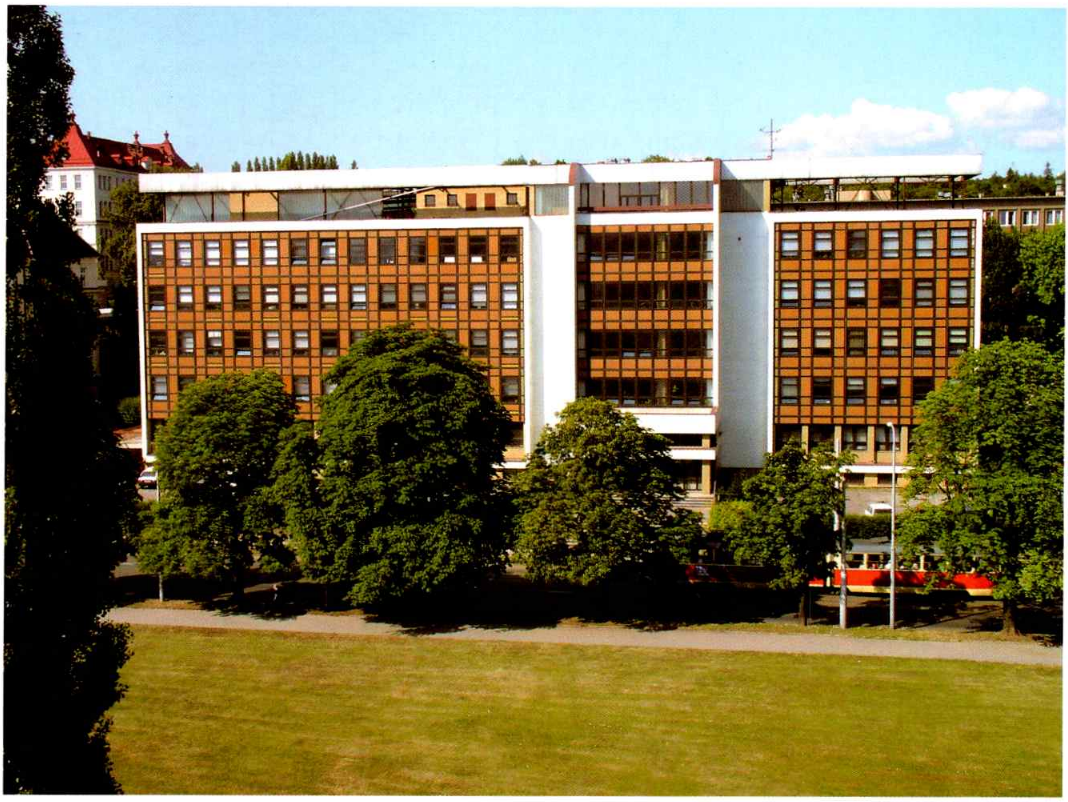
Preface

This brochure presents a review of the structure and past, present and future scientific, organizing and teaching activities of the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic based on the work done in the time from January 1999 to June 2003.

We believe that this publication will serve as the reference information about our Institute to the international scientific community, to the grant agencies and sponzors as well as to those who are interested in postgraduate forms of scientific education or in specialized studies in the field of analytical chemistry.

Brno, September 2003

Josef Chmelík
Head



The building of the Institute of Analytical Chemistry in Brno.

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Structure of the Institute

Management

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Webmaster

Ing. František Matulík
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Scientific Departments

Scientific research in the Institute of Analytical Chemistry is focused on the following topics, which are studied in the individual scientific departments of the Institute:

- **Department of Bioanalytical Instrumentation**
  Head: Ing. František Foret, CSc.
  +420 532 290 242, foret@iach.cz

- **Department of Biopolymer Analysis**
  Head: Ing. Josef Čáslavský, CSc.
  +420 532 290 216, caslavsky@iach.cz

- **Department of Electromigration Methods**
  Head: Prof. RNDr. Petr Boček, DrSc.
  +420 532 290 133, bocek@iach.cz

- **Department of Environmental Analytical Chemistry**
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- **Department of Liquid-Phase Separations**
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- **Department of Proteomics**
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- **Department of Supercritical Fluid Extraction and Chromatography**
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- **Department of Trace Element Analysis**
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The history of the Institute

commenced in 1956 when the Laboratory for Gas Analysis was established by transferring a small research group from the Institute for Petroleum Research in Brno to the former Czechoslovak Academy of Sciences. The establishment of the Laboratory had largely been made possible through the foresight of František Šorm, at the time the Scientific Secretary of the Czechoslovak Academy of Sciences. The leader of the original research group was Jaroslav Janák. He served as the first director of the Institute until 1980, and the Institute gained a widespread international recognition under his leadership.

Since 1956, the Institute has gradually grown in both staff and research topics. The growth has been marked by several formal milestones, the last of these being the establishment of the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic in 1991. The original subjects of gas analysis and gas chromatography in the fifties and sixties have naturally developed towards a broader field of separation methods. The Institute's scope has been extended to include high-efficiency liquid chromatography and isotachophoresis, and later also field-flow fractionation, supercritical fluid chromatography and extraction, capillary zone electrophoresis, and selected specific techniques of environmental analytical chemistry. The choice of models for study has shifted from substances typical of petroleum chemistry to substances of biological interest as well as to trace elements present in the environment. During the whole existence of the Institute, great emphasis has been placed on the development of modern analytical instrumentation.

The history of the Institute has benefited from an extensive „cross-pollination“ among the individual research teams and topics. In particular, this applies to the design and development of laboratory gadgets and instruments for analytical-scale separations, and to the design of „couplings“ between a separation method and a detection technique.

In 1991, the Institute has moved into new and more spacious premises. This event secured somewhat better conditions for the full development of the capabilities of the staff. In 1992, the number of employees of the Academy of Sciences of the Czech Republic was cut down significantly because of budget restrictions. As a result, some institutes of the Academy have been dissolved, including the former Institute of Nuclear Biology and Radiochemistry in Prague. However, the research group of trace inorganic analysis from that institute has become a detached workplace of the Institute of Analytical Chemistry. This event marked an important change in scope of the scientific program of the Institute, adding some selected methods of atomic spectrometry to the Institute's former program concerned entirely with analytical separations.

The most recent additions to the research program of the Institute include applications of mass spectrometry, and establishment of three new research groups:

Department of Bioanalytical Instrumentation on April 1, 2001, and Department of Proteomics and Department of Biopolymer Analysis on November 1, 2001. These applications will employ coupling of ESI-MS with separation techniques as well as the MALDI-TOF technique. The Department of Bioanalytical Instrumentation will primarily be concerned with the development of chip-based separation devices.

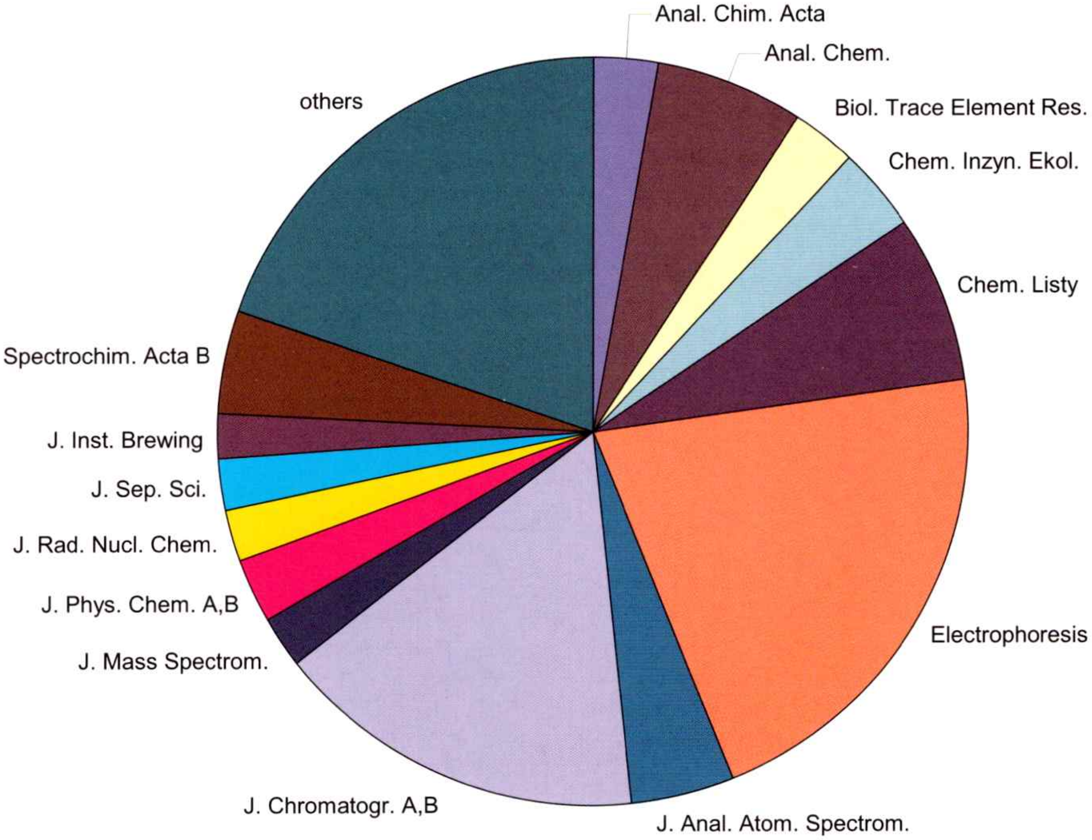
Currently, our efforts are aimed toward an optimum utilization of the potential of analytical chemistry in diverse branches of science and technology. We have been engaged in developments of theoretical background, applications, and instrumentation of separation and spectroscopic methods of analytical chemistry. The separation branch of our research includes methods employing electric field, sorption, fluid flow, force field, chemical reaction, and their combinations as the driving forces for separation. The spectroscopy branch of our research includes mass spectrometry, atomic spectroscopy, and developments of some optical spectroscopic techniques to be used for detection in the separation methods. As an important and inseparable by-product, the theoretical outlets also provide new, original knowledge applicable in other branches of science, primarily in biochemistry and physical chemistry. Applications pertain to a broad range of fields including medicine, environmental protection, foodstuff production, and high-purity materials.

Part of the staff of the Institute. The picture was taken just before the departure for Summer School of Modern Analytical Methods, Rejvíz, June 4-6, 2003.

Number of publications of the employees in individual scientific journals in 1999-2002.

Journal	**1999**	**2000**	**2001**	**2002**	**total**
Acta Univers. Agricult. Silvicult. Mendel. Brunensis			1		1
Analytica Chimica Acta	1	2		1	4
Analytical and Bioanalytical Chemistry				1	1
Analytical Chemistry	2	4	2	1	9
Annali di Chimica				1	1
Bioanaytical Chemistry		1			1
Biological Trace Element Research		2	2		4
Biomedical Chromatography	1				1
Bios Boissons Condit.	1				1
Canadian Journal of Microbiology		1			1
Chemia i Inzynieria Ekologiczna	1		3	1	5
Chemické Listy	2	2	1	5	10
Chemical Reviews		1			1
Coll. Czech. Chem. Commun.				1	1
Colloids and Synthesis A	1				1
Czechoslovak J. Phys.	1				1
Electrophoresis	4	10	5	11	30
Environmental Science & Technology				1	1
Food and Chemical Toxicology				1	1
Horticulture Science				1	1
Journal of Aerosol Science			1		1
Journal of Analytical Atomic Spectrometry		2	1	3	6
Journal of Chromatography A	6	6	8	2	22
Journal of Chromatography B			1		1
Journal of Colloid and Interface Science			1		1
Journal of Mass Spectrometry			3		3
Journal of Physical Chemistry Ä		1			1
Journal of Physical Chemistry B	2		1		3
Journal of Radioanalytical and Nuclear Chemistry				3	3
Journal of Separation Science (former HRC and JMS)		3			3
Journal of the American Chemical Society			1		1
Journal of the Institute of Brewing	1		1	1	3
Journal of Trace Microprobe Techniques		1			1
Klinická biochemie a metabolismus				1	1
Kvasný průmysl			1		1
Minerva biotecnologica			1		1
Monatshefte Brauwiss.	1				1
New Journal of Chemistry				1	1
Ochrana ovzduší			1		1
Pharmazie			1		1
Polycyclic Aromatic Compounds		1			1
Polyhedron				1	1
Proteomics			1	1	2
Rostlinná výroba		1		1	2
Spectrochimica Acta B	1	3		2	6
Toxicology Letters			1		1
Total	**25**	**41**	**38**	**41**	**145**

Publication frequency in scientific journals in 1999-2002



Contributions of the employees of the Institute of Analytical Chemistry to the impact factor production of the Institute in 1999 - 2002.

