efficiencies of individual steps of the generation process were determined using radiotracers. The unfavorable feature of generation of volatile compounds of Ag and Au is their slow release from the reacting solution reflected in broad signals. Therefore, the possibility of collection of volatile Ag compound on a quartz surface was investigated. A modified modular design of the quartz tube atomizer was employed. Fast volatilization and atomization of the analyte were realized. Preconcentration efficiency reached 95%. The limit of detection was 0.11 ng/ml, nine times better than in the on-line atomization mode. The accuracy of the method was tested using water reference materials. Department of Fluid Phase Separations was formed in July 2013 by combining former Department of Liquid Phase Separations and Department of Separations with Compressed Fluids. It is oriented to development of isoelectric focusing methods, chromatographic instruments, application of near-

and supercritical water as an agent to treat the siliceous surfaces, study of supercritical fluid chromatography as a tool to probe the partition behaviour of organics in biphasic systems composed of an ionic liquid and supercritical carbon dioxide, and study of solubilities of organic nonelectrolyte solids in pressurized hot water. The long-term program of theoretical and methodological development of isoelectric focusing (IEF) in various scales and separation formats continued with numerous studies including preparative divergent flow IEF without carrier ampholytes for separation of complex biological samples, divergent flow IEF for the separation and preparative analysis of peptides, a new solution IEF device for micropreparative separations of peptides and proteins, the design of a simple power supply for power load-controlled IEF, or the experiments with tapered capillaries to confirm earlier theoretical results indicating that separation efficiency of IEF in a capillary with a continuous taper should be superior to that in a constant-diameter capillary. Separations of microorganisms by electromigration methods largely capitalized on the strong theoretical and methodological background in the various variants of IEF. Recent original achievements have included the use of electromigration techniques and MALDI TOF MS in the separation of phenotypically indistinguishable *Candida* species, determinations of the isoelectric point differences in biofilm-positive and biofilm-negative strains of bacterial species, or the trace analysis of microorganisms in real samples by combination of a filtration microcartridge and capillary IEF. Also, the advent of supercritical water-etched fused silica capillaries has paved the way to unique separations of important microorganisms by both capillary IEF and capillary zone electrophoresis (CZE). IEF in tapered capillaries has been employed to improve the separation of several species of probiotic bacteria from cow's milk (*Lactobacillus* sp.), and also to accomplish the separation of several important phytopathogens of the *Dickeya* genus. In these studies, subsequent MALDI TOF MS fingerprinting of the separated zones was shown to be very useful in confirming the identities of the individual bacterial species. CZE in constant-diameter, surface-corroded fused silica capillaries allowed the separation of methicillin-resistant (MRSA) from methicillin-susceptible (MSSA) *Staphylococcus aureus* strains. Apart from improving the separation efficiency, the use of supercritical water-treated and modified capillaries has also led to reduction of the detectable amount of bacteria. To date, the electromigration separations of microorganisms in supercritical water-treated capillaries have proved to be useful in several application areas including food production (dairy industry), agricultural crop protection and human health (clinical microbiology). In particular, the fast and low-cost method to differentiate between MRSA and MSSA appears important because MRSA is a frequent cause of both nosocomial and community-related infections. The long-term experience in the design and construction of high-pressure equipment for analytical extractions gradually resulted in testing of near- and supercritical water as an agent to treat the surfaces of analytical separation devices made of fused silica or glass. An in-house-constructed high-temperature and high-pressure apparatus for the purpose has made it possible to treat both fused silica capillaries and the glass substrates for lab-on-a-chip applications. In fused silica capillaries etching with supercritical water can produce extensive changes in both internal diameter and the inner surface morphology depending on the operating conditions. The application of tapered capillaries results in enhanced separation efficiency of capillary IEF of proteins and bacteria. In turn, the constant-diameter capillaries with different degrees of the inner surface corrosion are very useful in CZE for unique separations of closely related strains of important bacteria. A simple thermodynamic model for prediction of aqueous solubility of amorphous silica as a function of temperature and pressure was developed as well. A simple and affordable HPLC platform to operate with micro- and nanocolumns was constructed that allows splitless nanocolumn gradient separations in either constant pressure or constant flow rate mode and provides a suitable springboard for further development toward portable HPLC setup. The development of chromatographic column technology has largely been focused on monolithic silica-based capillary columns for LC. Monolithic silica columns were prepared by the sol–gel method involving acidic hydrolysis of tetramethoxysilane in the presence of suitable porogen. Several aspects including

the effect of preparation temperature on the chromatographic properties of the monolithic silica skeleton, chemical modifications of the monolithic columns with suitable zwitterionic agents for application in hydrophilic interaction chromatography, and modification of the monolithic columns with liposomes to produce a chromatographic retention model system mimicking the interactions of pharmaceuticals with cell membranes were studied. The long-term program of using capillary column supercritical fluid chromatography as a tool to probe the partition behaviour of organics in biphasic systems composed of an ionic liquid and supercritical carbon dioxide continued with the systems involving butylmethylimidazolium methylsulfate and butylmethylimidazolium trifluoromethanesulfonate. Here, an extensive data base of experimental results was used to upgrade and test a generalized predictive correlation for solute partition coefficients in ionic liquid–supercritical carbon dioxide systems. In the field of pressurized liquid extraction of plant materials, a new procedure to evaluate the antioxidant activity of plant extracts was developed. The procedure involved an off-line combination of pressurized liquid extraction and electron paramagnetic resonance spectroscopy, with the latter method serving to monitor the activity of extracted antioxidants in scavenging the free radical probes added to the extracts. The studies included a comparison of relative merits of lower aliphatic alcohols and water as the extraction solvents. A comparative study of several methods to extract important quaternary benzo[c]phenanthridine alkaloids from plant material confirmed the competitive position of pressurized liquid extraction of plant materials when compared with classical extraction methods such as maceration or Soxhlet extraction. The long-term program of determination of high-temperature, high-pressure aqueous solubilities of heavy organic nonelectrolyte solids continued with the studies of ferrocene, selected organic electronic materials, and macrocyclic hosts (calix[6]arene and 4-*tert*-butylcalix[4]arene) as the solutes. No previous data on the aqueous solubilities of these compounds at elevated temperature and pressure have been available in the literature. In the theoretical aspect of this topic, a group contribution model was developed to correlate and/or predict the aqueous solubilities of organics over a wide range of temperature (~200 K). Department of Environmental Analytical Chemistry is oriented to the development of new methods and the instrumentation for the chemical analysis and/or characterization of environmental systems, minor and trace gaseous components, nano-particles and aerosols in the air, the composition of nanoparticles, chemical analyses in aquatic systems, soils and sediments with respect to organic and inorganic pollutants and their accessibility to plants, food chains, health risk for human being and other topics dealing with environmental protection. Methodology is based on separation and preconcentration techniques in tandem combination with novel specific detectors, GC and LC, MS, and AS methods. For controlled long-term nanoparticles inhalation experiments with small animals, a new instrumentation was set up and deposition of inhaled nano-particles in organs of small animals was studied. A study of formation, transport and chemical composition of fine particles and nanoparticles (metals, organic compounds) in heavily polluted regions of the Czech Republic was elaborated. The results acquired within frame of inhalation projects of small animals and sampling of air in heavily polluted regions followed by chemical analysis of fine particles and nanoparticles stimulated a public debate concerning the health risks of nanoparticles and fine particles from the point of quality of life of human population. A novel portable device for the analysis of energetic materials at trace concentrations in environment was developed. Photochemical processes of nitrous acid (HONO) that take place in the atmosphere were studied, and sources, interference within HONO measurements, intercomparison of data of techniques and instruments for the determination of HONO were revealed. International cooperation with partners in Spain within the study of photochemical processes of HONO in the atmosphere in EUPHORE for which a specially designed annular diffusion denuder for elimination of positive artifacts in carbonaceous aerosol sampling was developed, illuminated some questions arising from the presence of atmospheric oxidants in typical urban and semi-rural air. The department was involved also in projects focused on the study of effect of commercially produced TiO_2 nano-particles on human blood cells, and projects

dealing with a characterization of organs/tissues of mice that were exposed to different nanoparticles (CdO, PbO and MnO, Mn_2O_3). During the evaluated period, research productivity of researchers of the Institute of Analytical Chemistry constantly increased as can be easily revealed at web pages http://www.lib.cas.cz/arl/statistika/periodika/uiach-o/dA and http://www.lib.cas.cz/aagama/statistika/periodika/uiach-o. The average impact per article had increased regularly yearly from 2.3 in 1993-97 over 3.1 for 2006-10 to 3.6 for 2010-14. Scientific teams at the institute were relatively successful in national competitive funding and solved in average 25 projects yearly, 8 new projects per year. The most successful year was 2013 when research topics were supported by 30 grants. Individual departments were supported by purchase of instrumentation needed for research and method development, totally in the sum of 1 744 741 EUR. The most successful was the Department of Bioanalytical Instrumentation that gained 409 235 EUR from competitive funding and was also supported by the institute with 99 921 EUR and by the Academy of Sciences of the Czech Republic with 54 190 EUR. Total sum that was given to the Department of Fluid Phase Separations was 507 971 EUR enabling to modernize scientific equipment. Also Department of Environmental Analytical Chemistry was successful in gaining money from projects as well as from the institute total worth 206 538 EUR for purchase of instrumentation. The Department of Trace Element Analysis was supported significantly by the institute and Academy of Sciences with 312 582 EUR without any income from grants to invest. The institute is involved in two Centres of Excellence – the Centre of Advanced Bioanalytical technologies and the Centre for Studies and Toxicity of nanoparticles. All teams are involved in preparation of the new strategy of the Academy of Sciences. In addition to publishing activity and success in national and international competitive funding, some results, new methods and instrumentation were applied in various fields of industry, agriculture, environmental protection, health care and protection of cultural heritage.