Name	**1999**	**2000**	**2001**	**2002**	**total**
Babický		0,913	0,385	0,423	1,721
Boček	8,921	23,825	5,212	10,093	48,051
Broškovičová				1,033	1,033
Bucková		0,425			0,425
Čáslavský		0,724		0,713	1,437
Dědina	0,605	3,700		6,141	10,446
Dočekal		2,467	1,668	0,308	4,443
Dokulilová/Budinská			0,806	0,390	1,196
Foret			3,285	3,487	6,772
Gebauer	1,518	4,913	3,785	4,686	14,902
Horká		1,693	0,466	3,244	5,403
Chmelík	3,101	1,810	4,217	3,580	12,708
Janoušková			0,698		0,698
Kahle	1,145	0,829	0,466	0,168	2,608
Karásek	1,518	0,425		0,916	2,859
Klepárník	0,840	0,675	2,358	2,781	6,654
Krumlová			0,108	0,082	0,190
Křivánková	1,680	1,805		2,162	5,647
Lojková		0,425			0,425
Malá	0,840	0,675	1,427		2,942
Marek		2,467	1,653		4,120
Matoušek	0,605	3,700		4,793	9,098
Matulík				1,033	1,033
Mikešová	0,063	0,344			0,407
Mikuška		0,925	1,605	1,225	3,755
Otevřel				2,163	2,163
Pantůčková	2,358	2,766		1,081	6,205
Pařízek			0,931		0,931
Planeta	1,581	1,538	1,137	2,614	6,870
Plocková		1,276	1,164	1,033	3,473
Pól				0,916	0,916
Procházková	0,840	0,846			1,686
Přibyla		0,564			0,564
Rejtar	1,260	1,147			2,407
Roth	4,353	0,918	0,845	0,916	7,032
Řehulka			0,671	1,821	2,492
Selecká				0,898	0,898
Sklenská/Pešková			0,931	1,033	1,964
Šalplachta				0,067	0,067
Šlais	2,520	1,693	0,715	3,244	8,172
Šťastná	2,409	4,180	3,588	1,081	11,258
Varaďová Ostrá				0,067	0,067
Večeřa		1,350	1,263	2,258	4,871
Vejrosta	1,581	1,113		0,849	3,543
Vespalec	2,011	14,201	0,765	0,685	17,662
Vlčková				0,112	0,112
Vobecký	0,598	2,076	0,385	2,340	5,399
Zdráhal		0,871	0,448	0,625	1,944
Žídková		0,139	1,343		1,482
total	**40,347**	**87,418**	**42,325**	**71,061**	**241,151**

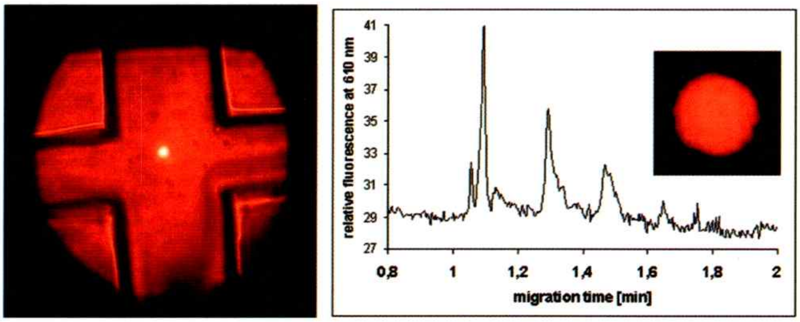
Department of Bioanalytical Instrumentation

Research scientists:	Ing. František Foret, CSc. (head)
	Ing. Karel Klepárník, CSc.
Research assistant:	RNDr. Zdeňka Malá
PhD. students:	Ing. Michal Spěšný
	Mgr. Petr Šenk
	Mgr. Marek Otevřel
	Ing. Jakub Grym
	Ing. Jana Křenková

The department was established in 2001 to promote research of new trends in bioanalytical sciences. The core research scientists have previously been involved in a number of projects dealing with DNA and protein analysis using capillary electrophoresis coupled to laser induced fluorescence and mass spectrometry. Current research is focused on **microfabrication, microfluidics and separations with LIF detection, mass spectrometric interfacing and single cell analysis.**

Current research topics

The success of microfabrication is most notable in electronics; however, the first successful applications of microfluidic devices have also been described. Shrinking the size of the devices and integration of several analytical steps not only reduces the cost of the analysis, but also allows the speed and efficiency of the analysis to be increased. This is well known in microcolumn separations where the reduction of the column diameter provides significant increase in the separation efficiency.

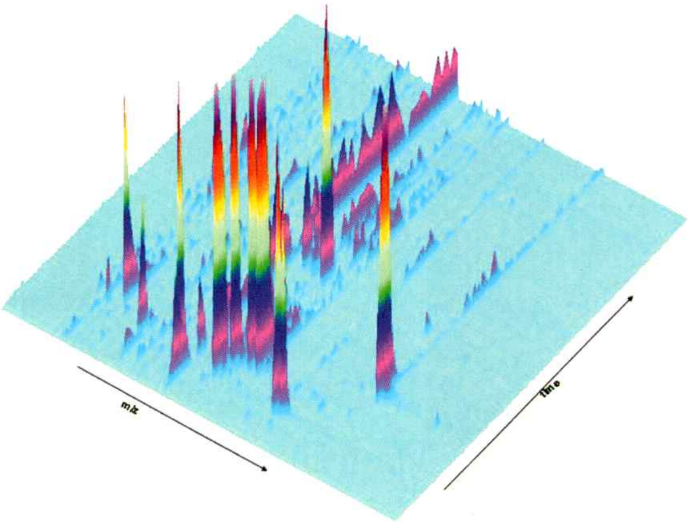


Single cardiomyocyte in the microchannel and analysis of its degraded DNA.

Current trends focus on microfluidics to speed-up and simplify the instrumentation for analysis of very small sample amounts. Since the department's inception, the work has been directed towards building the basic infrastructure for microfabrication including the construction or acquisition of a clean box, spin coater, bonding furnace, etc. At the same time collaborations with industrial partners (Gyros, AB, Sweden and ABI, USA) have been established, providing access to some of the more advanced technology.

In collaboration with the Institute of Pathological Physiology of Masaryk University, new analytical methodology is being developed for the detection of apoptosis induced by doxorubicin (important cytostatics) in individual cardiomyocytes. Based on previous experience with the single-cell analysis by capillary electrophoresis, planar structures of a microfluidic system integrated on a CD-like plastic disk have been used for analysis of individual cells. DNA fragments labeled by a fluorescent intercalating agent were separated in electric field and recorded as a laser-induced fluorescence signal using a confocal microscope equipped with CCD camera and photomultiplier tube.

Recently the department acquired an electrospray TOF mass spectrometer (donation of ABI, Framingham, MA, USA) and started working on on-line coupling with capillary separations. An example of a peptide analysis by CE-MS using a laboratory-constructed interface is given in Figure. This work is directed towards identifying the structure of new lytic enzymes released by therapeutically interesting bacteriophages in a joint research conducted with the departments of Analytical Chemistry and Molecular Biology at the Masaryk University in Brno. In another collaboration with the University Hospital in Brno and with support of our industrial partners we are developing a microfluidic platform for analysis of markers of metabolic disorders. A new interface for the direct interfacing of the microfluidic devices with mass spectrometry is currently under development.



CE/MS analysis of a peptide mixture

Future trends

Many analytical protocols rely on unequivocal identification of the analyzed compounds with mass spectrometry. Such analyses are required, e.g., in proteomics, drug development or for analysis of biomarkers and metabolites. The focus of the future research will be on development of microfabricated devices for analysis of minute amounts of samples with direct coupling to ESI/MS. An important part of this research will also be directed on finding new ways to enhance the ion transmission into the mass spectrometer. The main stress will be initially put on the electrospray ionization; however, new modes of the MALDI will also be investigated. Besides the mass spectrometry coupling the LIF detection will be used for the most sensitive analyses with the microfluidic devices. Here we expect to benefit from our extensive experience with CE-LIF and the availability of the new generation of solid state lasers.

Although advances in the LIF detection and mass spectrometry allow analyses, which were impossible to achieve just a few years ago, the sample preparation is going to be a dominant part of the analytical process in the years to come. This is especially true when attempting analysis of small amounts of complex biological samples. Thus, a significant effort will be spent on development of the prefractionation and sample enrichment techniques and procedures, including microscale separations (electrophoresis, chromatography). Additionally, miniaturized reactors with immobilized enzymes will be developed for integration into the microfluidic systems.

There is a growing interest in developing techniques for analysis of individual cells or cell organelles. As discussed before, this effort was partially succesful on the DNA level. Currently, the interest also includes proteins, peptides and small molecules in single cells. Given the sensitivity limits, such research is currently restricted to LIF or electrochemical detection. We anticipate that knowledge gained in our research will form the basis for succesful development of a system for cell handling using microfluidic devices with integrated enzyme reactors and mass spectrometric analysis. Such a system will be designed for monitoring of protein expression and detection of biomarkers.

Currently supported projects

Integrated genome and proteome analysis of therapeutically important bacteriophages

Grant Agency of the Czech Republic, grant No. 203/03/0515 (2003-2005)
Co-Investigator: František Foret

Coupling of microchip with mass spectrometry for high throughput population and selective screening of inborn metabolic disorders
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. S4031209 (2002-2005)
Principal Investigator: František Foret

Other specific activities

The core members of the department have teaching activities at the Masaryk University in Brno, University in Pardubice and Charles University in Prague. Since the department inception the results of the department have been presented as invited lectures at 10 international conferences.

The department head is a member of editorial boards of Electrophoresis (deputy editor), Journal of Chromatography and Journal of Separation Science. A special issue on miniaturization is being edited yearly for the journal Electrophoresis.

Industrial collaborations include Gyros AB, Uppsala, Sweden and Applied Biosystems, Framingham, MA, USA.

Academic collaborations have been established with the departments of Analytical Chemistry, Molecular Biology and Pathological Physiology of Masaryk University and with the University Hospital in Brno.

Additional informal collaborations are ongoing with several academic groups in Europe and the USA.

Department of Biopolymer Analysis

Research scientist:	Ing. Josef Čáslavský, Ph.D. (head)
Research assistants:	RNDr. Jana Plocková Ing. Karolína Benešová Ing. Pavla Kotlaříková
Undergraduate students:	Markéta Prajslerová Hana Staňková

The history of the Department of Biopolymer Analysis is quite short – this department was established on 1st of November 2001 as one of the successors of the former Department of Biopolymer Separation.

Current research topics

The most important research topic in the Department is entitled "The methods of organic compound analysis by non-traditional coupling of separation methods and mass spectrometry". The off-line coupling of supercritical fluid chromatography and MALDI-TOF mass spectrometry was found to be very useful for the analysis of complex mixtures like silicone oils, which are very difficult for other separation and detection methods. Very good results were also obtained in the field of analysis of heavy polycyclic aromatic hydrocarbons by coupling of HPLC with ion trap mass spectrometry using argentation electrospray ionisation. This method is based on the post-column addition of silver cations, which effectively ionise polyaromates in standard electrospray. Promising results were also obtained for the analysis of peptide mixtures by coupling of μHPLC and ion trap mass spectrometry with electrospray ionisation.

Methods of passive sampling of persistent organic pollutants in the environment by semipermeable membrane devices (SPMDs) represent another important field of research activities in the department. The research interest in the last 3 years was concentrated on the sampling of polyaromatic hydrocarbons (PAHs) in the atmosphere. On the basis of experimental results obtained, a model of organic compounds uptake by SPMDs was proposed and the first experimental values of sampling rates RS for PAHs were derived (these values are used for the evaluation of average PAH concentration during sampling period). This problem is quite complex due to physical state of PAHs in the atmosphere (some are almost exclusively in vapour phase, some are only in particulate phase, and some are distributed between them) and due to the influence of meteorological conditions, which is not quite clear yet.

Future trends

The research activities of the Department in the next few years will be focused on the application of non-traditional coupling of separation methods with mass spectrometry for the analysis of complex mixtures of organic compounds, which are difficult to analyse by traditional hyphenated methods. The off-line coupling of μHPLC and MALDI-TOF mass spectrometry is one of the most promising methods in this field, followed by the application of electromigration methods (especially capillary electrochromatography) with mass spectrometric detection.

In the field of passive sampling of organic pollutants using semipermeable membrane devices (SPMDs) the main research effort will concentrate on the process of the uptake of compounds adsorbed on particles by SPMDs from atmosphere, resulting in the improvement of reliability in the results obtained using these devices for passive air sampling. Also the influence of important meteorological parameters (temperature, wind speed) and some other factors like the shape of deployment device on the amounts of organic compounds sequestered by SPMDs will be studied.

Currently supported projects

Sampling of persistent organic pollutants by SPMDs
Grant Agency of the Czech Republic, grant No. 205/01/0836 (2001 – 2003)
Principal Investigator: Josef Čáslavský

Analysis of organic compounds by non-traditional coupling of separation methods and mass spectrometry
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A4031104 (2001 – 2005)
Principal Investigator: Josef Čáslavský

Other specific activities

- membership in the organization committee of the 2nd European Meeting on Environmental Chemistry (Josef Čáslavský)
- membership in the Association of Chemistry and the Environment (Josef Čáslavský – member of the scientific board)
- membership in the editorial board of Environmental Chemistry Letters (Josef Čáslavský – associate editor)

Department of Electromigration Separation Methods

Research scientists: Prof. RNDr. Petr Boček, DrSc. (head)
RNDr. Petr Gebauer, CSc.
Doc. RNDr. Ludmila Křivánková, CSc.
Mgr. Pavla Pantůčková, Dr.
Mgr. Marek Urbánek, Ph.D.
Doc. Ing. Radim Vespalec, DrSc.
Mgr. Andrea Procházková, Dr.
Research assistants: Ing. Michaela Březková
PhD. student: Ing. Hana Horáková

The department was established in 1972 as the Department of analytical isotachophoresis. In 1980 it was renamed the Department of electromigration separation methods. The head of the Department since its beginnings is Petr Boček except the period 1993 – 2001 when he was Director of the Iinstitute and the Department was headed by Ludmila Křivánková. The present staff is formed by experienced scientists renowned by the international scientific community. The scientific output of the department comprises over 250 scientific papers, chapters, and books on Analytical Capillary Isotachophoresis and Capillary Zone Electrophoresis, many of them with high citation counts.

Current research topics

The development of preconcentration and preseparation procedures

is aimed at reaching and utilizing the limits of the method of capillary zone electrophoresis (CZE) in the field of analysis of samples with complex matrices. The effects caused by the presence of transient isotachophoresis (ITP) known also as stacking effects can be utilized to increase separation efficiency and sensitivity of analyses provided that their principles are known. Selected results:

- description of the principles and properties of processes with stacking effects, including the behavior of the zone boundaries and of their self-sharpening properties at the moving-boundary method as well as at isotachophoresis and zone electrophoresis;
- description of the properties of a macrocomponent in real samples especially of biological origin where this component may under certain conditions act against the stacking effect of other sample components that play the role of leader or terminator of the transient isotachophoretic process;
- study of conditions of correct quantification of minor analytes from UV-detection peak height, defining the mutual influence of sample preparation and composition of the background electrolyte on the linearity/non-linearity of calibration curves as well as the correct quantitation when indirect UV absorbance detection is used.

The research of methodology of capillary zone electrophoresis investigates the behavior of analytes in electrolyte systems to define the conditions for reaching maximum separation efficiency and therefore also maximum sensitivity of the given method. Selected results:

- the study of effects in commonly used background electrolytes, as, e.g., phosphate buffers which behave in the neutral and weakly alkaline region as a binary electrolyte with respect to the co-ion. This results in the existence of two regions of zone symmetry and of one region of large dispersion of analyte zones;
- the definition, conception and terminology of the concept of selectivity, the optimum use of which allows the optimization of the conditions of a separation in a way that ensures the achievement of maximum efficiency of various types of analyses;
- the study of electrolytes containing multivalent weak acids or bases, the individual dissociated forms behave as mutually independently migrating co-ions. This introduces into the system a new center of symmetry and thus also a migrating system zone which negatively influences the separation and detection of analytes migrating nearby;
- the study of the effect of cetyltrimethylammonium bromide as an electrolyte additive influencing the electroosmotic flow which interacts with the surface of fused silica capillaries but also with substances present. In a concentration higher than the critical one this additive forms micelles and enables the separation of neutral components as well, at the same time, however, at concentrations slightly above the critical value, it may behave as a three-component additive and increase the number of potential system zones;
- the creation of a system to a comprehensive approach to the selection of a suitable electrolyte system for capillary electrophoretic analysis of a given sample. The system includes general rules based on a theoretical description of electrophoretic migration of analyte and system zones as well as a computer program evaluating published results for the same or similar analytes by means of artificial intelligence simulating the approach of an expert chemist.

The research of electrophoretic interaction phenomena and their utilization in the measurement of physico-chemical constants. An example are chiral separations of optically active isomers which are very important especially in pharmacy because the particular isomers differ in their physiological effect and their production requires maximum purity of the preparations. Selected results:

- the study of capillary zone electrophoresis separations with emphasis to a correct selection of working systems and to an optimization of separation conditions;
- the study of interactions of chiral selectors with enantiomers and other chiral analytes during electrophoretic separations and the calculation of stability constants from data obtained form electrophoretic analyses;

- research of biochemical activity of the alkaloids sanguinarine and chelerythrine and measurement of stability constants of complexes of these alkaloids with human and bovine serum albumin;
- the use of capillary electrophoresis for the development of chiral separation methods for sterically different forms of charged cluster boranes and their derivatives.

Future trends

The future research in the department will be focused on further increasing the sensitivity and selectivity of capillary electrophoretic techniques. This should bring new possibilities to directly apply capillary electrophoretic techniques in cases where laborious and time-consuming sample pretreatment are needed so far or where analytical methods are not established yet. Fundamental research will be aimed at processes that occur during the separation process in complicated systems where the sample contains a complex matrix with varying concentration ratio of components of the order up to one million, or the separation medium contains additional ion pairing or complexing agents. Behavior of analytes in such complicated systems will be studied both theoretically and experimentally and attention will be paid to properties of zones and zone boundaries with the aim to predict zone properties and to select the optimum composition of the separation medium. Attention will also be paid to the methodology of micropreparation of sample components in order to identify them by mass spectrometry or other methods. Methodology and instrumentation of new concentrating and fractionating principles will be developed to be on-line combined with capillary electrophoresis. Theoretical considerations will also investigate the problem of optimization, validation and critical evaluation of the expeditiousness of the methods and techniques developed. Besides the above topics, the research in the application area will be focused at chiral separations, their optimization and search for new selectors.

Currently supported projects

Development of new procedures for highly reliable analytical control of technologies, materials, foods and environment and for medical diagnostics with the use of modern instrumentation
Grant Agency of the Czech Republic, grant No. 203/02/0023 (2002-2004)
Co-Investigator: Ludmila Křivánková

System phenomena in capillary electrophoresis
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A 4031103 (2001-2005)
Principal Investigator: Petr Boček

Mapping of electrolyte systems for electrophoresis
Grant Agency of the Czech Republic, grant No. 203/01/0401 (2001-2003)
Principal Investigator: Petr Boček

Other specific activities

Petr Boček is Senior Deputy Editor of the international journal Electrophoresis (Wiley) and member of the editorial boards of Journal of Chromatography (Elsevier), Journal of Capillary Electrophoresis (ISC Technical Publications), Analytical Biochemistry (Academic Press) and Journal of Biochemical and Biophysical Methods (Elsevier). Ludmila Křivánková is member of the editorial board of Electrophoresis. Petr Boček is permanent member of the scientific committee of international symposia on isotachophoresis – ITP.

Department of Environmental Analytical Chemistry

Research scientists:	Ing. Zbyněk Večeřa, CSc. (head) Doc. RNDr. Bohumil Dočekal, CSc. RNDr. Pavel Mikuška, CSc. Ing. Anna Broškovičová, PhD. RNDr. Zbyněk Zdráhal, CSc.
Research assistant:	Ing. Jana Sklenská
Technician:	Zlatka Týnová
External graduate student:	Ing. Veronika Smetková

The Department of environmental analytical chemistry was established in 1990 in response to growing public interest in environmental problems in the Czechoslovak Republic. The first activities were focused on analyses of real samples, mainly determination of PCBs and PAHs content in soils; studies of decomposition of organic compounds using low-temperature plasma; research of selected halogenated hydrocarbons (volatile organic compounds and chlorinated biphenyls) in ecosystems as well as study of negative influence of traffic on environment.

Later, the knowledge of the research team was explored within the projects supported by the Agency of the Ministry of Health named „Application of perfluorocarbons for the lungs ventilation in the therapy of respiratory distress syndrome in neonates“ and „Application of nitric oxide in therapy of respiratory distress of neonates“, and the Agency of the Ministry of Defense “ Study of presence PCB and PAH within army bases”.

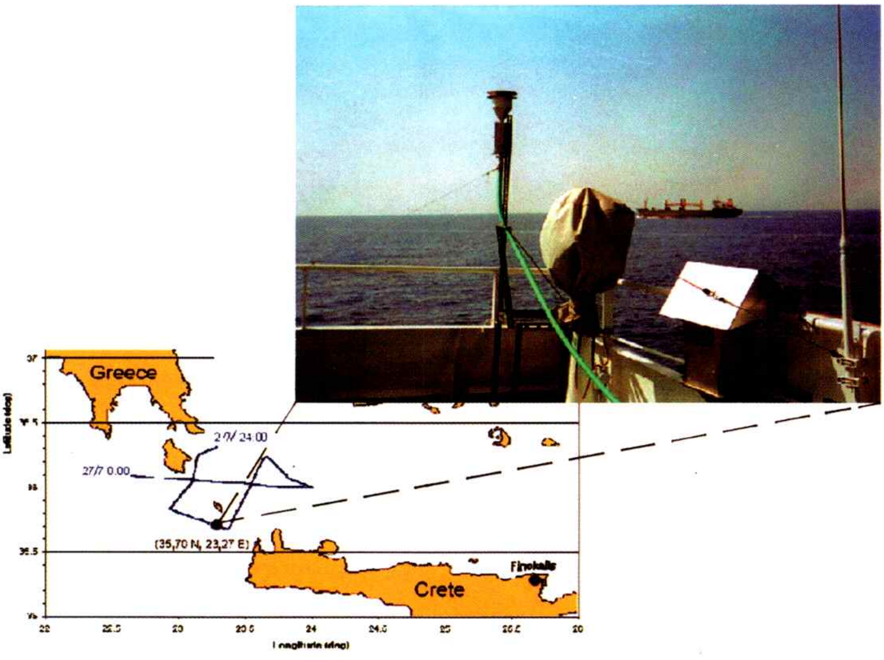
Since 1993, the research activity has been shifted from the practically oriented research (quantitative determination of trace concentrations of organic and inorganic pollutants by existing analytical instrumentation methods) towards new enrichment techniques and progressive instrumentation for determination of important pollutants of an inorganic nature (e.g. nitrogen dioxide and ozone) as well as organic compounds at ppt (v/v) levels in ambient air, what lead to greater selectivity and reduction in analysis time of compounds of interest.

An important part of the research was theoretically goal-directed to fundamental understanding of the mass transport across the gas-liquid interface, and equilibrium processes between gas and liquid phases and their analytical exploitation.

In 1990, a Laboratory designed for trace element analysis was established as an integrated part of the Department. It is equipped with clean-room facilities and clean benches (class 100 according to US Standard 209b), and modern instrumentation for atomic absorption and emission spectrometry, special devices for sample preparation and decomposition, and other specific dedicated tools.

Current research topics

During the years 2000-2003, part of the research activity stemmed from the exploitation of "unconventional" enrichment techniques such as wet effluent diffusion denuders and aerosol enrichment unit. Fully automated devices were developed for the determination of reactive nitrogen species and ozone, within very different meteorological conditions. These original pieces of equipment have been used for monitoring of "background concentration" (10-100 ppt (v/v)) of the compounds of interest in air, and in studies of the origin, transport, and interchanges among oxo-compounds of nitrogen (NO, NO2, HNO2, HNO3) in continental air as well as in on-boat measurement carried out in the Mediterranean area (INCO-Copernicus project "Subgride Scale Investigations of Factors Determining the Occurrence of Ozone and Fine particles").

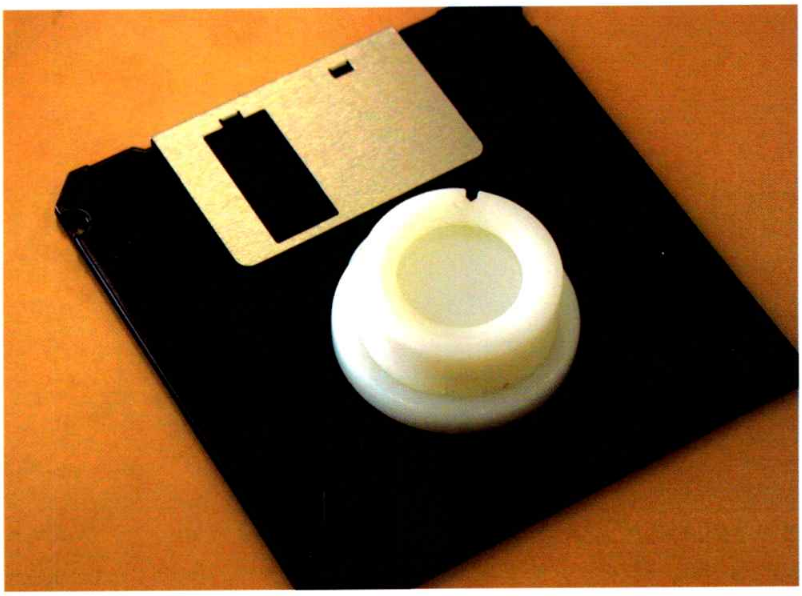


Boat measurement carried out in the Mediterranean area

A new analytical system based on the chemiluminescence with alkaline solutions of luminol was designed for the determination of nitric acid in aqueous solutions at 10-9 M level. The unique process of UV reduction of nitrate to peroxynitrous acid in water, and diffusion based collection of trace gases into an absorption liquid not only offers a discrimination from concurrently present aerosol particles, but also provides extreme selectivity, high-time resolution and unprecedented sensitivity in the determination of nitrous and nitric acids in ambient air.