Cooperation with universities was fruitful for both sites – by transfer of the newest achievements in the research to students within lectures during teaching at universities in Brno, Prague and Pardubice, and by involving the students to the research work at the institute. An intensive cooperation was realized also with CEITEC, the scientific center in the fields of life sciences, advanced materials and technologies supported by structural funds of European Union. International reputation of the institute is at high level, each year renowned scientist from around the world are coming to give lectures at the institute and to take part at the international conference Central European Conference on Electrophoresis that has expanded the scope to International Interdisciplinary meeting on Bioanalysis and is organized by the institute each year in Brno.

Research Report of the team in the period 2010–2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

Scientific team	Department of Bioanalytical Instrumentation

In the past five years the department continued in its activities focused on bioanalysis research with the stress on instrumentation, microfluidics and nanotechnologies. During this period the department has also absorbed the former department of glycomics and proteomics as recommended during the evaluation in 2010. While several workers of this former department are still on maternity leave, the consolidation of the department after the merger can be now considered complete. As seen from the age structure of the group, the main part of the team are students working at the department on their PhD. The following text summarizes some of the most important activities of the department since the previous evaluation.

Instrumentation

Instrumental research has been one of the most important directions of the department work and the major part was directed towards the CE/MS interfacing. Coupling of hydrodynamically closed large bore capillary isotachophoresis with electrospray mass spectrometry has been previously difficult since most of the current designs rely on applying flow (pressure driven) at the coupling point. The vertical ITP analyser with coupled columns doesn't provide means for combination with the current commercially available ESI interfaces. In collaboration with the manufacturer of the capillary isotachophoretic analyzer an interface allowing attachment of the hydrodynamically closed system to electrospray mass spectrometer was developed. The work has already resulted in an awarded patent and the instrument is on the market. This was a joint project with colleagues from Villa Labeco, Slovakia.

In another project related to the electrophoresis-MSI coupling a joint work with our colleagues from Italy was published on simultaneous analysis of cocaine and its metabolites in urine by capillary electrophoresis -electrospray mass spectrometry using a pressurized liquid junction nanoflow interface. Here the interface for electrospray ionization mass spectrometry has been constructed for use with capillary zone electrophoresis. The design was based on a pressurized system requiring both the injection and detection points to be kept at elevated pressures. The system was applied to the analysis of drugs of abuse and related metabolites in urine.

In cases when sample components do not readily ionize a labelling protocol for MS analysis is necessary. For example, analysis of glycoproteins is at the forefront of biology related research. In an ongoing project new means of cationic labelling of oligosaccharides for electrophoretic preconcentration and separation with contactless conductivity and MS detection are under development. The published work describes the development of a separation protocol for fast separations of oligosaccharides using capillary electrophoresis with conductivity detection. A new cationic labelling protocol was developed allowing isotachophoretic preconcentration of the sample with a potential for high sensitivity ESI/MS characterization.

The current state-of-the-art of the CE/MS technology has been published in an invited review paper bringing innovative views on the topic described in the past several years -On-line CE/ESI/MS interfacing: Recent developments and applications in proteomics Special attention was paid to the new technology advancements discussing the potential for applications in proteomics.

Besides mass spectrometry, optical detection is an important technology, especially in high sensitivity miniaturized systems. While the optical alignment may be often critical and need precise fabrication new technologies may be useful in designing optomechanical parts. 3D printing was successfully tested for this purpose and the results were published in the paper: Fluorescence detector for capillary separations fabricated by 3D printing. This paper describes the use of a new fabrication technology – 3D printing – for preparation of a versatile high sensitivity fluorescence detector. Since the computer design files developed in this work are universal for all 3D printers and can be freely downloaded from the journal web site, anybody interested in using inexpensive, yet highly sensitive, fluorescence detector the can easily replicate the system for his/her own work.

Analysis of common biology related samples typically involves larger amount of material corresponding to hundreds of thousands or more cells. Thus the information about the chemical content is an average corresponding to the sample as a whole. As the analytical technology evolves there is an increasing interest in quantitative chemical analysis on the single cell level. When dealing with ultimate sensitivity requirements, such as in detection of bioluminescence originating from single cells, one has to use the most sensitive detection mode available, such as the photon counting. In this work a new system for analysis of caspase 3 in single apoptotic cells using chemiluminiscence with single photon counting detection has been designed and some of the results were published in the paper: Bioluminescence determination of active caspase-3 in single apoptotic cells

Microfluidics and nanotechnology

This part of the research has a strong collaborative part. In the first joint project with colleagues from IBP, Brno the results were published in the paper: Fabrication and characterization of solid mercury amalgam electrodes for protein analysis. Electrochemistry of biopolymers is an important emerging field of electrochemistry commonly employing mercury electrodes. The protocol for preparation of thin film microelectrodes was developed and characterized using vacuum sputtering, and photolithography. In the final step electroplating was used for creation of thin film mercury amalgam electrodes. This technology practically eliminates the risks associated with toxic mercury since the designed miniaturized electrode system, with the electrode array defined by photolithography and patterned photoresist consume only pmols of mercury. The related instrumentation was developed during this work (nA – pA regulated constant current source) together with protocols for electrogeneration of the mercury amalgam and its characterization by electron microscopy. The collaborators from IBP have applied the prepared electrodes for electrochemical studies of proteins using their newly developed H-peak technique.

Electrochemical reactions on a nanoscale can be also monitored optically as described in our joint project with colleagues from Germany: Detection of electrochemiluminescence from floating metal platelets in suspension. Here the thin film technology and photolithography has been employed for formation of structured free floating metal nanoparticles. The nanoparticles have been tested as nanoelectrodes for generation of electrochemiluminiscence in a capillary format.

In part of our ongoing research, started during the past five years, the work has been focused on the use of inorganic luminescent semiconductor nanocrystels, as described in the paper: Conjugation reactions in the preparations of quantum dot-based immunoluminescent probes for analysis of proteins by capillary electrophoresis. The use of semiconducting inorganic nanocrystals as luminescence labels for detection of proteins and DNA is a new trend for achieving high sensitivity and selectivity in bioanalysis. In this work (Capillary electrophoresis immunoassays with conjugated quantum dots) conjugation reactions suitable for preparation of quantum dot-based immunoluminescent probes for analysis of proteins by capillary electrophoresis were described. In a continuation of this research the work has been extended to test the laboratory synthesized quantum dots for DNA mutation detection using FRET technology and high temperature DNA melting. This work is being conducted with our industrial partners from Genomac, Prague.

Besides quantum dots applications the work has also been focused on the use of other types of nanoparticles for both detection and selective preconcentration purposes. In the former direction a technique for preparation of photodeposited silver nanoparticles for on-column surface-enhanced Raman spectrometry detection in capillary electrophoresis was developed. Nanoparticle surface enhanced Raman spectrometry is viewed as a potentially powerful on-line detection/identification technique for analyses of extremely small amounts (pmol-attomol) of biological samples separated by capillary electrophoresis. This work described a laser sintering method for creation of a nanoparticle rich detection zone inside a 50 micron capillary channel and its use for on-line measurement of the SERS spectra of the separated zones.

The use of inorganic nanoparticles for selective enrichment of phosphorylated peptides has been described in the paper: Nanoparticle-modified monolithic pipette tips for phosphopeptide enrichment. Characterization of protein phosphorylation is necessary for understanding of the cell signalling processes. Selective enrichment of phosphopeptides is of a key importance during the analyses. New polymeric monolithic material has been developed with embedded iron nanoparticles with high affinity to phosphoproteins allowing highly selective analyses of minute sample amounts. The material has been immobilized in disposable pipette tips or in capillary columns for flow-through operation.

During joint experiments with collaborators at DCU in Ireland an interesting phenomenon was observed in fused silica capillaries coated with a transparent protection/cladding layer. The propagation of UV radiation, originally intended for detection purposes, has caused photopolymerization at the inner capillary wall far from the radiation entrance. This phenomena has been utilized for surface initiated photopolymerization of porous monolith at the capillary wall and described in a joint paper: Evanescent wave-initiated photopolymerisation as a new way to create monolithic open-tubular capillary columns: use as enzymatic microreactor for on-line protein digestion. In general, photopolymerization techniques are being developed for surface modifications of microfluidic channels. This works exploited the light-guiding property of a fused silica capillary for surface initiated polymerization of an acrylic polymer using UV LED source. The UV radiation, propagating inside the silica material, created evanescence wave at the capillary inner surface allowing controlled surface photopolymerization impossible to achieve by other means.

Flow through microreactors with immobilized enzymes are becoming an important tool for characterization of biopolymers. The processing speed depends on the density of active enzyme sites accessible during the substrate flow. In a recently published work (Oriented immobilization of peptide-N-glycosidase F on a monolithic support for glycosylation analysis) a genetically engineered peptide-N-glycosidase F enzyme (incorporating a gluthathion transferase sequence for binding) was used for oriented immobilization on the surface of the monolithic material of the microreactor providing a substantial increase of the density of the active enzyme sites for extremely fast reactions. This was a joint project with colleagues from Hungary.

Analysis of glycoproteins is at the forefront of biology related research. After the glycan release the separation of individual oligosaccharides, often after the chemical labelling for sensitive detection, is the key step of the analytical protocol. For this purpose a new separation protocol was developed for very fast separations of fluorescently labelled glycans using an automated microfluidic system, originally designed for DNA and SDS protein electrophoresis. This technique allows decreasing the time of analysis by an order of magnitude in comparison to the standard capillary analyzers. The results of this joint project with colleagues from Germany, Australia and Hungary have been described in a paper: Chip-based CE for rapid separation of 8-aminopyrene-1,3,6trisulfonic acid (APTS) derivatized glycans.

International interdisciplinary conference on bioanalysis – CECE (www.cece.org)

The CECE conference has become a yearly constant in our work. With over 100 participants (over 170 in 2015) it is a unique event for learning new trends in bioanalysis and finding new scientific contacts. The conference abstracts are indexed on the WOS.