In the period of 2000-2003, an important part of the research effort has also been focused on the development of the methodology and instrumentation for continuous monitoring of non-methane hydrocarbons (NMHCs) in forest air, based on wet effluent diffusion denuder and large volume injection technique (LVI). The developed devices are advantageous for the implementation in cases when fast analyses of NMHCs and automation are of paramount importance, primarily it is usable for the monitoring NMHCs fluxes in forest air.

During the period of 2000-2003, first steps for deep understanding of the role of aerosols in atmospheric processes were done. The three-year of GA AV (2000-2003) project deals with the development of special modification of the Venturi scrubber with pre-impactor, and/or cyclone allowing continuous sampling of atmospheric aerosols of inorganic nature. The aerosol collector provides selective sampling of fine aerosol fraction, and with on-line detection devices can provide in-situ automated analysis of selected aerosol species in real time.



Device for Diffusive Gradient in Thin film (DGT) technique

The main goal of activities in the Laboratory designed for trace element analysis (B. Dočekal´s group) was to develop new methods for the characterization of bioavailability and toxicity, especially of heavy metals in ecosystems, soils, invertebrates and plants. Fractionation/speciation studies, complementary to the conventional operationally and functionally defined methods, were performed on sewage sludge contaminated soils by Diffusive Gradient in Thin film technique (DGT) that can provide very good surrogate of bioavailability of heavy metals. Comparative measurements were carried out on invertebrate specimens and crop plants growing on these soils. Development of new analytical methods for determination of very low concentration of essential and toxic elements at ppb and sub-ppb level in biological and clinical materials is also in the scope of the research activities in the Laboratory. The Laboratory also participated in activities concerning generation and atomization of volatile analyte species for atomic spectrometry methods, primarily supported and organized in close cooperation with the detached group of the Department of Trace Element Analysis (J. Dědina, Prague). The main interest is focused on the investigation of an in situ collection of hydrides and other volatile compounds of analyte, covalent hydrides and carbonyl compounds. The principal objective of this investigation is to develop new powerful methods for the determination of very low concentrations (down to the sub-ppb level) of detrimental elements in materials for modern technologies, for advanced ceramics and microelectronics, that may play a decisive role in the quality of final products. New approaches of overcoming the sample contamination, reduction of sample treatment are developed. A very promising approach is to apply direct sample introduction and analysis. For this purpose, new prototypes of devices are designed and tested.

Future trends

The major goal of the department is shifting to the search for sources of and the definition of the environmental effect of selected groups of organic (biogennic compounds, C1-C6 aldehydes, and pesticides) and inorganic pollutants (NO, NO2, HNO2, HNO3, PAN, NH3, and OH radicals) that are distributed in the gas phase, and/or contained in aerosols. The research in this area will arise from exploitation of conventional analytical techniques, new analytical practices (tandem GC-MS, GS/MS/MS and LC-MS with large injection procedure) and "unconventional" enrichment techniques such as wet effluent diffusion denuders and aerosol enrichment unit.

The studied subjects will include understanding the basic principles behind the long-term effects of increased carbon dioxide levels on the formation, accumulation and emissions of isoprene, monoterpenes and other reactive volatile compounds produced by coniferous (Picea abies (L.) Karst) as well as deciduous forest. The results can help us to explain the role of forests under „Global warning“ climate changes.

Further, problems related to environmental chemistry of aerosols will also be explored, and emphasis will be placed on the following topics:

- origin, transport, and interchanges among oxo-compounds of nitrogen (NO, NO2, HNO2, HNO3, PAN, NH3) in air, their relation to production and composition of aerosols, and the linking relations to acidification processes.
- study of the microstructure of aerosols and the actual chemical composition of

urban aerosol. Contributions of natural sources (lignine, cellulose, hemi-cellulose) to the aerosol content.

- study the influence of elevated concentrations of NO2 on the chemistry of new nitro compounds with a high mutagenic potential.
- study of the source of humic-like substances in air.

In the near future, the group will also be focused on the development of methods for analysis of environmental samples, and instrumental prototypes designed on electric focusing for preconcentration of aerosols linked with atomic emission spectroscopy, as a tool for in-time monitoring of specific sources of fine aerosols. Original equipment for monitoring of "background concentration" of the compounds of interest in air, and sampling devices for aerosol analysis will also be designed and built.

The main goal of activities in cycling trace metals in sustainable management of agricultural soils, from the point of view of food safety and quality, will be the development of new methods for characterization of bioavailability and toxicity, especially of heavy metals in ecosystems, like soils, invertebrates, plants etc. New analytical methods for the determination of very low concentration of essential and toxic elements at ppb and sub-ppb levels in biological and clinical materials are also in the scope of the research activities in this laboratory. Especially, the development of procedures for contamination free sample preparation is of great interest.

Investigation of new pollutants hazardous to health and for mechanisms of their transport and distribution in ecosystem is still a perennial element of the environmental analytical chemistry.

Currently supported projects

Emission of biogenic compounds from Picea abies (L) Karst under long-term increased amount of carbon dioxide

Grant Agency of the Czech Republic, grant No. 526/03/1182 (2003-2005)
Principal Investigator: Zbyněk Večeřa

Development of new aerosol collector for continuous sampling of fine fraction of atmospheric aerosols

Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A4031105 (2001-2003)
Principal Investigator: Pavel Mikuška

Subgride Scale Investigations of Factors Determining the Occurrence of Ozone and Fine particles

INCO Copernicus EVK2-CT-1999-0052 SUB-AERO, 5th FP (2000-2003)
Supervisor: Zbyněk Večeřa

Characterization and Sources of Carbonaceous Atmospheric Aerosols
Belgian grant, SPSD II EV/02/11(2000-2005)
Supervisor: Zbyněk Večeřa

Development of techniques for generation and atomization of volatile analyte compounds for trace and ultratrace element and speciation analysis by atomic spectrometry methods
Grant Agency of the Czech Republic, grant No. 203/01/0453 (2001-2003)
Member of the research team: Bohumil Dočekal

Other specific activities

- **cooperation with foreign research institutions**

  The Scientific and Technical Research Council of Turkey, Bursa Test and Analysis Laboratory, Turkey, Prof. S. Gucer

  Ghent University, Department of Analytical Chemistry, Institute for Nuclear Sciences, Belgium, Prof. W. Maenhaut

  N.C.S.R. Demokritos, Athens, Greece, Dr. K. Eleftheriadis

  Technical University of Crete, Chania, Department of Environmental Engineering, Greece, Prof. M. Lazaridis

  Dept. Environmental Sciences, University of Parma, Italy, Prof. N. Marmiroli
- **teaching activities**
  - basic courses in atomic spectroscopy – Institute of Analytical Chemistry, Technical University Prague (B. Dočekal)
  - courses for postgraduate students in atomic spectroscopy - organized by Ioannes Marcus Marci Spectroscopic Society (B. Dočekal)
- **membership in editorial boards**
  - member of the Advisory Board of the journal Analytical and Bioanalytical Chemistry (merger of Fresenius J. Anal. Chem, Analusis, Química Analítica – Springer Verlag) (B. Dočekal)
- membership in societies
  - vice-president (B. Dočekal) of the Ioannes Marcus Marci Spectroscopic Society
  - vice-president of the Czech aerosol society (Z. Večeřa)
  - member of the Committee for Environment at the Academy of Sciences of the Czech Republic (Z. Večeřa)

Department of Liquid Phase Separations

Research scientists:	Doc. RNDr. Karel Šlais, DrSc. (head)
	RNDr. Vladislav Kahle, CSc.
	Ing. Marie Horká, CSc.
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Technician:	Olga Smolová
PhD. students:	Mgr. Marta Zeisbergerová
	Ing. Vratislav Košťál
Undergraduate students:	Zdeňka Šišková
	Markéta Šrámková

The department, which was known as the Department of liquid chromatography until 1993, when it was renamed the Department of liquid phase separations due to involving in capillary electrophoretic techniques and hybrid techniques including electrochromatography, chromatofocusing as well as other chromatographic techniques including capillary liquid chromatography and thin layer chromatography. Since that time, a number of the Department's papers have also dealt with isotachophoresis, zone electrophoresis and isoelectric focusing.

Current research topics

Capillary electrochromatography (CEC)

Capillary electrochromatography (CEC) can be considered a hybrid of capillary electrophoresis and high performance liquid chromatography (HPLC). The growing interest in this technique is reflected in an increasing number of relevant scientific publications. CEC uses an electrically driven flow to transport the solutes through the chromatographic column. Separation can be achieved by differential interaction with the stationary phase, differential electromigration, or a combination of both.

A simple, easy-to-assemble gradient generator for capillary electrochromatography was suggested. Using a homologous mixture of alkylphenones and a macroporous polyacrylic separation matrix, we demonstrated the feasibility of either stepwise or continuous gradients to be performed in a reproducible manner. Also, a microprocessor controlled gradient elution system suitable for capillary electrochromatography has been developed and tested for electrochromatography on a monolithic polyacrylic column under gradient conditions.

Capillary electrochromatography (CEC) using polymer-based monolithic stationary phase has been developed as a promising method for the determination of lignans of Schisandra chinensis. The columns were prepared by in situ copolymerisation of acrylamide, N,N‘-methylenebisacrylamide, vinylsulfonic acid and lauryl acrylate in presence of poly(ethylene glycol) as a porogenic agent. The results of quantitative CEC analyses were compared with those obtained by reversed-phase HPLC, the levels of schizandrin, gomisin A, gomisin N and wuweizisu C determined by CEC were in good agreement with those determined by HPLC.

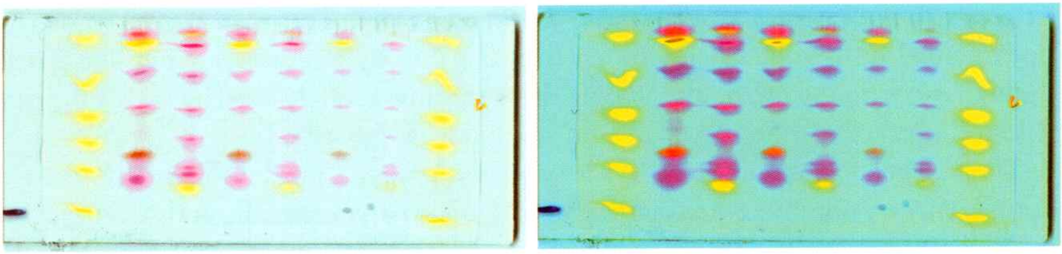
Capillary zone electrophoresis (CZE)

Pyrenebutanoate as the amphiphilic fluorescent compound was suggested as a buffer additive in capillary zone electrophoresis (CZE) for a dynamic modification of several protein samples. Using deuterium lamp UV excitation for the on-column fluorometric detection, minimum detectable amounts in the amol-range of the proteins sampled on the CZE capillary were achieved.

The asymmetric triangle (fronting or tailing) concentration profiles and their broadening are the typical results of the electromigrational zone dispersion characterizing a system of the analyte in the background electrolyte (BGE). We have suggested the parameter named the relative velocity slope, S-BGE,S-X, which was introduced as a quantity characterizing the peak broadening and the asymmetry. S-BGE,S-X vs. analyte ionic mobility diagrams are suitable for comparison of BGEs of a given pH and conductivity composed of electrolytes of different pK(a)s and ionic mobilities. The concept of S-BGE,S-X diagrams is verified by capillary zone electrophoresis of the model analytes, which involve (i) the series of sulfobenzoylated poly(ethylene glycols) as examples of the strong electrolytes with different ionic mobilities and (ii) the series of monobasic phenols as weak electrolytes with different pK(a)s and similar ionic mobilities. It follows from both theoretical predictions of peak symmetry and their experimental verification that the optimum composition of BGEs is determined mostly by the suitable ionic mobility of the counterion in dependence on the ionic mobility of the analyte. It was proved that the low-conductivity BGEs based on low-molecular carrier ampholytes are at best only comparable with the properly chosen monobasic electrolytes.

Isoelectric focusing (IEF)

Isoelectric focusing (IEF) is a technique for separation, focusing, and characterization of amphoteric analytes such as proteins. The principle of the method involves the focusing of an amphoteric molecule at that point in the system, where the local pH value corresponds to the isoelectric point (pI) of the ampholyte. To characterize the analyte, the pH at the place of focusing should be known. pH is most often evaluated with the help of the reference substances. They may have different names, e.g., pI markers, isoelectric point markers, IEF standards, IEF markers, pH markers, internal markers, test substances for IEF, etc. Their use may be universal: they are applicable to gel plates, in preparative and chip channels and also in capillary modes of IEF. Until now, the derivatized proteins, which are not only unstable but also difficult to separate (due to their large molecular mass) from bioactive molecules after preparative IEF, have been used as pI markers. Though a number of other compounds were developed and suggested in the past, they are still far from the optimal ones.



Gel 50x105x0.4 mm, model 111 mini IEF cell (BioRad), 3% (v/v) Bio-Lyte 3/10 ampholyte (Bio-Rad) in polyacrylamide gel (5%T, 3%C), constant power 0.6 W, run 1 hour, scanning after focusing by UMAX Astra 3400 scanner (UMAX Technologies Inc.), left - real colors, right – software-modified colors. Lane 1 and 8 - yellow pI markers : pI - 4.0, 5.3, 6.4, 7.5, 8.5, 10.1, Lane 2, 4 (2 x diluted), 6 (4 x diluted) - Mixture A (approximate pI - color at pI): - 2.7-orange (not visible) , 3.4- purple, 4.2-yellow, 5.2-violet, 6.3-red, 7.8-brown, 8.9- purple; Lane 3, 5 (2 x diluted), 7 (4 x diluted) - Mixture B - 2.7-red, 3.4-orange, 4.2-red, 5.2-violet, 6.3- red, 7.1-purple, 8.4-purple, 8.7- purple, 9.2-yellow.

One of our research activities is directed toward the development of low-molecular-mass good ampholytes with potential use as model compounds in electrophoretic focusing techniques. Currently, several potentially useful compounds have been developed; their detailed characteristics and applicability in electrofocusing techniques are examined.

Recently, a series of low-molecular-mass fluorescent compounds excitable in near UV region with suitable acidobasic and electrophoretic properties was suggested as pI markers for IEF with UV photometric and UV excited fluorometric detection. The experimental setup of cIEF with UV excited fluorometric detection and properties of new UV induced fluorescent pI markers were given. Examples of separation of new pI markers together with derivatized proteins by cIEF with photometric or fluorometric detection have been presented.

We also prepared a series of low-molecular-mass fluorescent ampholytes with narrow pI range. These fluorescein-based ampholytes are detection-compatible with argon laser-induced fluorescence (LIF) detection. The selected properties, important for their routine use as fluorescent pI markers, were examined. The pI values of new fluorescein-based pI markers were determined by capillary isoelectric focusing (CIEF) using currently available low-molecular-mass pI markers for CIEF with photometric detection. The examples of CIEF with fluorometric detection of new compounds together with fluorescein isothiocyanate (FITC) derivatized proteins were presented.

The visual indication of actual gradient course is advantageous in IEF. Even under current status of development of colored pI markers they may be useful for some applications. Especially, the visual observation of focusing process, e.g., on a gel plate, may help to fix the time needed to complete the focusing, to demonstrate the focusing for students, to quickly check the gradient profile, to locate the invisible spot without staining, etc. The applications in capillary, chip and FFE forms of IEF also come into consideration. When sampling on a gel plate, micrograms are sufficient to generate well observable marker spots, see Figure. They can conveniently be documented on-line by a camera or, after the run, by a scanner. When the gel is treated by a staining procedure, the markers are immediately washed out of the gel. The solutions of markers are stable on shelf at least for weeks.

The sol-gel techniques of the FS capillary preparation developed by us is a simple way to minimize the interactions of microorganisms, E. coli, C. albicans, S. epidermidis and E. faecalis, and proteins with the capillary wall during CIEF and to achieve a high separation efficiency and good reproducibility. The relative standard deviations in retention times by CIEF of all analytes, low-molecular-mass and fluorescent pI markers or microorganisms and fluorescein labeled proteins, respectively, were lower than 2 %. This indicates the stability of the sol-gel coating of the capillaries. The sol-gel technology is compatible with on-column fluorescence detection with 490 nm excitation wavelength. This technique of the capillary coating is possible to use for the identification and quantification of microorganisms by CIEF.

Future trends

Capillary electrochromatography (CEC) is a quickly developing high performance separation method based on combination of principles of capillary liquid chromatography (LC) and capillary electrophoresis (CE). The method enables separation of analytes with the selectivity typical to LC while the high efficiency and simplicity of instrumentation corresponds to CE. Now it is obvious that CEC is a promising analytical tool in many important application areas. Future projects aim to contribute to the development of CEC in the following areas:

- development of the integrated sample injector and mobile phase gradient generator.
- preparation, characterization and analytical application of polymeric CEC stationary and pseudostationary phases based on crosslinked gels and solutions of linear polymers.
- development and application of microchip structures suitable for CEC.
- study of the processes governing sample injection and separation for the purpose of their optimization.
- development of photometric and fluorometric detection methods for microchip structures.
- development of new CEC applications for analysis of real samples in pharmacology and clinical biochemistry.

The identification of micro-organisms usually requires a long time of analysis. The use of efficient electrophoretic techniques for the separation and identification of micro-organisms is a possible solution for a fast microbiological analysis. The separation techniques employing fluorescence detection are sensitive and selective so they are often applied for trace analysis of biological samples. The future project suggests the use of the fluorescent amphiphilic compounds for the detection of biopolymers on the basis of their mutual interactions in the separation medium which leads to the formation of soluble and detectable aggregates. Our preliminary experiments show the possibility of the dynamic modification of proteins for UV and fluorometric detection (amol) in CZE. Therefore, we expect positive results from this project in the area of a fast, including proteomic, identification of microorganisms without previous isolation with the final aim of the analyses of real samples.

Currently supported projects

Capillary electrochromatography in linear and planar microstructures using polymer beds

Grant Agency of the Czech Republic, grant No. 203/02/1447 (2002-2004)
Principal Investigator: Vladislav Kahle

Apparatus for automation of electrochromatographic and electrophoretic analysis

Grant Agency of the Academy of Sciences of the Czech Republic, Program for the support of the targeted research and development, grant No. S4031201 (2002-2005)
Principal Investigator: Vladislav Kahle

Using of the fast electrophoretic separation with the high sensitive fluorometric detection for the identification of microorganisms

Grant Agency of the Academy of Sciences, grant No. A4031302 (2003-06)
Principal Investigator: Marie Horká

Department of Proteomics

Research scientists: RNDr. Josef Chmelík, CSc. (head)
Research assistant: Mgr. Richard Čmelík
PhD. students: Ing. Jana Cabálková
Ing. Marcela Dokulilová
Ing. Karel Mazanec
Mgr. Pavel Řehulka
Ing. Jiří Šalplachta
Ing. Markéta Štikarovská
Ing. Zdeněk Tůma

Department of Proteomics was established in November 2001 after a short break as a continuation of the Department of Separation of Biopolymers founded by Josef Chmelík in 1990.

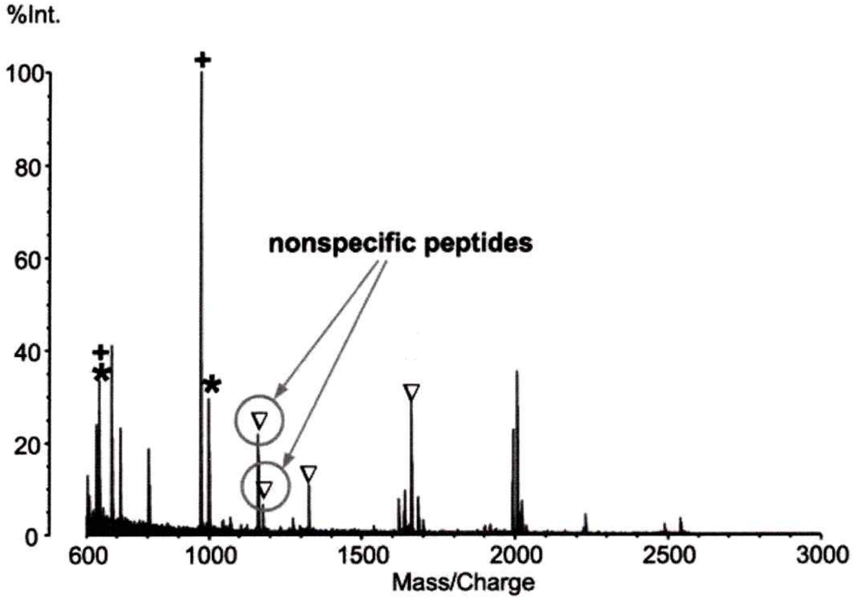
Therefore, other topics are studied beside proteomics (e.g. carbohydrates, synthetic polymers, particles). The Department originally consisted of four workers including two PhD. students. The others joined the Department during fall 2002.

Current research topics

The main research area is proteomics. Special attention is paid to the investigation of the proteome of barley. The other research directions are analysis of carbohydrates and other polymers by mass spectrometry and characterization of particles and cells. The methodology is based on various combinations of separation methods and mass spectrometry (MS) for characterization of polymers, and field-flow fractionation is used for particle analysis.

Proteins

Proteins are the major functional molecules of life. MS has become a powerful method for the determination of molecular mass, peptide sequence, identification of post-translational modifications, characterization of noncovalent protein interactions, detection of the different species involved in dynamic equilibrium, quantitative assessment of mixture heterogeneity, and investigation of protein folding. A very important implementation of MS in protein chemistry is protein identification. Nowadays, proteomics represents an important tool for the understanding of processes in living organisms. Proteomic methodology used in the Department consists of a combination of suitable separation techniques (e.g. liquid chromatography, electrophoresis), mass spectrometry measurements and data evaluation using bioinformatics tools. The most important current research topic is the investigation of barley grain proteome. The aim is to contribute to understanding of malting processes and to selection of malting barley cultivars.



MALDI-TOF mass spectrum of the peptides from an in-gel digest from the band C:

+ *denotes the peptides from subtilisin-chymotrypsin inhibitor CI-1A*

* *denotes the peptides from subtilisin-chymotrypsin inhibitor CI-1B*

▽ *denotes the peptides from nonspecific lipid-transfer protein 1 precursor*

Carbohydrates

Carbohydrates are the most widely distributed naturally occurring organic compounds on the Earth. They are basic organic substances from which most other organic compounds are synthesized. According to their molecular size, they are divided in to three categories (mono-, oligo-, and polysaccharides). Each of these categories requires different methods for analysis. Suitable techniques for characterization of mono- and disaccharides are electromigration techniques and chromatography. Because of the complexity of natural carbohydrate mixtures, it is advantageous to use for determination of the molecular mass of oligo- and polysaccharides various separation techniques (e.g. CE, HPLC, asymmetrical FFF and SEC) in combination with MS. We found that MALDI-TOF MS is a useful method for size determination of oligosaccharides (e.g., starch hydrolysates) in combination with SEC and can also be used for detection of primary structure of oligosaccharides.

Synthetic polymers

Synthetic polymer materials play very important roles in everyday life as well as industry. Several techniques such as GPC, GC, NMR and MS methods have been used to characterize synthetic polymers. MALDI-MS became a new method for polymer characterization. MALDI as a „soft“ ionization process that produces (quasi)molecular ions from large nonvolatile molecules, such as synthetic polymers, with minimum fragmentation is ideally suited for polymer analysis. We used MALDI-TOF MS for characterization of molecular mass distribution of various polymers (polystyrenes, polyethyleneglycols etc.). The most successful was the characterization of silicone oils by combination of MALDI-TOF MS and supercritical fluid chromatography.

Particles and cells

The investigation of particle size and characterization of their distribution is important for their further use. Gravitational field-flow fractionation (GFFF) is the experimentally simplest and cheapest technique utilizing the Earth‘s gravitational field as the external force field that causes settlement of particles towards the channel accumulation wall. However, this technique is a powerful tool for characterization of micron-sized particles including polymer latexes, glass beads, and living cells. The size distribution of starch granules (the principal component of barley kernels) was found as an important parameter for characterization of malting quality of barley varieties.