Research Report of the team in the period 2010–2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

Scientific team	Department of Electromigration Methods

Characteristics of main research directions

Research in the department of Electromigration methods is oriented to the development and improvement of progressive chemical methods for trace analysis and analysis of complex samples by capillary electrophoresis (CE) and other electromigration techniques. The classical research field of the so-called “Brno electrophoresis school” comprising theory, methodology and new approaches to techniques utilizing electromigration stacking, combination of isotachophoresis (ITP) and zone electrophoresis (CZE) and focusing methods was considerably extended in the time period 2010-2014. In the beginning of 2010, the department obtained CEMS instrumentation which allowed to include the research of application of electrophoretic stacking principles in CE-ESI-MS analyses. Besides this, a novel perspective research direction was opened, comprising methods based on electromembrane extraction (EME) and microextraction techniques. The results of the individual research directions are summarized below.

Theory and methodology of CE

A new separation principle in CE was described [1] consisting in focusing of weak non-amphoteric ionogenic substances and their transport to the detector on an inverse electromigration-dispersion profile with pH decreasing towards the anode or cathode for focusing of anionic or cationic analytes, respectively. The theoretical principles of this technique were formulated and the conditions under which analytes are focused on the profiles of the mentioned type were defined and experimentally verified.

A very important fundamental phenomena was investigated, which is brought about by omnipresent carbonates in the electrophoresis background electrolytes when alkaline medium is required and detection is performed by conductivity and indirect UV detection [2]. Computer simulations and experiments have shown that the absorption of carbon dioxide from air can cause formation of moving system zones and system boundaries that negatively influence the migration of analytes. The higher the pH of the BGE, the stronger these effects and the broader their spectrum, involving (i) changes of effective mobilities and selectivity due to changes in pH of the BGE, (ii) occurrence of additional peaks, dips or more complex disturbances in the detection signal, (iii) modification of the separation process due to temporary interactions with sample components. The proposed theoretical approach allows prediction of behavior of individual systems and elimination of problems with qualitative and quantitative evaluation of results of analyses.

Our research aimed at sample preseparation and selective preconcentration of trace components in body fluids via on line combination of ITP and CZE resulting in the development of a new method for determination of mefolinate, a metabolite of folic acid, in serum, blood and urine [3].

Electrolyte systems for the combination of CE-MS

An innovative method was developed for easy and sensitive analyses by CE with ESI-MS detection [4], which is based on the theory of extended ITP. It is well known that ESI-MS is compatible with very limited range of background electrolytes, and, thus, it is very difficult to reach the required selectivity and to use stacking in order to enhance sensitivity. The introduced theoretical model of extended ITP offers powerful on-line analyte stacking in ESI-compatible electrolytes by adding a controlled concentration of the leading ion to the terminating zone. The method allows advanced tuning of selectivity within a wide range of analyte mobilities by changing the composition of both leading and terminating zones, and description of the selectivity of a system by simple diagrams. The potential of the method was demonstrated on the example of analysis of thiabendazole with a detection limit of 0.1 nM (20 ng/L) and its determination in orange juice.

A general strategy for sensitive analyses by capillary ITP with ESI-MS detection was formulated [5,6]. It is based on the extended model of ITP migration created in multicomponent systems with moving sharp boundaries and on the newly defined concept of zone-related boundary mobility [6]. In moving-boundary ITP systems, both the leading and terminating zones can be formed by various concentration ratios of the same substances. This approach effectively compensates the limited amount of available system components compatible with ESI-MS detection. The presented theory, together with recommended ESI-compatible substances for of electrolyte system composition, allow advanced selectivity tuning for cationic and anionic ITP within a broad range of analyte mobilities by changing the composition of leading and/or terminating zones. The method offers very sensitive analyses by powerful stacking of the analytes on an ITP boundary which was demonstrated on the example of anionic analysis of the drugs ibuprofen and diclofenac in waters with detection limits on the analyte concentration level 0.1 nM (20-30 ng/L) [5]. Further research of new combinations of electrolyte systems and new ways of improving selectivity and sensitivity of electrophoretic configurations resulted in development of new approaches to architecture of ITP systems. Based on the formulated of general principles of moving-boundary ITP [6], system configurations were developed that allow both accumulation of trace substances at a sharp boundary and their separation by generating more than one such boundary [7]. The limited amount of available system components compatible with the used detection technique is effectively compensated by using multicomponent systems including either spacer substances or systems with multiple moving-boundary subsystems.

Electromembrane extractions

During initial considerations regarding EMEs, literature surveys showed that electromigration of ionic species across phase interfaces, especially across supported liquid membranes (SLMs), may be very selective and may open new horizons in analytical separations. In EME, a short segment of porous polypropylene hollow fibre is usually impregnated with an organic solvent to form the SLM and constitutes a low cost, single use, disposable extraction unit. The research therefore first started with reliable monitoring of the selectivity of SLMs. A universal method was developed to follow the performance of a SLM by using CE with capacitively coupled contactless conductivity detection (CE-C^4D) [8]. The method has proven to be a useful tool for the determination of SLM selectivity. The CE-C^4D method has evidenced for the first time that large proteins, such as human serum albumin, are efficiently retained on all examined SLMs and that transfer of other matrix components and the analytes is strongly SLM dependent. Obviously, to apply a

correct SLM in particular sample pretreatment, rapid determination of the transfer of analytes and matrix compounds across the SLM is necessary, which requires the use of an analytical method with universal detection technique [8].

Further research was focused on new analytical application potential, which may be opened by electric field enhanced transport across chemically tailored liquid membranes. Several new selective and sensitive analytical methods were developed, based on EMEs. A method that serves for sample clean-up and preconcentration of heavy metal cations in complex samples was presented [9]. Heavy metal cations are transferred from aqueous solutions across a thin wall of a hollow fibre into aqueous acceptor solution. Acceptor solution is then directly used for injection into CE instrument. Applications of this method were demonstrated on determination of zinc in drinking water and powdered milk samples.

New contributions to the development of instrumentation for clean-up and preconcentration of samples with complex matrices by EME were described [10-13]. EME was applied to determination of endogenous and therapeutic concentrations of lithium in body fluids [10] and also to analysis of essential amino acids in body fluid [11]. Some of these amino acids (e.g. phenylalanine and branched chain amino acids) are markers of inborn metabolic disorders. Trace determination of perchlorate (an inhibitor of iodide uptake to thyroid gland) was also developed in drinking and environmental water samples [12]. Based on theoretical considerations, a new approach to the application of EME was proposed and tested. Stabilized constant
d.c. current was applied during extraction instead of constant d.c. voltage, which offered significantly better repeatability of the extraction process [13]. This improvement was demonstrated on analyses of basic drugs and amino acids in standard solutions and body fluid samples and presents a basis for a more generous and wider application of this newly developing extraction technique. The effects of selected operational parameters on efficacy and selectivity of EME were also investigated and useful ways to their optimization and fine tuning were found by proper choice of the composition of electrolyte solutions and by addition of highly selective SLM modifiers [14,15].

A new methodology was developed using miniaturized EME across free liquid membrane (FLM) [16-18]. FLMs are stable phase interfaces, their dimensions can be easily determined and manipulated and shapes of FLMs can also be altered. FLMs enable selective micro-extractions of analytes of interest from complex samples and the extraction process can be visualized in real time. These characteristics enable direct processing of raw biological samples, preconcentration of analytes and might help understand the fundamental principle of EMEs across liquid membranes.

An analytical method, which used simple micro-electrodialysis (micro-ED) procedure for pretreatment of complex biological samples was presented [19]. Proteins and other high molecular mass matrix components from biological samples were efficiently retained on ultrafiltration membrane with defined molecular weight cut-off value, whereas ions with size smaller than the pore size of the membrane passed through into an acceptor solution and could be subsequently analyzed using CE-C^4D. Both solutions were aqueous and less than 1 microliter or real sample was required for micro-ED, which is advantageous in non-invasive sample withdrawal techniques. Applicability of the method was demonstrated on determination of major inorganic cations in blood serum and plasma, and in untreated whole blood.

Novel microextraction techniques

The research of microextraction techniques resulted in both improvement of the instrumentation and important practical applications. Various methods for down-scaled and rapid extractions of biological fluids were presented and directly combined with CE [20-25]. These include methods for efficient pretreatment of complex biological samples by using: (i) direct coupling of disposable open-tubular ion-exchange precolumns to CE, (ii) direct coupling of SLM extractions to CE and (iii) direct coupling of micro-dialysis to CE. Moreover, the open-tubular precolumns and extraction devices with SLMs were designed for their direct use in commercial CE instruments. In the first approach, disposable open-tubular ion-exchange precolumns were connected to analytical separation capillary. Matrix components from complex samples (e.g. proteins from body fluids) were retained on the active sites of the precolumn and small analytes (e.g. inorganic ions) migrated freely into the separation capillary [20]. Further, a simple sample clean-up device, based on disposable SLMs, was developed for clean-up of complex samples [21]. The SLM was inserted between the donor (blood serum, plasma, etc.) and acceptor aqueous solution and formed a selective interface between the two solutions. Analytes were selectively transported through the SLM by diffusion [21] or by electromigration [22], whereas matrix components were eliminated by this SLM. The developed methods could be applied to on-line determination of major as well as minor components of complex samples. The method of selective transport of analytes across SLMs and in-line coupling to CE was extended for use in commercial instrumentation [23, 24]. A disposable extraction device was proposed and assembled, which may be placed into a conventional microvial vial of CE instrument, and, thus, it is compatible with injection system of Beckman PACE [23] and/or Agilent [24] instruments. The devices were used for automated pretreatment and analysis of formate in undiluted whole blood and serum samples [24] and of basic drugs in various undiluted human body fluids [23]. All analytical procedures except for filling the pretreatment device with donor and acceptor solutions, i.e., extraction across SLM, injection of the extracted sample and CE-UV determination of analytes, were performed fully automatically. The pretreatment device required only microliter volumes of blood sample and organic solvent per extraction and the overall analytical process including blood withdrawal, filling the SLM device with respective solutions, extraction of blood sample, injection into separation capillary and CE separation of analytes from other compounds was much faster than for standard analytical methods. The method was proved useful by direct determination of elevated formate concentrations in undiluted serum samples of a methanol intoxicated patient [24]. Due to its compatibility with currently commercially available CE instrumentation, disposability of extraction devices, minimum sample handling/consumption, and short extraction/analysis times, the developed method might be attractive for rapid diagnosis of methanol poisoning in clinical and toxicological laboratories.

Last but not least, in-line coupling of micro-electrodialysis to CE was proposed and examined [25]. The system proved to be useful as fast and simple method for analysis of formate in minute volumes of human blood for rapid diagnosis of methanol poisoning.

Other scientific outputs

An inherent part of department´s output were a number of well-cited invited review papers [26-35]. Most of them [29-35] were invited reviews for the special biannual issue of Electrophoresis that have impact on formation of the view of the scientific community on particular topics of separations based on electromigration.

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Research Report of the team in the period 2010–2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

Scientific team	Department of Trace Element Analysis

The general target of research of the team is

A. to develop promising aspects of generation, preconcentration and atomization of volatile compounds for trace elemental and speciation analysis by atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), inductively coupled plasma mass spectrometry (ICP-MS), and

B. to employ methods of trace elemental and speciation analysis based on AAS, AFS and ICP-MS for applications mainly in biomedical and environmental fields.

The research of the team was in recent five years supported by the following grant projects: “Improvement of Cryogenic Trapping Arrangement for Arsenic Speciation by Hydride Generation -Atomic Absorption Spectrometry”, Grant Agency of the Charles University, Project No. 133008, 2008-2010 (PI M. Svoboda)

"Analytical Laboratory for Development of Biomarkers of Environmental Exposures to Arsenic"; Gillings Innovation Labs project, UNC School of Public Health (USA), 2008-2011, (Czech PI J. Dědina)

"Speciation analysis of toxicologically important As species: development of instrumentation and methodologies"; Czech Science Foundation, Project 203/09/1783, 2009-2011 (PI J. Dědina)

"Quartz trap for ultratrace determination of lead and tin – optimization, detector comparison and mechanistic study"; Czech Science Foundation, Project 206/11/P002, 2011-2013 (PI J. Kratzer)

”Development of methods of arsenic speciation analysis for toxicological research applications”; Ministry of education, youth and sports of the CR, program VES 12- LH Kontakt II for cooperation with USA Project LH12040, 2012-2014 (PI T. Matoušek)

”Method optimization for determination of toxicologically relevant arsenic species in biological matrices”; Cooperation of ASCR and CONACYT, Mexico, Project 004MX2011, 2012-2014. (Czech PI T. Matoušek)

"Development and optimization of new analytical methods for speciation analysis of arsenic"; ASCR internal program for international collaboration support, project M200311271, 2012-2013 (PI S. Musil)

"Journal Grant for International Authors (sponsoring international scientific visit in Trace Element Speciation Laboratory, Scotland, UK)"; Royal Society of Chemistry; 2013 (PI S. Musil)

"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)

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

The main research areas resolved in recent five years were:

(i) speciation analysis of toxicologically important metabolites of arsenic;
(ii) other applications of trace elemental analysis in biological and environmental matrices;

(iii) development of novel atomizers of volatile compounds;

(iv) new approaches to preconcentration of volatile compounds by in-atomizer collection;
(v) generation of volatile compounds of transition metals.

Speciation analysis of toxicologically important metabolites of arsenic Methodologies based on selective hydride generation (HG), mainly interfaced to cryotrapping (CT) and to AAS, ICP-MS and AFS, as well as liquid chromatography with ICP-MS detection, for the speciation analysis of As metabolites in biological and other matrices were developed. These newly developed methods were validated and implemented in practical studies.

In the vein of development of the CT procedure it was found that dryer tubes based on tubular Nafion membranes caused significant losses of dimethylarsine and complete loss of trimethylarsine. Since Nafion membrane dryers have been commonly used in analytical atomic spectroscopy, implications for trace and speciation analysis of arsenic are discussed. A dryer based on sodium hydroxide pellets with appropriate size is proposed as an alternative, safe for all arsines [1]. This research has been completely performed by our team.

A number of other important results was reached, the most significant is the development of a HG-CT method (with AAS, AFS or ICP-MS detector) for the analysis of As species in homogenates of animal tissues. The method developed is unique since it makes possible, in contrast to other so far published procedures, a direct determination of trivalent methylated As species in tissues without extraction or digestion. This is practically the only method capable of analysis of unstable trivalent arsenicals in tissues, effectively pushing the frontiers of arsenic toxicology.

The HG-CT-AAS method based on selective hydride generation directly from sample slurry was developed and validated for liver tissue. The limits of detection below 6 ng As/g of tissue make this method suitable even for studies examining low exposures to As [2]. Our team completely developed the method, which was then transferred to
U.S. laboratory, where it is routinely used. Because of availability of suitable samples, presented analyses of liver tissue were performed there. That is why corresponding author is from the cooperating U.S. laboratory.

Results of a study of stability of methylated trivalent arsenic metabolites in cells and tissues performed by the HG-CT-AAS method suggested that, unlike urine, samples of tissues or cells collected in human population studies provided suitable material for the quantitative, oxidation state specific analysis of As species, assuming that these samples were properly handled and stored prior to the analysis [3]. Our team performed completely the analytical method development and analyzed the tissues and cell samples. Our collaborators in USA who provided us with all the samples performed comparative analyses of the same samples prior to shipping them to our laboratory.

A method based on HG-CT with ICP-MS suited for As speciation analysis at ultratrace levels or in very small biological samples was developed [4]. To our best knowledge, this

is, together with the methods with AFS detection mentioned below, the only method for arsenic speciation analysis at sub-ppt levels in the world. Method was applied to analysis of water samples containing < 1 ng/ml of total arsenic and microsamples of cells. Method opens new possibilities in toxicology of chronic exposure to arsenic. Our team completely developed the method and performed all the presented analyses. Pilot experiments were performed by a team member in Canada, our collaborators from USA and Mexico provided us with the test samples of urothelial cells collected in their field study. The method was also transferred to U.S. laboratory, where comparative analyses were performed on the same samples, and where it is presently routinely used for toxicological studies. For example, it was implemented for an in-vitro study of mechanism of arsenic induced diabetes [5] and for a population study of associations between arsenic species in exfoliated urothelial cells and prevalence of diabetes [6]. A member of our team was involved in setting the method and training the personnel in the collaborating laboratory as well as adjusting the method for specific types of samples analyzed in those studies.

In order to develop instrumentation and methodology of HG-CT-AFS, a new shielding unit of the atomizer was designed [7]. The respective patent was fully granted to our institute in recognition to the contribution of our team members to the development of the shielding unit. Together with the new approach to remove moisture from gases introduced to the atomizer (discussed above), these measures resulted in an excellent performance of the HG-CT-AFS method [8]. Detection limits reached were at least one order of magnitude better than those reported in the literature for the HGCT-AFS approach and two orders of magnitude better than for our HG-CT-AAS method. It is suitable for analysis of water samples with a very low content of arsenic as well as for arsenic toxicological studies in biological microsamples [8]. Our team completely developed the AFS instrument, constructed the advanced flame-in-gasshield atomizer, developed the analytical method and performed the presented analyses of water samples and bladder exfoliated cell samples. Comparative analyses were performed with ICP-MS instrumentation by collaborators in USA who also provided us with the bladder exfoliated cell samples collected from residents in Mexico.

An established method based on ion-pairing HPLC-ICP-MS, used and published by several groups of authors, was compared to HG-CT-AAS. Serious analytical artifacts were proved in real biological matrices. In fact, unstable trivalent As species were lost in the HPLC-ICP-MS analysis of enzymatic arsenic methylation assays and urine. [9]. Member of our team initiated this study, which was then experimentally performed by our collaborators in USA.

The method of selective HG with ICP-MS/(MS) was developed for determination of inorganic arsenic (iAs), the most toxic arsenic species, in foodstuff [10]. It uses the selectivity that HG can offer by using high concentrations of HCl to volatilize almost exclusively iAs leaving all organoarsenic species in solution. It enables to measure iAs without a prior step of species separation by chromatography and is a feasible option for large scale screening of relevant foodstuff samples [10]. As stated in the paper, a member of our team, S. Musil, and Á. H. Pétursdóttir contributed equally to this work. S. Musil developed completely the method of selective HG-ICP-MS/(MS) and measured the majority of real samples by this method during his postdoc at the University of Aberdeen. Á. H. Pétursdóttir prepared the real samples and measured them with HPLC-(HG)-ICP-MS for comparison. The other authors participated eitherin comparative analyses or were supervisors of Á. H. Pétursdóttir.

Selective HG with ICP-MS for determination of iAs in rice samples was compared with the

"classical" approach to the determination based on HPLC-ICP-MS [11]. S. Musil contributed partly to this application of the method that was described above. He gave advice when using the HG-ICP-MS/(MS) method and consulted the results.

Other applications of trace elemental analysis in biological and environmental matrices

Our team members contributed to a variety of projects by performing trace elemental and speciation analysis for various analyte/matrix combinations.
Our team member participated in studies of solubility and sorption processes that influence diel cycles in trace element concentrations and microbial effects on the release and precipitation of arsenic forming the realgar deposits in (sub)surface waters in natural As geochemical anomaly [12,13]. The role of our team member was to perform arsenic speciation analysis in water samples.

Capabilities of the HG-CT-AAS method for analysis of inorganic and methyl-substituted arsenic species in N-methylglucamine antimonate (methylglucamine), a drug used for treatment of Leishmaniasis disease, were explored. This method was chosen because of its inherent simplicity and low investment and running costs. The accuracy was assessed by the comparison of the determined inorganic arsenic content with the total arsenic content determined by ICP-MS [14]. The collaborating Brazilian laboratory provided samples and performed the ICP-MS measurements. All the other work was performed by our team members. D.P. de Moraes, the doctoral student from Universidade Federal de Santa Maria (Santa Maria, Brazil), who worked in our team fully under a supervision of our team members, was engaged in spectrometric experiments.