Future trends

a) For characterization of complex protein mixtures, the use of separation techniques and their combinations with MS measurements is necessary. Proteins can be fractionated into several groups by a variety of different separation methods. The experiments will be designed to separate proteins according to their size, hydrophobicity, charge, isoelectric point, or affinity.

b) Beside the determination of chemical composition, MS can be also used for structural studies. Information on the protein conformation can be gained from MS analyses of deuterated proteins or the accessibility of the specific amino acid residues to the soft chemical modifiers. Information on inter-distances of surface amino acids can be derived from reactions of bifunctional reagents with proteins, which causes cross-linking of accessible amino acid residues, which is evidenced by the presence of specific peptides in the mass spectrum.

c) An interesting opportunity is brought by electrospray ionization mass spectrometry (ESI-MS). It represents a convenient tool to reveal and describe the conformation of proteins. The folding states of proteins are monitored by charge state distributions that they produce during the measurement. Varying the conformational state of the protein is accompanied by a shift of the charge distribution of the mass spectra. It is generally observed that an unfolded protein leads to the formation of higher charge states than the same protein in its native state. Several factors have been shown to influence ESI charge-state distribution, including molecular conformation, acid-base chemistry both in solution and in the gas phase, solvent properties, and instrumental parameters. However, the correlation between the pattern of charge distribution in the mass spectra and the conformation in solution has been confirmed for various proteins. The conformational dependence of protein ESI-MS data originates from high specific sensitivity of this technique to tertiary contacts in the protein, the secondary structure is rather less important. In the absence of conformational changes, the ESI charge state distributions of proteins are known to undergo only very minor changes. Its high sensitivity to changes in protein tertiary structure is of particular relevance for the detection and characterization of folding intermediates. Certain proteins, after being subjected to denaturation, are able to refold into their native forms when the solution conditions are restored; others are incompletely renatured. ESI-MS can be effectively distinguished from both the native and the different refolded states. The same problem of exact conformation will be solved for native and appropriate nascent recombinant protein. Nevertheless, as compared with unfolding, the refolding processes have not been intensively analyzed using electrospray ionization.

d) Starch is a polysaccharide composed exclusively of D-glucose and it is one of the three most abundant organic compounds found on the Earth. Starch is found in the leaves of higher green plants where it serves to store the chemical energy obtained from the light energy of the sun in the process of photosynthesis, and it is found in amyloplasts, where it exists as water-insoluble granules. In many studies reported so far, the photosynthesis and growth of broad-leaved and coniferous species have been stimulated by long-term exposure to elevated CO2 concentrations. The conclusions of these studies were that with increasing atmospheric CO2 concentration, biomass production in forest ecosystems will increase, and so will the amount of starch. It will be interesting to compare the composition of carbohydrates isolated from plants grown in normal atmospheric CO2 concentration and in long-term exposure to higher CO2 concentration.

e) For evaluation of the accuracy of molecular mass distributions of polymers determined by a MS technique, it is advantageous to compare these results with molecular mass distributions measured by other methods (e.g., separation methods). One has to consider the physico-chemical properties of the polymer used for determination of molecular mass distribution by a given technique. It is necessary to consider the sensitivity of the technique employed, and to realize what measured data mean (number- or weight-average data). This research will be concentrated on studies of synthetic polymers (determination of chemical composition, molecular mass and its distribution) by MALDI TOF MS and separation methods. The most direct way to optimize mass spectral analysis by MALDI TOF MS will lead to exploring new matrices, solvents, and ionizing agents, and to optimize conditions of measurement (matrices concentrations, sample preparation) using standard monodisperse polymers in the first step. It will be followed by minimization of mass discrimination effects of broad polymer distributions by combination of MALDI TOF MS with separation methods such as SFC, SEC, and ThFFF. During whole research special emphasis will be put on study of the biodegradable polymers.

f) The characterization of particle size by field-flow fractionation will be focused on the investigation of cells (blood cells, yeasts, etc.) and important living processes (e.g., apoptosis). Special attention will be paid to an on-line coupling of the GFFF channel with a particle-size detection system.

Currently supported projects

The effect of size distribution of starch granules on the technological quality of barley

Ministry of Agriculture of the Czech Republic, grant No. QD 1005 (2001-2004).
Co-Investigator: Josef Chmelík

Analysis of gluten and investigation of quality of gluten-free foods as raw materials

Ministry of Agriculture of the Czech Republic, grant No. MZ- QD 1023 (2001-2005)
Principal Investigator: Josef Chmelík

Analysis of compounds of organic origin by using non-traditional couplings of separation methods and mass spectrometry

Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A4031104 (2001-2004)
Collaboration

Development of new processes for highly reliable analytical control of technology, materials, and environmental, and for medical diagnostics using modern instrumentation

Grant Agency of the Czech Republic, grant No. 203/02/0023 (2002 – 2004)
Collaboration

Characterization of macromolecules and particles of biological and industrial interest

AIP, Kontakt 5, University of Bologna (2002 – 2004)
Principal Investigator: Josef Chmelík

Emissions of biogenic compounds from Picea abies [L.] Karst under the long term-influence of increased carbon dioxide levels

Grant Agency of the Czech Republic, grant No. 526/03/1182 (2003 – 2005)
Collaboration

Other specific activities

Several members of the Department have teaching activities at Masaryk University, Mendel University and Technical University in Brno. The department head is a member of the Scientific council of Technical University in Brno.

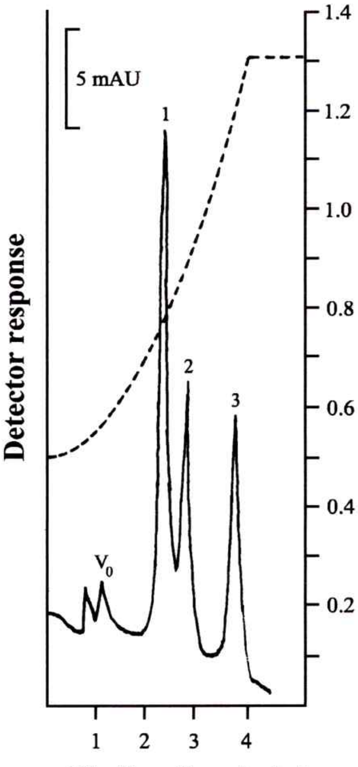
Scientific collaborations have been established with several departments of Masaryk University, Mendel University and Technical University in Brno, Charles University in Prague, Pardubice University, University of Bologna, University of Vienna, University of Limoges, University of Lund and the Research Institute of Brewing and Malting. Informal collaboration is ongoing with several academic groups in Europe, USA and Korea.

Department of Separation of Biopolymers

The Department was founded by Josef Chmelík in 1990 and in 2001 it was split in the Department of Proteomics and the Department of Biopolymer Analysis.

Research topics

The main research was separation of biopolymers (proteins and polysaccharides including development of new separation techniques, e.g. dynamic capillary isoelectric focusing) and particles. An important part of the activity was devoted to combinations of separation methods with MALDI-TOF MS. In the area of particle separation, the main attention was paid to programming of the field force in gravitational field-flow fractionation. This technique was applied to separation of latexes, silica gels, starch granules and cells.



Optimization of separation in gravitational field-flow fractionation. Fractogram of a model silica mixture obtained under the conditions of parabolic flow-rate gradient. The course of the flow-rate gradient is described by the dashed line. V0 denotes the peak corresponding to void volume and the peaks 1, 2, and 3 refer to the silica 1 (porous, 10-μm), 2 (porous, 5-μm), and 3 (non-porous, 1.6-μm), respectively.

Supported projects

Development of rapid methods for assessing the quality of starch particles from various cereal species for purposes of agriculture and food industry European Commission, INCO Copernicus ERB ICT15-CT98-0909 (1998-2001)
Principal Investigator: Josef Chmelík

Investigation of elution modes in gravitational field-flow fractionation and application to separation of particles and cells
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A4031805
(1998 - 2001)
Principal Investigator: Josef Chmelík

Department of Supercritical Fluid Chromatography and Extraction

Research scientists: Doc. RNDr. Michal Roth, CSc. (head)
Ing. Pavel Karásek, Ph.D.
Ing. Josef Planeta, Ph.D.
Ing. Jaroslav Pól, Ph.D.
Research assistants: RNDr. Milena Mikešová
Ing. Elena Varaďová Ostrá

In the late eighties, Jiří Vejrosta and Michal Roth from the Institute's former Department of Gas Chromatography focused their attention on the supercritical state of fluids and on the possibility of employing supercritical carbon dioxide in separation techniques. This interest and the associated research efforts have resulted in the establishment of the Department of Supercritical Fluid Chromatography and Extraction in 1990. The department has always numbered about 6 persons, and the research has simultaneously been carried out in two directions. While the group around Jiří Vejrosta focused their attention on experiments, i. e., on the development of instrumentation and methodology for supercritical fluid chromatography and extraction, Michal Roth focused on theory, i. e., on the thermodynamic description and application of supercritical fluid chromatography to estimation of thermodynamic properties. Both research directions influenced, complemented and benefited from each other throughout the whole period of the existence of the Department.

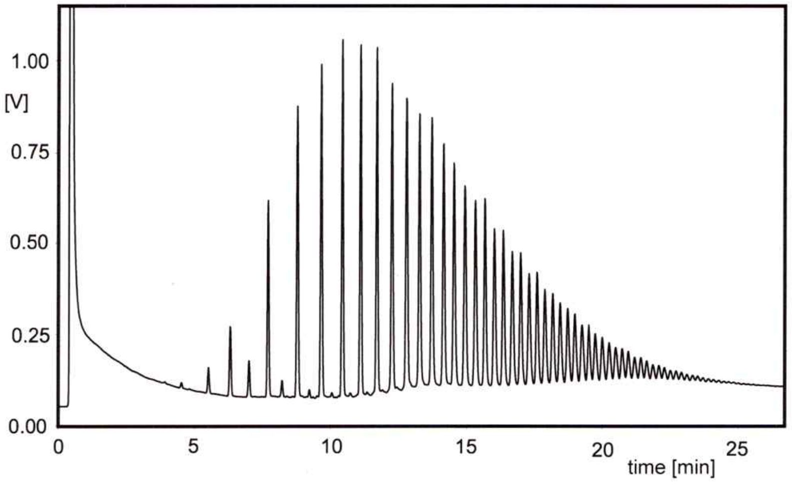
Current research topics

- **Supercritical fluid chromatography (SFC)**

The activities in SFC have involved instrumentation developments as well as applications of SFC to thermodynamic property measurements. Some recent results include the following: A new method for the preparation of micropacked capillary columns by slurry packing in supercritical CO2 led to very efficient and stable columns suitable for special SFC and HPLC separations. Separation performance of the resultant columns more than matches that of the columns produced by reputed commercial companies. The figure below shows a typical example of the column performance.

Further, a novel, non-traditional type of on-line connection of a supercritical fluid chromatograph to mass spectrometer was proposed, designed and experimentally tested. The purpose of this design has been described in more detail in the Department of Proteomics section.

In the field of thermodynamic applications of SFC, methods of estimation of second cross virial coefficients and Krichevskii parameters in asymmetric alkane–CO2 mixtures were developed. The term "asymmetric" refers to the large difference in molecular size between the solute (alkane) and the solvent (CO2). SFC can provide thermodynamic data on highly asymmetric solute–solvent systems that are difficult to study by traditional techniques of phase equilibrium measurements. Very recently, the research on ionic liquids as stationary phases in SFC separations was started. The motivation for this project comes from the rapidly growing importance of ionic liquids as nonvolatile, emission-free, environmentally benign solvents.



Separation of silicone oil (PDMS, 2% v/v solution in CH2Cl2) by supercritical fluid chromatography in a packed capillary column (ODS, 5 µm, 15 cm, 250 µm i.d.) at 80°C with an 8-35 MPa pressure ramp.

- **Supercritical fluid extraction (SFE)**

Initial research efforts in SFE were focused on batch-type extractions of solid samples with supercritical carbon dioxide, and the group developed an extractor that was later marketed by the SEKO-K company. Both on-line and off-line connections were studied of SFE to analytical separation techniques such as GC and HPLC. The instrumental developments resulted in an original solution of the problem of analyte trapping after SFE. A novel type of analyte collection device was designed that employed an expansion of supercritical mixture into hot solvent vapor to achieve a quantitative collection of analytes in the liquid solvent. This design takes advantage of the condensation of organic solvent vapor on the nanoparticles of the solid solute nucleating from the expanding supercritical mixture. The applications of the SFE of solid samples included a successful separation of lignans from the seeds of Schisandra chinensis, a Chinese medical plant, as well as others.

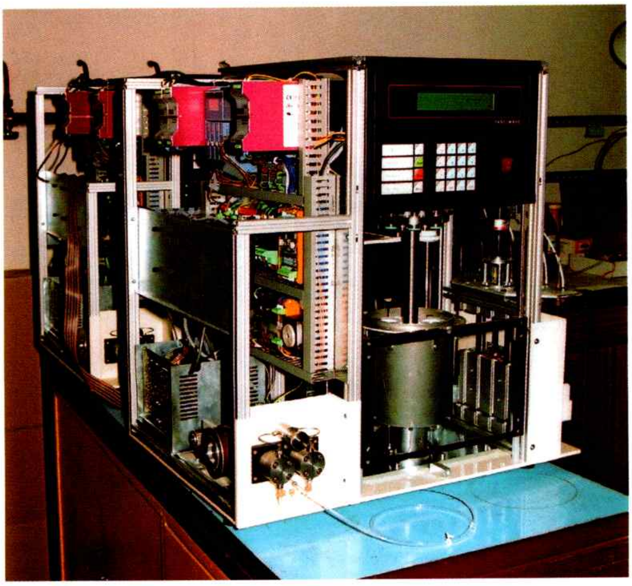
As regards the liquid sample matrices, an automated apparatus for direct continuous SFE of aqueous matrices by supercritical carbon dioxide was developed and used for extraction of model and real aqueous samples. The direct SFE of model aqueous samples were used for measurement of partition coefficients (K) of substituted phenols between the aqueous and the supercritical phase, and a significant influence of the analyte molecule structure on the analyte partition coefficient in a water-supercritical carbon dioxide system was confirmed.

Applications of direct SFE to real aqueous samples have hitherto included wine and beer. Over 100 wine samples of 21 vine varieties were extracted by direct SFE and by a more conventional procedure combining solid phase extraction and SFE. Multivariate statistical processing of the resultant GC records showed that the direct SFE provided a considerably better discrimination among the individual wine varieties. The explanation for this result comes from the additional analyte–sorbent partitioning steps that detract from the information content of the GC records from the combination of the solid phase extraction and SFE.