Se concentrations were determined in human skeletal remains of prehistoric populations by in situ trapping of Se hydride by electrothermal atomization AAS [15]. Our team member worked out the analytical method, performed the analyses and verified their accuracy by means of a radioactive indicator 75Se incorporated in tissues of laboratory animals.

Our team contributed to an investigation of electrochemical selenium hydride generation [16]. Two members of our team performed determination of selenium hydride generation efficiency in various electrochemical cells by employing 75Se radioactive indicator. They also visualized the spatial distribution of the analyte in the apparatus and tracked analyte losses.

Our team also proved that ICP-MS was superior in terms of sensitivity, accuracy, and temporal resolution over the current method employing the potassium-binding fluorescent probe for quantification of changes in intracellular potassium concentration (20-140 mM) due to the action of a pore-forming toxin, the adenylate cyclase toxin (CyaA) from the pathogenic bacterium Bordetella pertussis [17]. Our team developed the ICP-MS method and analyzed the potassium concentrations in the cell lysates, while our collaborators from Institute of Microbiology of the ASCR, v.
v. i. cultivated the cells, prepared the toxin experiments and performed the analyses with fluorescent probe.

Development of novel atomizers of volatile compounds

In the vein of development of novel atomizers of volatile compounds for AAS, an investigation of dielectric barrier discharge (DBD) plasma sources was started. Our goals are complex: optimization of atomization conditions for individual analytes, subsequent validation of analytical applications, as well as understanding the mechanisms of atomization.

Our first study aimed to an ability of three plasma-based ambient pressure ion sources (including DBD) to desorb and ionize organic analytes for ambient mass spectrometry. Desorption and ionization mechanisms were studied by emission spectrometry and ion current measurements. They were found to be identical for all three plasma sources with ionization occurring via proton transfer from protonated water clusters to analyte molecules [18]. This research was performed by a team member during his postdoc stay in a prestigious laboratory (NRC Canada). Corresponding author of this work is a foreign supervisor of this postdoc stay. The experience of our team member gained during his postdoc stay abroad enabled to open a new field of research of our team and boosted the ongoing fruitful cooperation with NRC group in Canada. As the follow-up Bi hydride was employed as a model volatile compound to explore perspectives of DBD atomizers for AAS [19]. All features of DBD atomizers were compared with those of conventional quartz tube atomizers (QTA). Silanization of the inner DBD surface was essential improving sensitivity (3 times). DBD can be used for the routine determination of Bi, providing satisfactory repeatability and accuracy. This is the first report in the literature comparing performance of DBD atomizers with the conventional QTA. All the experiments were planned and carried out by our team. The role of other coauthors was as follows: J. Boušek constructed the atomizer and its power supply source and members of the prestigious laboratory (NRC Canada),

R. Sturgeon and Z. Mester, provided access to their facilities which are not available in our lab.

New approaches to preconcentration of volatile compounds by in-atomizer collection

Generation of volatile compounds makes analyte preconcentration easy -by collection in atomizers for AAS.

Using our quartz device for hydride collection, termed "trap-and-atomizer device", our team developed, verified and applied a procedure to determine Sb in acetic acid leachates from pewter cups [20]. M. Dessuy, who worked in our team fully under a guidance of our team members and who was engaged in spectrometric experiments, was a doctoral student visiting from Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, supervised there by M.G.R. Vale and B. Welz.

In a series of papers [21] [22-24], investigation of preconcentration of Sn and Pb in the trap-and-atomizer device followed by the development and application of resulting analytical methods was reported. Mechanisms of preconcentration and analyte atomization were studied and explained with the help of AAS and ICP-MS detectors as well as of autoradiography and gamma radiometry. Also the capacity of the quartz surface was estimated and nature of bismuth interference during lead preconcentration was elucidated. All this research has been completely performed by our team including the sophisticated preparation of Pb radioactive indicator [22].

Subsequently, members of our team developed and patented [25] a modular device which makes possible to use a sapphire tube as the atomizer. Using Bi hydride as a convenient model for volatile compounds, no significant difference between quartz and sapphire regarding atomization as well as in atomizer collection was found and virtually the same analytical characteristics were achieved. The use of sapphire tubes has a great potential for several applications including in-atomizer collection of volatile compounds of transition metals [26]. All the work was solely performed by our team members.

Spectral interferences at analytical lines of As and Se present a serious problems for in-atomizer preconcentration of these two elements. Therefore, absorption by molecular oxygen and gaseous water in the vicinity of As 193.7 nm and Se 196.0 nm analytical lines in quartz atomizers was studied by both line source atomic absorption spectrometry and high-resolution continuum source absorption spectrometry. Oxygen absorption has a structured spectrum whereas water has a pseudo-continuum structure in the studied range. The influence of relevant experimental parameters such as atomizer temperature, carrier gas flow rate, and oxygen/water concentration on absorption by oxygen/water molecules was specified [27]. The work was done by two of our team members with B. Dočekal from the Department of Environmental Analytical Chemistry of our Institute and with U. Heitmann from Institute for Analytical Sciences, Department of Interface Spectroscopy, Berlin, Germany.

Generation of volatile compounds of transition metals

Regarding generation of volatile compounds of transition metals, systems for generation of silver was optimized with the help of radiotracers and transmission electron microscopy: interferences of Au on chemical vapor generation of silver were described and explained. Transport losses were practically eliminated to lead to detection limit as low as 1 ng/ml [28]. The construction of the new quartz atomizer design with a heated inlet arm for efficient transport was developed solely by our team. The separation of radioactive indicator 111Ag from an irradiated target nuclide and all the measurement were also performed by us. The only co-author of the paper who does not belong to our team, O. Benada, participated in transmission electron microscopy experiments.

The next transition metal studied was gold. Individual steps of Au volatile compound (VC) generation were traced using radiotracers [29]. The main benefit is the assessment of the influence of individual generation conditions, namely of the carrier gas flow. It can serve to an ample improvement of analytical procedures based on VC generation of Au. It was proved that Au VC were nanoparticles. This is, together with our previous analogous finding on Ag VC, the only positive identification of nanoparticles as VC of transition metals [29]. The nanoparticle presence was determined by means of transmission electron microscopy with the help of O. Benada from Institute of Microbiology of the ASCR, v. v. i. All the other work was completely performed by our team members. Y. Arslan, the doctoral student from Middle East Technical University in Ankara (Turkey), who worked in our team fully under a supervision of our team members, was engaged in spectrometric experiments. O. Ataman was Ankara supervisor of Y. Arslan.

The unfavorable feature of generation of volatile compounds of Ag and Au is their slow release from the reacting solution reflected in broad signals. Therefore, the possibility of collection of volatile Ag compound on a quartz surface was investigated. A modified modular design of the quartz tube atomizer was employed. Fast volatilization and atomization of the analyte were realized. Preconcentration efficiency reached 95%. The limit of detection was 0.11 ng/ml, nine times better than in the on-line atomization mode. The accuracy of the method was tested using water reference materials [30]. All the work was solely performed by the members of our team.

Other scientific outputs

The state of the art of the generation of volatile compounds for analytical atomic spectrometry was presented in the prestigious Wiley's Encyclopedia of Analytical Chemistry. Theory, instrumentation, methodology, interferences and approaches to speciation analysis are treated together with a critical discussion of advantages and limitations of the technique [31]. A considerable part of the covered material, namely that treated in the chapter Atomizers/Detectors, is based on research of our team. This output was completely prepared by our team member. As mentioned above, a substantial part of the covered material was published by us. The major chapter, treating atomization and detection, is decidedly dominated by our publications. One team member contributed together with five other authors to the IUPAC Technical Report devoted to the mechanisms of chemical hydride generation [32].

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Research Report of the team in the period 2010–2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

Scientific team	Department of Fluid Phase Separations

In 2010–2014, the scientific activities of the Department of Fluid Phase Separations (including the former Departments of Liquid Phase Separations and of Separations with Compressed Fluids till June 2013) resulted in numerous original publications and several reviews in impacted journals. The papers fall within several subfields of analytical separations, and most of the publications are discussed below:

Development of isoelectric focusing methods – methodology and instrumentation

The long-term program of theoretical and methodological development of isoelectric focusing (IEF) in various scales and separation formats continued with several studies including preparative divergent flow IEF without carrier ampholytes for separation of complex biological samples [1], divergent flow IEF for separation and preparative analysis of peptides [2], a new solution IEF device for micropreparative separations of peptides and proteins [3], the design of a simple power supply for power load-controlled IEF [4], or the design of electrolyte system for fast preparative focusing in wide pH range based on bidirectional isotachophoresis [5]. Also, the experiments with tapered capillaries [6] confirmed earlier theoretical results indicating that separation efficiency of IEF in a capillary with a continuous taper should be superior to that in a constant-diameter capillary under the same applied voltage.