In SFE of beer samples, the aim has been to optimize the working conditions and to isolate and quantify selected groups of analytes, namely, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and fatty acids. Yet another application of direct continuous SFE involved SFE–HPLC determinations of "green" natural insecticides of the pyrethrine group from Chrysanthemum cinerariaefolium flowers, and of β-carotenes from fruits and vegetables.

- **Pressurized fluid extraction (PFE)**

PFE, offering some distinct advantages over conventional low-pressure techniques of liquid extraction, is the most recent addition to the research program of the department. The activities of the group in this field have so far been focused on the development of original automated extractors. Several models of fully automated, laboratory-scale apparatuses for pressurized fluid extraction were designed and constructed. The most recent addition to the family of laboratory-scale PFE extractors is shown in the figure below.



Automated apparatus for pressurized fluid extraction (cover panels removed).

Both model samples and real matrices (soil, food) were extracted by means of the apparatuses and the recoveries of target analytes (polycyclic aromatic hydrocarbons, polychlorinated biphenyls) were compared with the results obtained by other extraction techniques.

Future research trends

In SFC, the microcolumn technology developments will concentrate on application of sol-gel sorbents and on exploring the possibility of deactivation of SiO2 particles in low-viscosity supercritical fluids as opposed to conventional deactivation in liquid solvents. The experience gained in the successful design and testing of an off-line SFC–MALDI interface will be utilized in the planned development of an HPLC–MALDI interface. Thermodynamic measurements by SFC will focus on the systems containing room-temperature ionic liquids (RTILs) as stationary phases. The combinations of RTILs and supercritical CO2 provide very promising biphasic solvent/reaction medium systems compatible with sustainable development and "green" chemistry. In applications of these systems, partition coefficients of the target solutes between the two phases will be needed, and SFC provides a potential route to such data. Preliminary results obtained with the [bmim][PF6] ionic liquid testify to the feasibility of this intention.

In SFE, extractions of solid sample matrices will be suppressed, leaving more room for direct continuous SFE of aqueous media. Analytical applications involving diverse aqueous samples (beverages, nutraceuticals, non-human pharmaceuticals) as well as thermodynamic studies of analyte partitioning between the two phases in water–CO2 system will employ the automated experimental setup developed before. Thermodynamic explanation will be sought of the efficiency differences among various techniques of analyte trapping after SFE of aqueous solutions.

In PFE, the previous extensive efforts invested into instrumentation development will be put to use in analytical applications. One line of applications will make use of conventional solvents for PFE (ethanol, acetone, isopropanol) and their mixtures in analyses of food products and environmental samples. The other line, requiring additional instrumentation developments, will concern the use of high-temperature (subcritical, "hot") water as a "green" solvent for PFE. The analytical applications of PFE will be complemented with the development of auxiliary thermodynamic models for correlation of the experimental results.

Currently supported projects

Research and development of the methodology and instrumentation for direct extraction of aqueous matrices by supercritical carbon dioxide

Grant Agency of the Academy of Sciences of the Czech Republic, grant No. S4031110 (2001-2003), Principal Investigator: Michal Roth

Supercritical fluid chromatography as a source of thermodynamic data: from conventional systems to neoteric systems with ionic liquids
Grant Agency of the Czech Republic, grant No. 203/02/1093 (2002-2004)
Principal Investigator: Michal Roth

Analytical pressurized solvent extraction: from organic solvents to subcritical water
Grant Agency of the Czech Republic, grant No. 203/03/0859 (2003-2005)
Principal Investigator: Michal Roth/Pavel Karásek

Thermodynamic models for applications of pressurized fluids in analytical separations
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. IAA4031301 (2003-2006)
Principal Investigator: Michal Roth

Development of new processes for high reliable analytical control of technology, materials, and environmental, and for medical diagnostics using modern instrumentation
Grant Agency of the Czech Republic, grant No. 203/02/0023 (2002 – 2004)
Collaboration

Analysis of compounds of organic origin by using non-traditional couplings of separation methods and mass spectrometry
Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A4031104 (2001-2004)
Collaboration

Other specific activities

The department head had teaching activities at the Masaryk University in Brno. Academic collaborations have been under way with the Faculty of Chemical Technology of the University of Pardubice, and informal cooperations also with several university groups in Europe and the USA. The development of automated apparatuses for pressurized fluid extraction has involved a continued cooperation with commercial companies, namely, Applied Separations (Allentown, Pennsylvania, USA) and SEKO-K s. r. o. (Brno, Czech Republic).

Department of Trace Element Analysis

Research scientists:	RNDr. Jiří Dědina, CSc. (head) RNDr. Vlasta Korunová Mgr. Tomáš Matoušek, PhD. RNDr. Jiřina Sysalová, CSc. Ing. Miloslav Vobecký, CSc.
Research assistant:	Mgr. Helena Líšková
Technicians:	Jana Čeledová Anna Selecká
Undergraduate students:	Olga Grossová Jan Kratzer

The development and application of atomic absorption spectrometry (AAS) and nuclear & radioanalytical methods for trace element analysis has always been our principal aim. Before the middle of 1993, the department belonged to the Institute of Nuclear Biology and Radiochemistry, ASCR. Until 2000, we were engaged mainly in three fields:

Hydride generation and atomization for atomic absorption spectrometry (AAS).

We developed a mathematic description of currently employed hydride generation methods. This made possible an efficient optimization of experimental parameters of individual hydride generation approaches used for routine analysis. We proposed the radical theory of hydride atomization in quartz tube atomizers, which is now generally accepted. Further, elucidation of the analyte transfer in these atomizers made it possible to account for the observed effects of various experimental parameters on the analytical signal. This is extremely valuable for optimization of experimental set-up. Furthermore, in cooperation with our Italian colleagues, we designed a unique hydride „flame-in-gas-shield“ (FIGS) atomizer for atomic fluorescence spectroscopy (AFS) making it possible to improve limit of detection considerably. We also studied hydride atomization in graphite furnaces and found it to be analogous as in quartz tube atomizers.

Application of AAS for trace and ultratrace elemental analysis.

Ultratrace determinations of the elements typically present in high concentrations in the environment can be accurately performed only in a specially equipped ultratrace laboratory. Running such a laboratory, we developed and employed a number of methods for trace and ultratrace determinations in biological, environmental and industrial materials using AAS.

Nuclear analytical methods, nuclear chemistry.

We contributed significantly to the methodical development of instrumental neutron activation analysis (INAA) and applied it most notably to the analysis of lunar samples (Apollo 11, Apollo 12). Later, we oriented our research towards biological and environmental problems. For the trace selenium determination in biological materials a new nondestructive technique was developed based on the gamma-gamma coincidence spectrometry of induced 75Se radionuclide.

Current research topics

Generation and atomization of volatile compounds for trace element analysis and for speciation by AAS and AFS.

Generation of a volatile compound of the analyte involves analyte preconcentration and separation from the sample matrix resulting in a superior sensitivity. It is therefore ideally suited for trace and ultratrace elemental analysis. Volatile hydrides are the most popular compounds generated, even though this technique is confined to limited number of elements. However, generation of useful volatile compounds of other elements is gaining importance as well. The analytical process based on generation of volatile compounds for atomic spectrometry methods includes two independent steps: generation of the compound and its atomization/detection. To make use of the full potential of this approach promising techniques for both steps have to be sought and optimized with respect to sensitivity, accuracy and overall performance.

Currently, reduction of analyte by tetrahydroborate in acidic medium is employed almost universally for hydride generation (HG). The inherent disadvantage of the BH4-/acid reduction is the risk of contamination of the reducing agent, which often controls the limit of detection (LOD) of the determination. The essential advantage of the electrochemical HG, which is an alternative to the BH4-/acid reduction, is that it does not require beside the acid any other reagent. Analyte concentration in blanks can be therefore made very low. This makes electrochemical HG promising for ultratrace elemental analysis notably in connection with a collection method. Recently, we used 75Se radiotracer to optimize relevant experimental parameters of electrochemical HG to determine SeH2 generation efficiency (72 ± 2%) in an electrolytic cell of novel design (Pb wire cathode) and to track ways in which the analyte can be lost. A substantial fraction of the lost analyte was found in the waste solution. In contrast, radiotracer experiments for using another novel design (granular Pb cathode) proved there was no analyte lost to the waste. However, SeH2 generation efficiency was not improved due to analyte losses at the surface of Pb granules. We also explored electrochemical HG of SeH2 in connection with in-situ trapping in graphite furnaces (GF). We described a novel experimental approach utilizing a silica capillary for the sampling of both, hydride as well as Pd modifier, to the graphite atomizer. The achieved LOD was around the same as for BH4-/acid reduction since it was dominated by contamination of the reagent acid in this case.

Further, we studied the extent and mechanism of interferences in the electrochemical HG of SeH2 for the Pb wire cathode. In accord with previous reports, we found the extent of the interferences more serious than for the BH4-/acid reduction. All the investigations of electrochemical HG have been performed in cooperation with Department of Analytical Chemistry of the Faculty of Sciences, Charles University in Prague (Dr. P. Rychlovský).

The same acid/BH4- system as used for the generation of hydrides has been employed for generation of volatile forms of transition and noble metals. In principle, the volatilized analyte can be detected by any method of atomic spectroscopy. Despite the efforts of several groups, this promising technique still suffers from relatively low generation efficiency in the lower ten percentile. Recently, we employed generation of volatile form of Ag as a model for some other analytes such as Au, Cu, Pt and Rh. By using INAA we determined Ag generation efficiency to be 7%.

AFS has become very popular especially for speciation analysis (around 30 papers in the last 5 years). For AFS, the almost exclusively used hydride atomizer is the miniature diffusion flame (MDF). Recently, we found the mechanism of hydride atomization and of free atom removal from the observation volume of MDF and FIGS atomizer (see History of the Department). All the knowledge makes possible to understand the influence of all relevant experimental parameters (including the design of the shielding unit of the FIGS) on the observed sensitivity and blank noise.

For AAS, the currently used atomizers of volatile compounds are conventional quartz tubes (QTA) and GF with in-situ trapping. We devised a new atomizer named multiple microflame quartz tube atomizer (multiatomizer) to overcome fundamental disadvantages inherent to poor linearity of calibration graphs and pronounced atomization interferences. The multiatomizer combines the favorable features of the miniature flame atomizers, namely good linearity of analytical graphs and high resistance to interferences, with the excellent sensitivity of the conventional QTA. Subsequently, we improved the design of the multiatomizer to reach better linearity (no calibration curvature due to processes in the atomizer) and one to two orders of magnitude better resistance against interferences with no loss either in sensitivity or in LOD compared to the QTA. However, the resistance towards atomization interferences (which is comparable to that when using in-situ trapping - see below) is still substantially worse than observed with the MDF or FIGS atomizers for AFS.

One of the inherent advantages of the generation of volatile compounds is that the analyte can be easily preconcentrated either in a special collection device (usually by cryogenic trapping) or directly in the atomizer. The cryogenic collection is time consuming and a lot of effort is involved. In contrast, in-atomizer trapping is the most convenient way of analyte collection.

Until recently, the only approach to in-atomizer trapping was in-situ trapping in commercial graphite furnaces. By using radiotracers, we showed that the vital parameter of the technique, trapping efficiency, was complete for hydrides in a broad range of all relevant experimental parameters. The main drawback of the in-situ trapping is the relatively serious trapping interferences observed in the presence of other (than analyte) hydride forming elements in the sample. Recently, we investigated the mechanism and the control of trapping interferences. We found that the interference extent is given by a trapping capacity of the surface - the higher the capacity the lower the extent of the interference.

It should be underlined that commercial GF are unsuitable for volatilization either to conventional QTA or to multiatomizer for AAS or to any atomizer for AFS. This was the reason we started to explore alternative approaches to in-atomizer trapping. One of them is to collect hydride on a quartz surface. A successful trapping of plumbane in a bare quartz tube was announced last year by the group of prof. Ataman. In the collaboration with this group, we started to explore the same approach to trapping of SbH3 in our laboratory. Our preliminary results are rather encouraging: trapping efficiency around 65% and 40 times improvement of the limit of detection - down to 4 pg ml-1.

Application of radioanalytical and radiochemical methods in life sciences

Anthropogenic contribution of bromine into the environment has lead to extensive exposition of mammals including man. Increasing amounts of bromine in the food chain are connected with the application of brominated hydrocarbons in the pre-planting fumigation of soils and post-harvest fumigation of commodities as grain, spices, nuts, fruits and tobacco. One of the organs in which an interaction of chemically allied halogens could occur is the thyroid gland. Interaction of bromine with iodine was studied in the rat thyroid gland under the conditions of different bromide intake.

INAA and radioative indicators have been employed to study the interaction of bromine with iodine in the thyroid gland.