Development of isoelectric focusing methods – separations of microorganisms

Separations of microorganisms by electromigration methods largely capitalized on the strong theoretical and methodological background in the various variants of IEF as mentioned above. Because of amphoteric nature of cell membrane, microorganisms display their effective isoelectric points that make them amenable to IEF. Our recent original contributions included, e.g., the use of electromigration techniques and MALDI TOF mass spectrometry in the separation of phenotypically indistinguishable *Candida* species [7,8], the determinations of the isoelectric point differences in biofilm-positive and biofilm-negative strains of bacterial species [9] and differentiation between the biofilm-positive and biofilm-negative strains [10], or the trace analysis of microorganisms in real samples by combination of a filtration microcartridge and capillary IEF [11]. Other applications of electromigration methods to separate and/or characterize microorganisms and related objects included, e.g., separation of attogram amounts of terpenes by capillary zone electrophoresis with fluorometric detection [12], purification, separation and identification of subtypes of swine and equine influenza viruses by electromigration techniques with UV and fluorometric detection [13,14], characterization of *Agrobacterium* species by capillary isoelectric focusing [15], combinations of electromigration methods with mass spectrometry to characterize important bacterial strains [16–18] or filamentous fungi [19], or the use of capillary isoelectric focusing in separation of ampholytic antibiotics and bacteria from different matrices [20]. Also, a method of dynamic labeling of diagnostically significant microbial cells in cerebrospinal fluid by red chromophoric non-

ionogenic surfactant for capillary electrophoresis separations was also developed [21]. Thereby, the potential of electromigration methods in rapid and efficient separations of numerous species of microorganisms was convincingly demonstrated. Also, the advent of supercritical water-etched fused silica capillaries (see below) brought a new impetus and paved the way to unique separations of important microorganisms by both capillary IEF and capillary zone electrophoresis. Isoelectric focusing in tapered capillaries was employed to improve the separation of several species of probiotic bacteria from cow's milk (*Lactobacillus* sp.) [22], and also to accomplish the separation of several important phytopathogens of the *Dickeya* genus [23]. In these studies, subsequent MALDI TOF mass spectrometric fingerprinting of the separated zones was shown to be highly useful in confirming the identities of the individual bacterial species. Capillary zone electrophoresis in constant-diameter, surface-corroded fused silica capillaries allowed the separation of methicillin-resistant (MRSA) from methicillin-susceptible (MSSA) *Staphylococcus aureus* strains [24]. Apart from improving the separation efficiency, the use of supercritical water-treated and modified capillaries led also to lowering of the detectable amount of bacteria. To date, the electromigration separations of microorganisms in supercritical water-treated capillaries proved to be useful in several application areas including food production (dairy industry), agricultural crop protection and human health (clinical microbiology). In particular, the fast and low-cost method to differentiate between MRSA and MSSA appears important because MRSA is a frequent cause of both nosocomial and community-related infections. These results were mostly obtained in cooperations with the Department of Microbiology, Faculty of Medicine, Masaryk University, Brno, with the Division of Diagnostics, State Phytosanitary Administration, Olomouc, or with the National Authority for Nuclear, Biological and Chemical Protection, Brno. The role of collaborating institutions largely consisted in bacterial sample preparation and also in performing comparative molecular tests such as PCR for identification of bacterial strains, if applicable. All electromigration experiments and MALDI TOF fingerprinting, if applicable, were carried out at the department.

Application of near- and supercritical water as an agent to treat the siliceous surfaces

The long-term experience in the design and construction of high-pressure equipment for analytical extractions gradually resulted in testing of near- and supercritical water as an agent to treat the surfaces of analytical separation devices made of fused silica or glass. An in-house-constructed, high-temperature, high-pressure apparatus for the purpose made it possible to treat both fused silica capillaries [25] and the glass substrates for lab-on-a-chip applications. Within the period 2010–2014, most results of this original activity concerned fused silica capillaries. However, now it is already certain that etching with supercritical water is also very effective in preparation of glass chips. In fused silica capillaries, etching with supercritical water can produce extensive changes in both internal diameter and the inner surface morphology, depending on the operating conditions. Both kinds of the changes can be put to a direct use in capillary electromigration methods. The application of tapered capillaries results in enhanced separation efficiency of capillary IEF of proteins [6] and bacteria [22,23]. In turn, the constant-diameter capillaries with different degrees of the inner surface corrosion appear to be very useful in capillary zone electrophoresis, providing for unique separations of closely related strains of important bacteria as mentioned above [24]. As an auxiliary tool, a simple thermodynamic model to predict the aqueous solubility of amorphous silica as a function of temperature and pressure was also developed [26].

Development of chromatographic instruments – miniaturization

Among the efforts in the field of miniaturized chromatographic instrumentation, the most important result was the design and construction of a simple and affordable HPLC platform to operate with micro- and nanocolumns [27,28]. The system allows splitless nanocolumn gradient separations in either constant pressure or constant flow rate modes, and it provides a suitable springboard for further development toward portable HPLC setup.

Development of chromatographic column technology

The development of chromatographic column technology was largely focused on monolithic silica-based capillary columns for liquid chromatography. Monolithic silica columns were prepared by the sol–gel method involving acidic hydrolysis of tetramethoxysilane in the presence of suitable porogen. The publications on this topic concerned several aspects including the effect of preparation temperature on the chromatographic properties of the monolithic silica skeleton [29], chemical modifications of the monolithic columns with suitable zwitterionic agents for application in hydrophilic interaction chromatography [30], modification of the monolithic columns with liposomes to produce a chromatographic retention model system mimicking the interactions of pharmaceuticals with cell membranes [31], or comparative study of two silica-based monolithic capillary columns modified with different zwitterions and coupled to tandem mass spectrometry for separation and identification of nucleobases, nucleosides, and nucleotides [32]. The latter two studies [31, 32] were accomplished in cooperation with the Department of Chemistry and the Faculty of Pharmacy, University of Helsinki (Finland). The roles of the cooperating institutions in Finland consisted in the preparation of stable forms of liposomes [31] and in the experiments involving the tandem mass spectrometry [32] whereas the column preparation and chromatographic separations with UV detection were carried out at the department.

Supercritical fluid chromatography as a tool to probe the partition behavior of organics in biphasic systems composed of an ionic liquid and supercritical carbon dioxide

The long-term program of using capillary column supercritical fluid chromatography as a tool to probe the partition behavior of organics in biphasic systems composed of an ionic liquid and supercritical carbon dioxide continued with the systems involving 1butyl-3-methylimidazolium methylsulfate [33] and 1-butyl-3-methylimidazolium trifluoromethanesulfonate [34]. Our extensive data base of experimental results was employed to upgrade and test a generalized predictive correlation for solute partition coefficients in ionic liquid–supercritical carbon dioxide systems [35]. These efforts may be useful in the design of applications of tunable biphasic solvent systems composed of an ionic liquid and supercritical carbon dioxide.

Pressurized liquid extraction of plant materials

In the field of pressurized liquid extraction of plant materials, the most important result was the development of a new procedure to evaluate the antioxidant activity of plant extracts. The procedure involved an off-line combination of pressurized liquid extraction and electron paramagnetic resonance (EPR) spectroscopy, with the latter method serving to monitor the activity of extracted antioxidants in scavenging the free radical probes added to the extracts [36,37]. The studies were completed in cooperation with the Food Research Institute in Bratislava (Slovakia) and they also included a comparison of relative merits of lower aliphatic alcohols and water as the extraction solvents. The role of the cooperating institution in Bratislava consisted in performing all EPR spectroscopic experiments whereas the extractions of plant materials were carried out at the department. Also, a

comparative study of several methods to extract important quaternary benzo[c]phenanthridine alkaloids from plant material was completed in cooperation with the Department of Biochemistry, Faculty of Medicine, Masaryk University [38]. This study fully confirmed the competitive position of pressurized liquid extraction of plant materials when compared with classical extraction methods such as maceration or Soxhlet extraction. The maceration and Soxhlet extraction experiments were performed by the cooperating institution whereas the pressurized liquid extractions were carried out at the department.

Solubilities of organic nonelectrolyte solids in pressurized hot water including the development of predictive thermodynamic models for solute solubility

The long-term program of determination of high-temperature, high-pressure aqueous solubilities of heavy organic nonelectrolyte solids continued with the studies of ferrocene [39], selected organic electronic materials [40], and macrocyclic hosts (calix[6]arene and 4-*tert*-butylcalix[4]arene) [41] as the solutes. To our knowledge, no previous data on the aqueous solubilities of these compounds at elevated temperature and pressure were available in the literature. In the theoretical aspect of this topic, a group contribution model was developed to correlate and/or predict the aqueous solubilities of organics over a wide range of temperature (~200 K) [42]. At the time of this writing, this program was already completed and closed.

Other contributions to bioanalysis by the department members while staying abroad

In this section, the publications co-authored by the department members during or after their long-term stays abroad are briefly discussed. In connection to her long-term cooperation with Professor Jennifer E. Van Eyk at the School of Medicine, Johns Hopkins University, Baltimore, MD, USA, Miroslava Šťastná co-authored several articles on various bioanalytical topics including identification and functionality of proteomes secreted by rat cardiac stem cells and neonatal cardiomyocytes [43], analysis of protein composition of rabbit aqueous humor following two different cataract surgery incision procedures [44], a review on secreted proteins as a fundamental source for biomarker discovery [45], investigations on the secretomes of the cells comprising the heart [46], a review on analysis of protein isoforms [47], and a chapter on optimized method to identify the proteomes secreted by cardiac cells [48]. Vratislav Košťál, former doctoral student in the department, co-authored two reviews on analysis of isolated organelles [49,50] among his other work while on his postdoctoral stay with Prof. Edgar A. Arriaga at the Department of Chemistry, University of Minnesota, Minneapolis (MN), USA.

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Research Report of the team in the period 2010–2014

Institute	Institute of Analytical Chemistry of the CAS, v. v. i.

Scientific team	Department of Environmental Analytical Chemistry

The key activities of the Department of Environmental Analytical Chemistry were research and development of new methods and the instrumentation for the chemical analysis and/or characterization of environmental systems, minor and trace gaseous components, nano-particles and aerosols in the air, the composition of nano-particles, chemical analyses in aquatic systems, soils and sediments with respect to organic and inorganic pollutants and their accessibility to plants, food chains, health risk for human being and other topics dealing with environmental protection. The principles of chemical analyses were based on separation and preconcentration techniques in tandem with developed novel specific detectors.

The Department of Environmental Analytical Chemistry was very successful in national competitive funding (The Czech Science Foundation -GACR, Ministry of Environments, Ministry of Culture, Ministry of Defense) in the period 2010-2014. The total funding for research of the Department of Environmental Analytical Chemistry within the period 2010-2014 was 45.8 mil. CZK. The national competitive funding (24.4 mil. CZK) and funding from contract research (1.95 mil. CZK) created 58.8% of the total funding of the Department of Environmental Analytical Chemistry. The department was equipped with the new instrumentation gaining from money of the projects as well as from the institute budget in total worth 5.1 mil CZK in the period 2010-2014.

The scientists of the Department of Environmental Analytical Chemistry were/are principal investigators of projects:

- "Gel techniques for characterization of environmental systems" (GACR, P503/10/2002, 2010-2013)
- "Study of transport of inhaled nano-sized particles (Pb, Cd) and their allocation in organs (GACR, P503/11/2315, 2011-2013)
- "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" (GACR, P503/14/25558S, 2014-2016) or the co-investigators of the projects:

- „The effect of ground level of ozone on forest ecosystem" (Ministry of Environment of the Czech Republic, SP/1b7/189/07, 2007-2010)

- "Determination of chemical and toxicological properties of dust particles and investigation of their origin" (Ministry of Environment of the Czech Republic, SP/1a3/148/08, 2008-2010)

- "Morphology, chemical and toxicological characterization of road dust and particulate matter including sources apportionment" (Ministry of Environment of the Czech

Republic, SP/1a3/55/08, 2008-2010)

• “Mechanisms of toxicity of biofuel particulate emissions“ (GACR, P503/13/1438S, 2013-2015)”.

The Department of Environmental Analytical Chemistry was involved as the co-investigator in Centre of Excellence – “Centre for studies on toxicity of nanoparticles” (GACR, P503/12/G147, 2011-2017) and in preparation of the new strategy of The Czech Academy of Sciences – AV21 (http://www.cas.cz/strategie/04-ucinna-premenaa-skladovani-energie.html)

The team developed within a funding contract research (Ministry of Defence of the Czech Republic, the grant 0901 8 7150 R. University of Defense, Brno) a portable device for fast analysis of explosives in the environment (L. Čapka et al., J. Chromatogr. A, 2015). 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 soils extracts in less than 8 minutes. The co-operation with the University of Defense in Brno also resulted in the development of new sensitive flow-injection method for the determination of nitrate in water. Nitrate is on-line photolytically converted to peroxynitrite by absorption of UV light, peroxynitrite is subsequently determined by the chemiluminescent reaction with luminol. The detection limit of nitrate is 7 × 10−10 M. Results were published in a paper by P. Mikuška et al. (Int. J. Environ. Anal. Chem., 2014).

The team also participated in nitrous acid (HONO) measurement in the frame of international campaign named FIONA (Formal Intercomparison of Observations of Nitrous Acid) that took place at Instituto Universitario Centro de Estudios Ambientales del Mediterráneo (CEAM) in Valencia (Spain) in May 2010 under participation of 17 institutions or universities from Europe and USA. Within the campaign 19 different instruments for detection of HONO were compared considering sensitivity of HONO detection and interferences for both simulated laboratory conditions and real air samples.

The part of the research of the Department of Environmental Analytical Chemistry was aimed on the development of analytical methods for the determination of compounds of interest in air, water and atmospheric aerosols.

Analysis of chemical composition of atmospheric aerosols was focused on toxic compounds (i.e., polyaromatic hydrocarbons, PAHs) or tracers of main emission sources like traffic (i.e., hopanes, steranes), biomass burning (i.e., monosaccharide anhydrides, PAHs) or coal combustion (i.e., hopanes, PAHs), and content of metals, ions, elemental carbon (EC) and organic carbon (OC) in aerosols. Developed methods were utilized during the comparison of chemical composition of submicron atmospheric aerosols (size fraction PM1) in two towns, Brno and Šlapanice, resulting in the identification of biomass burning and coal combustion in winter and traffic in summer as main emission sources of aerosols collected in these towns. This work was performed in the frame of the project “Determination of chemical and toxicological properties of dust particles and investigation of their origin” (Ministry of Environment of the Czech Republic, SP/1a3/148/08, 2008-2010). Results published in two papers (K. Křůmal et al., Atmos. Environ., 2010 and 2013) provided for the first time the basic information concerning the concentration of monosaccharide anhydrides (levoglucosan, mannosan, galactosan), hopanes and steranes in PM1 or PM2.5 aerosol samples measured in Brno and Šlapanice in winter and summer seasons. Moreover, results deepened knowledge about the concentration of PAHs in studied localities. In parallel, N. Kubátková (student of Brno University of

Technology, Faculty of Chemistry) developed during her diploma thesis (graduated in 2011) a method for the simultaneous determination of saccharides, methoxyphenols, resin acids and monosaccharide anhydrides in atmospheric aerosols. All these compounds serve as tracers of wood or biomass combustion and various biological sources. Moreover, methoxyphenols allow to distinguish a combustion of soft and hard wood. ¨

In the project of Ministry of Environment of the Czech Republic (SP/1a3/55/08, 2008-2010, "Morphology, chemical and toxicological characterization of road dust and particulate matter including sources apportionment") we dealt with chemical and toxicological characterization of resuspended dust in Brno and Ostrava and with identification of dust sources. Results were published in one paper (in a non-impacted journal) and a methodology was proposed.

In the frame of these two projects of Ministry of Environment of the Czech Republic we developed a new sensitive method for a semi-continuous analysis of water-soluble fraction of particulate metals. The system combines the continuous sampling of atmospheric aerosols into deionized water using an Aerosol Counterflow Two-Jets Unit and on-line chemiluminescent detection of water soluble fraction of metals in collected aerosols. The detection system was optimized for the determination of Fe3+, Cu2+ and Co2+ in atmospheric aerosols. The method was published in a paper by M. Vojtěšek et al. (Int. J. Environ. Anal. Chem., 2012).

Street dust with focus on mercury was a subject of interest of other study where total and bioaccessible mercury contents in resuspended dust in Brno were compared. The content of bioaccessible mercury in all samples throughout the year was below the limit of quantification, which indicates that resuspended street dust did not increase the daily mercury intake by the population in studied area. The results were published in a paper by P. Coufalík et al. (Bull. Environ. Contam. Toxicol., 2014).

We participated also at the measurements of number size distributions and chemical composition of submicron aerosols that has been performed at the Eastern part of Mediterranean as part of an extensive measurement campaign to study photo-oxidants and aerosols (SUB-AERO Project, ENVK2-1999-00052).The measurements were made at the Finokalia station on the island of Crete (Greece) and onboard the research vessel "Aegaeon" (2000-2003). Part of results was published in a paper by
V. Ždímal et al. (Water Air Soil Pollut., 2011).

Within the co-operation with the Department of Chemistry, Faculty of Science, Masaryk University Brno, a new method was developed for the measurement of size and number concentration of aerosol particles generated by laser ablation of various standard materials (ceramic, metals, glass) using an optical aerosol spectrometer Welas equipped with two different sensors. The concentration of ablated particles was measured in two size ranges (10–250 nm and 0.25–17 μm), and the influence of sample properties and its composition on the size and concentration of aerosol particles generated by nanosecond laser ablation at 213 nm was investigated. The results showed a significant dominance of particles smaller than 250 nm in comparison with larger particles, irrespective of the kind of material. In parallel, chemical composition of ablated particles was analyzed by means of ICP-MS. Additionally, the structure of the laser generated particles was studied after their collection on a filter using a Scanning Electron Microscope (SEM) and the particle chemical composition was determined by an Energy Dispersive X-ray Spectroscope (EDS). Results were published in 2 papers (M. Holá et al., Spectrochim. Acta B, 2010 and M. Holá et al., Talanta, 2010). Alternatively, the spectrometer Welas and laser ablation

inductively coupled plasma mass spectrometry were applied to the study of the interaction of molten LiF-NaF salts with three candidate structural materials for nuclear reactor-transmutor cooling circuit. The results were published in a paper by T. Vaculovič et al. (J. Anal. At. Spectrom., 2012).

During the project “Mechanisms of toxicity of biofuel particulate emissions“ (GA CR, P503/13/1438S, 2013-2015) a toxicity and chemical composition of exhaust emissions from gasoline and diesel cars powered by traditional gasoline or diesel fuel and alternative biofuels were studied. Analyses of chemical composition in particulate emissions were aimed to toxic compounds (i.e. polyaromatic hydrocarbons and polyaromatic nitro-hydrocarbons), tracers of traffic emission (i.e., hopanes, steranes, alkanes, pristane, phytane), and metals both the total concentration, and the concentration of their bioavailable fraction.

In the frame of Ph.D. thesis of A. Kořínková (student of Brno University of Technology, Faculty of Chemistry) we improved the collection efficiency of previously developed aerosol collector for sampling of small-sized aerosol particles. The work was carried out 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).

Within the fruitful co-operation with Belgian partner (prof. W. Maenhaut) a specially designed annular diffusion denuder for simultaneous removal of organic gaseous compounds and atmospheric oxidants in carbonaceous aerosol sampling (P. Mikuška et al., Anal. Chim. Acta, 2012) was developed. The annular diffusion denuder is compatible with the collection of aerosols on 47-mm diameter quartz fiber filters at a flow rate of 16.6 L min-1. The composition of denuder “coating” was optimized to ensure high collection efficiency over long-term field operation. The use of this denuder enables to sample carbonaceous aerosols on filters without positive sampling artefacts from volatile organic compounds and interferences from atmospheric oxidants. The annular diffusion denuder has been applied successfully for the sampling of carbonaceous aerosols during

The cylindrical wet effluent diffusion denuder (CWEDD) was employed for the measurement of uptake coefficients of formaldehyde, acetaldehyde and nitrous acid

(K. Motyka et al., Talanta, 2011). The theoretical approaches describing the collection of analytes in the CWEDD (calculated according to Gormley-Kennedy equation and with respect to Henry constant) were compared with experimental data for various absorption liquids. The CWEDD can be an alternative tool for the determination of the uptake coefficient. Obtained uptake coefficients were in good agreement with data found in literature.

An important part of our attention was also paid to the determination of selected gaseous pollutants in air, such as ozone, peroxyacetyl nitrate and nitrous acid. Ozone study was performed at the experimental station in Bílý Kříž (Beskydy, Czech Republic) where daily ozone deposition flux to a Norway spruce forest was measured using the gradient method. Results were in good agreement with a deposition flux model. In addition, net ecosystem production (NEP) was measured by using Eddy Covariance and correlations with O3 concentrations at 15 m a.g.l., total deposition and stomatal uptake were tested. Total deposition and stomatal uptake of ozone significantly decreased NEP, especially by high intensities of solar radiation. The results were published in a paper by M. Zapletal et al. (Environ. Pollut., 2011).

A new method for the rapid and sensitive determination of peroxyacetyl nitrate (PAN) in air based on a chemiluminescence reaction (CL) with an alkaline solution of luminol in the chemiluminescence aerosol detector was developed in the frame of diploma thesis of L. Bružeňák (student of Brno University of Technology, Faculty of Chemistry). The PAN is chromatographically separated from nitrogen dioxide and ozone in a packed column and the eluted PAN is detected via the direct reaction with the luminol solution. The limit of detection is 14.9 ng/m3 (3 ppt) of PAN. The detection of PAN via the direct CL reaction with the luminol solution is the most sensitive CL method for the determination of PAN in air that has been reported to date of publication of our paper. Alternatively, the PAN after separation is thermally converted to NO2 which is detected by the chemiluminescence reaction with a luminol solution. The limit of detection is 50 ppt of PAN and 50 ppt of NO2. The alternative approach affords the simultaneous determination of PAN and NO2.The time resolution is 3 min. The results were published in a paper by P. Mikuška et al. (Chem. Pap., 2014).

Next to above mentioned experiments two short reviews in Czech impacted journal Chemické Listy focused on the application of molecular organic markers for identification of emission sources of atmospheric aerosols (K. Křůmal et al., Chem. Listy, 2012) or to the composition, sources and detection of water soluble organic compounds that are very important part of atmospheric aerosols (A. Kořínková et al., Chem. Listy, 2014) were reported.

The next part of the research activity was oriented to development of novel approaches in application of diffusive gradient in thin film technique for characterization of environmental systems. The research program was supported by the Czech Science Foundation (project No. P503/10/2002, “Gel techniques for characterization of environmental systems“). Within the frame of this project, novel possibilities of metals speciation and fractionation analysis in aquatic, sediment and soil systems by means of modified diffusive gradient in thin film techniques (DGT) were developed for assessment of bioaccesibility and mobility of elements. For this purpose, new DGT probes based on sorption gels for specific immobilization of ecologically and toxicologically important elements were applied. These probes were also designed for measurement of depth profiles in sediments. Processes in sediments, release and/or immobilization of metals can be investigated by these probes in combination with diffusive equilibrium technique (DET). A new type of probes was developed for characterization of soils, for estimation of accessible pools of metals, metals fractionation, for description of kinetics of exchange processes, for the effect of moisture content etc. The performance of new DGT probes was also studied in combination with complementary pot experiments on model crop plants. It was also shown, that the DGT technique can be modified for application in characterization of various types of aerosols (urban, nanoparticles) to estimate potential toxicity of aerosol particulate matter including kinetics of mobilization mechanisms. It has been shown that these novel approaches are useful and powerful tools for characterization of environmental systems, and simple in routine practice. The results related to development of new DGT probes were published in papers (M. Gregušová, B. Dočekal Anal. Chim. Acta, 2011, B. Dočekal, M. Gregušová, Analyst, 2012, M. Gregusova, B. Docekal, Anal. Chim. Acta, 2013, H. Dočekalová, V. Kovaříková, B. Dočekal, Chem.Spec. Bioavail., 2012., H. Dočekalová, P. Škarpa, B. Dočekal, Talanta, 2015) in cooperation with Brno University of Technology (BUT) Faculty of Chemistry and Mendel University in Brno, Faculty of Agronomy, PhD and diploma students (M. Gregušová, J. Trávníčková) were involved in research projects supported by the Czech Science Foundation specified above.

The scientists of the environmental analytical chemistry department participated in project “Essential oils as tool for saving and increasing of culture heritage on paper“ (Ministry of Culture of the Czech Republic NAKI 63, DF11P01OVV28). The aim of the project is using essential oils (EOs) to protect damaged books and other archives on paper basis that are influenced by microbial activities. The antimicrobial properties of 15 essential oils for 17 microorganisms were determined. The most effective EOs were Lavandula angustifolia, Cymbopogon nardus, Citrus aurantifolia, Juniperus communis, Myrtus communis and Cinnamomum zeylanicum, and the components Lavandula angustifolia (limonen, eucalyptol and ocymene) with the most antimicrobial properties were chosen for detailed study in libraries and chanceries. The first results will be published in paper by K. Křůmal et al. (Chem. Pap., 2015).

The significant part of the research activity was oriented to problems associated with formation, transport and chemical composition of fine particles and nano-particles in heavily polluted regions of the Czech Republic, long-term nano-particles inhalation experiments with small animals (mice), and the study of allocation of inhaled nanoparticles in their organs (“Study of transport of inhaled nano-sized particles (Pb, Cd) and their allocation in organs“ and „Centre for studies on toxicity of nano-particles” GACR, P503/11/2315 and P503/12/G147).

The unique exposure system for whole body inhalation experiments with small animals for chronic as well as for acute exposure studies to nano-particles under strictly controlled conditions was constructed. The inhalation exposure system is assembled of four chambers from a glass and stainless steel and makes possible long-term inhalation procedures (up to 100 days, 24 hours/day, 7 days a week) with up to four discrete groups of mice population (up to 60 individuals each) or of rats population (up to 20 individuals each) under controlled illumination, temperature, relative humidity and concentration of nano-particles up to ca 107 particles/cm^3. The size distribution of nano-particles in the dose-concentration chambers was measured continuously using a Scanning mobility particle sizer analyzer (SMPS 3936 L72, TSI) in 5 minute intervals.

In vivo experiments we studied transport of inhaled nano-particles composed of nonbiogenous elements Cd and Pb (in form of PbO, CdO), and their allocations in mice (Mus musculus var. Alba) organs. Moreover, we studied the toxicity of PbO and CdO nano-particles that were selected as products of technological processes and because of their presence in ambient aerosol. Nano-sized particles of CdO and PbO were synthesized via aerosol route in a hot wall tube flow reactor using metallic Cd, and/or Pb. The acute (3 day) and chronic (60 and 90 days) inhalation experiments with nanoparticles concentration of 5 x 106 - 5 x 105 particles/cm^3, and size in the range 10-60 nm were carried out. Amount of Cd and Pb was determined in individual mice organs with atomic absorption spectrometry. The concentration of Cd and Pb in target organs increases with duration of exposure to CdO (PbO) and with increasing CdO (PbO) nanoparticles concentration in experimental cages. The concentration of Cd (Pb) in target organs, obtained within the long term experiment, decreases in the order kidney, liver, spleen and brain, whereas Cd concentration in organs obtained within the short term experiment decreases in the order spleen, liver and kidney. The observation indicates that process of cadmium and lead translocation among organs is executed probably by blood transport. In contrast, due to differences in mass of individual organs, the overall content of accumulated cadmium and lead in the individual organs during the long term experiment decreases in the order liver, kidney, spleen and brain. Inhalation of cadmium oxide and lead oxide nano-particles induces significant mass increase of lungs.

Next to inhalation experiments with nano-particles PbO and CdO, the inhalation experiments with nanoparticles MnO. $Mn2O_3$ and TiO_2 were also proceed. Nano-sized particles of $MnO.Mn_2O_3$ and TiO_2 were synthesized via aerosol route in a hot wall tube flow reactor using a thermal decomposition of metal organic precursors, manganese(II)acetylacetonate, and/or titanium tetra-izo-propoxide. The acute (3 day) and chronic (60 and 90 days) inhalation experiments run both with $MnO.Mn_2O_3$ (concentrations 3 x 106 and 5 x 105 particles/cm^3, size range 5-70 nm) and with nanoparticles of TiO_2 (90 days, concentrations 3 x 106 and 5 x 104 particles/cm^3, and size range 3-70 nm).

During the inhalation experiment, when the experimental group of mice was exposed to manganese oxides nano-particles ($MnO.Mn_2O_3$) and/or nano-particles of TiO_2 the mass of selected internal organs of mice from both exposed and control group was assessed. It has been proved that inhaled nano-particles ($MnO.Mn_2O_3$) are able to influence the mass of internal organs of mice. Statistically significantly lower mass of kidneys, liver and spleen and higher mass of pancreas have been found in the exposed group compared to the control group. However, the concentration of Mn in organs did not change significantly with exception of lungs and brain. The concentration of Mn in lungs decreased within clearance period when inhalation was interrupted. Clearance period had evidently no effect on the concentration of Mn in liver, kidney and spleen and it was statistically insignificant for brain. It can be assumed that blood cells contained more than 65 % of Mn present in whole blood.

Amount of Ti in target organs obtained within the long term inhalation experiments, and immunological analyses of mice organs are presently under evaluation.

The first results related to inhalation of nano-particles performed within a period 2010-2014 were published in the papers by K. Celá et al. (Cells Tissues Organs, 2014) and L. Bláhová et al. (Anal. Bioanal. Chem., 2014). Results related to „nano-projects„ obtained during the analyses of organic compounds in PM1 aerosols sampled in winter 2012 in Ostrava were published in paper by P. Mikuška et al. (Atmos. Environ., 2015).

The results acquired within the frame of inhalation projects with small animals and sampling of air in heavily polluted regions followed by chemical analysis of fine particles and nano-particles stimulated a public debate concerning the health risks of nano-particles and fine particles from the point of quality of life of human population at least in the Czech Republic.

The Department of Environmental Analytical Chemistry co-operates for a long-term period in horizontal basis of the Institute with the Department of Trace Element Analysis on the development of novel and powerful miniature devices useful in atomic spectrometry methods for collection of volatile species of analytes. Fundamental processes of trapping of volatile hydrides and other volatile species of elements were studied employing atomic spectrometry and radio-nuclide techniques. New electrothermal collection devices based on graphite and refractory metals were designed and performance of these devices was investigated with respect to their analytical properties and application. Influence of all the relevant experimental parameters, including geometry of the device, gas flow patterns, composition of the gaseous phase, modification of the collection surface, collection capacity, mutual and spectral interference effects was studied.

The latest results related to the investigation of spectral interference effects of traces of oxygen obtained within a period 2010-2014 were published in the paper by J. Kratzer et al. (J. Anal. At. Spectrom., 2011).