Considering the chemical similarity of bromine and iodine, goitrogenic effects of bromine should be considered, namely under iodine deficiency. The consequences of this effect are rather serious, especially for children in the period of early postnatal development. Effects of a high bromide intake in lactating rats on the performance of the dams and on the prosperity of the young were studied. Very high intake of bromide in the dams in the course of the lactation period (about 220 mg Br per day per dam) caused a marked decrease in the body weight increments in the sucklings survived and their general condition was very poor. In the dams, there were two striking consequences undoubtedly caused by high bromide intake: stagnation in the extent of diet consumption and water in the course of the nursing period, and a drop in the production rate of mother´s milk. Bromide ions easily penetrated into the rat milk and via mother´s milk were transferred in a large extent to the sucklings. The amount of bromide in mother´s milk depended on the bromide concentration in the drinking water taken by the dams. With the mean daily bromide dose of 220 mg, about 54 % of the chloride ions in the milk were replaced by bromide. A rise in the concentration of both halogens caused also an increase in the concentration of sodium in the milk.

Trace and ultratrace elemental analysis – applications

The development of quality control materials for the determination of selected trace elements in air pollution studies was performed. Three types of test samples were prepared for proficiency testing: (1) filters loaded with PM10 fraction of urban air particulate matter (APM) using high-volume air samplers, which were subsequently divided into smaller sections, (2) a bulk sample of APM collected in an automobile tunnel in Prague, and (3) simulated air filters loaded with APM using a wet deposition process. Homogeneity of the test samples was studied using instrumental neutron activation analysis, proton induced X-ray emission and atomic absorption spectrometry, and inductively coupled plasma optical emission spectrometry and mass spectrometry. Sufficiently homogeneous samples were prepared by all three procedures. The simulated air filters appeared to be the most suitable test samples for proficiency testing. We also investigated elemental As determination and speciation in body fluids by graphite furnace AAS and by HG AAS.

Future trends

The general target of our research in the near future is to develop promising aspects of generation and atomization of volatile compounds for trace element analysis and for speciation by atomic spectrometry methods, namely by AAS and AFS. Our explicit aims are detailed below. Radiometric measurements of appropriate radiotracers will play a key role in our experiments. Therefore, special attention will be paid to radiotracer preparation and application. The required radiotracers will be prepared by nuclear reactions, by neutron irradiation in a nuclear reactor or in a charged particle accelerator, with subsequent radiochemical separation and isolation. To obtain information on the distribution of radionuclides on various surfaces, the advanced image plate autoradiography will be used since it yields dramatically better performance than the conventional approach utilizing the photosensitive film.

- Our recent findings indicate that there is potential to further improve LOD for AFS when using FIGS for atomization of volatile compounds. Therefore we intend to optimize optical and electronic train of AFS spectrometer to achieve this goal. Subsequently, we will look for ways to miniaturize AFS atomizers for better performance for speciation analysis.
- In order to further improve performance of the multiatomizer, namely its resistance towards atomization interferences, a novel design of the atomizer has to be adopted. The idea is that the present design of the multiatomizer (a limited number of hand-made small apertures, in the wall of the quartz optical tube, serving to introduce multiple flows of oxygen to the observation volume of the atomizer)

cannot provide what is required for the optimum performance: to have the whole observed volume filled with hydrogen radicals. Various designs of the atomizer will be tested to approach the ideal.

- We will search for ways to reduce the extent of interferences for in-situ trapping in commercial graphite furnaces. Since the performance of the most often used modifiers (Pd or Ir) has already been specified, other modifiers will be tested to achieve higher trapping capacity and, consequently, better resistance towards trapping interferences. Also the performance of the graphite filter atomizer, designed by Katskov, for in-situ trapping will be investigated.
- Because of the promising results of our recent investigations, we intend to continue our study of the miniature collection devices. We will focus our attention on the Rh modified W-coil and on miniature quartz traps.
- To further develop electrochemical hydride generation, our recent results will be taken as a base to test the possibilities of several prospective aspects of electrochemical hydride generation.
- Finally, we intend to improve efficiency, precision and robustness of the generation of volatile forms of transient and noble metals.

Currently supported projects

Development of promising techniques of generation and atomization of volatile compounds for (ultra)trace element analysis and for speciation by atomic spectrometry methods
Grant Agency of the Czech Republic, grant No. 203/01/0453 (2001-2003)
Principal Investigator: Jiří Dědina

Other specific activities

Organization of conferences:

14th Radiochemical Conference, Mariánské Lázně (2002), vice-chairman of Conference Committee (M. Vobecký)

Current cooperation with foreign research institutes:

Istituto per i processi chimico-fisici del C.N.R. in Pisa, Italy - Dr. D‘ Ulivo, Dr. L. Lampugnani

Department of Analytical Chemistry, University of Umeå, Umeå, Sweden - Prof. W. Frech

Institute for Environmental Chemistry, NRC, Ottawa, Canada - Dr. R. Sturgeon

Department of Chemistry, Middle East Technical University, Ankara, Turkey - Prof. Y. Ataman, D. Korkmaz

Centro de Energia Nuclear, Piracicaba, Brazil - Prof. F. Krug

Comisión Nacional de Energía Atómica, Buenos Aires, Argentina - Dr. P. Smichowski

Technikon Pretoria, Pretoria, South Africa - Prof. D. Katskov

Cooperation with commercial companies:

RMI s.r.o., Lázně Bohdaneč, Czech Republic

Membership in international organizations:

The International Association of Bioinorganic Scientists (M. Vobecký)

Teaching activities:

Department of Analytical Chemistry, Faculty of Science, Charles University in Prague; J. Dědina - external lecturer and supervisor of two undergraduate students

Faculty of Nuclear Science and Physical Engineering, Czech Technical University in Prague; M. Vobecký - external lecturer and consultant of a post-graduate student

Overview of specific activities

Membership in Editorial Boards of international journals:

Electrophoresis	Petr Boček – Senior Deputy Editor František Foret – Deputy Editor Ludmila Křivánková
Journal of Chromatography A	Petr Boček František Foret
Journal of Separation Science	František Foret
Journal of Capillary Electrophoresis	Petr Boček
Analytical Biochemistry	Petr Boček
Journal of Biochemical and Biophysical Methods	Petr Boček
Analytical and Bioanalytical Chemistry	Bohumil Dočekal
Environmental Chemistry Letters	Josef Čáslavský

International scientific meetings organized by the Institute:

14th Radiochemical Conference, Mariánské Lázně (2002), vice-chairman Miloslav Vobecký

Brno Analytica 1999 (Gradient separation methods), chairman Josef Chmelík

Brno Analytica 2001 (Characterization of starch granules), chairman Josef Chmelík

Brno Analytica 2003 (Proteomics and MALDI-TOF/TOF-MS), chairman Josef Chmelík

Participation in international research on the basis of international contracts concluded by the Czech Republic with foreign entities:

Czech-Italian scientific and technological cooperation „Characterisation of macromolecules and particles of biological and industrial interest“, 5/2002, University of Bologna

Membership in non-governmental international scientific organisations:

Josef Čáslavský – membership in the Advisory and National Representatives Board of the European Association Chemistry and the Environment

Miloslav Vobecký – membership in the International Association of Bioinorganic Scientists

Josef Chmelík – membership in the IUPAC Project for analytical chemistry of polymers (TGM1999-021-1-400)

Joint projects with foreign research and development organisations:

Petr Boček – Dr. Salvatore Fanali (CNR – Istituto di Cromatografia, Roma)
Mapping of electrolyte systems for capillary electrophoresis
Agreement CNR/Academy of Sciences CR – Cooperative Programme 2001/2003, No. 4

Long-term cooperation with Barnett Institute, Boston, USA (Prof. Barry L. Karger) in the research of biopolymer analysis – a series of the Institute employees visited the Barnett Institute within the framework of short- and long-term stays.

The agreements with the company Gyros, AB, Uppsala, Sweden about joint research of microfabricated devices and with the company Applied Biosystems, Framingham, USA - research of the bioanalytical use of mass spectrometry.

Cooperation with several foreign laboratories [University of Limoges (FR), University of Bologna (IT), University of Ferrara (IT), University of Lund (S), University of Patras (GR), Institute of Biochemical Physics (RU)] within the framework of the project *Development of Rapid Methods for Assessing the Quality of Starch Particles from Various Cereal Species for Purposes of Agriculture and Food Industry*, EU INCO Copernicus, Proposal No. ERB 3512 PL 979070, Contract No. ERB IC15-CT98-0909 (principal investigator J. Chmelík).

Two projects with “Istituto per i processi chimico-fisici”, Pisa (IT) under the agreement between the Academy of Sciences (CR) and CNR (IT):

New atomizers for atomic absorption and fluorescence spectrometry (1998-2000)

Optimization of ”flame-in-gas shield” atomizer for atomic fluorescence spectrometry (2001-2003)

Cooperation with the University of Parma (IT) within the framework of the project:

Cycling of Trace Metals in Sustainable Management of Agricultural Soils, EU INCO Copernicus, Proposal No. ERB 3512 PL 971161, Contract No. ERB IC15-CT98-0124.

Cooperation with the Norwegian Institute of Air Research (N) within the framework of the project

Subgrid Scale Investigations of Factors Determining the Occurrence of Ozone and Fine Particles SUB-AERO, EU INCO Copernicus, No. EVK2-CT-1999-0052 SUB-AERO.

Cooperation with the University of Ghent (Belgium) within the framework of the project Characterization and Sources of Carbonaceous Atmospheric Aerosols (Belgian-Czech project financed by means of EU, No. SPSD II EV/02/11).

Long-term stays abroad:

Jana Cabálková	University of Lund (S), 6 months (2003)
František Foret	Barnett Institute, Boston (USA), 1991-2001
Tomáš Matoušek	Institute for National Measurement Standards (INMS), National Research Council of Canada, 24 months (2001-2003). The stay was invited by the INMS and the costs were granted by the inviting institution. The stay was focused on the analytical use of generation of volatile forms of transition and noble metals.
Jaroslav Pól	University of Siegen (D), 8 months (2001), analytical SFE of aqueous media
Jaroslav Pól	University of Helsinki (FI), 6 months (2003-2004), analytical extraction with hot (subcritical) water, analytical applications of reactions in supercritical water
Andrea Procházková	University of Bern (CH), 6 months, electromigration methods (1999-2000)
Tomáš Rejtar	Barnett Institute, Boston (USA), 1999 – capillary zone electrophoresis and mass spectrometry
Pavel Řehulka	University of Vienna (AT), 9 months (2000-2002)
Pavel Řehulka	University of Münster (D), 6 months (2003)
Miroslava Šťastná	National Institutes of Health, Bethesda (USA), 3 years (1998-2001), electrophoresis of proteins

Membership in advisory boards and commissions of foreign scientific institutions:

František Foret	external member of the Commission for PhD. degree granting – University of Alberta, Edmonton, Canada (2003)

Long-term stays of foreign scientists in the Institute:

Andrea Zattoni (PhD. student - University of Bologna, IT), 2000-2001 – gravitational field flow fractionation

Deniz Korkmaz (Middle East Technical University, Ankara, TR), 2002 – collection of volatile compounds for atomic spectrometry in miniaturized quartz device

Ivan Kalinichenko (A. V. Dumansky Institute of Colloid and Water Chemistry, National Academy of Sciences of Ukraine), 2000-2001 – chemiluminescent reaction in aqueous solutions

Dr. Alessandro D'Ulivo (C.N.R, Istituto per i processi chimico-fisici, Pisa, IT), total 5 months during 1999-2003 – development of new hydride atomizers

Joint projects with foreign universities and organizations:

University of Bern (CH)
  development of preconcentration and preseparation methods for CZE focused on clinical biochemistry
Eindhoven University of Technology (NL)
  methodology of selection of BGE systems for CE
University of Vienna (AT)
  cooperation in the research of barley proteome
University of Chunchon (South Korea)
  non-formal cooperation in the research of thermodynamic measurements in supercritical fluid chromatography
University of Bochum (D)
  non-formal cooperation in the research of thermodynamic measurements in supercritical fluid chromatography
Applied Separations, Inc., Allentown, PA (USA)
  cooperation in the development of laboratory extractors for pressurized solvent extraction
National Academy of Sciences of Ukraine
  development of the methodology of determination of trace concentrations of nitric acid in aqueous matrix
Bursa Test and Analysis Laboratory, The Scientific and Technical Research Council of Turkey, Bursa (TR)
  method development for analysis of environmental materials
Middle East Technical University Ankara (TR)
  collection of volatile compounds
  for ultratrace atomic absorption spectrometry analysis
University of Umea (Sweden)
  research of basic processes in atomizers for atomic absorption spectrometry
National Research Council, Institute for National Measurement Standards Chemical Metrology, Ottawa (Canada)
  generation of volatile forms of transition and noble metals as a method for sample input in atomic absorption spectrometry

Educational activities:

Accredited doctoral programmes:

Doctoral programme „Analytical Chemistry“ at the Palacky University in Olomouc.

Diploma theses written at the Institute, or supervised by the Institute’s researchers:

1999 – 5 theses
2000 – 4 theses
2001 – 5 theses
2002 – 5 theses
2003 – 8 theses

PhD. and habilitation theses written at the Institute:

Dočekal, Bohumil: Direct analysis by atomic absorption and atomic emission spectrometry (habilitation thesis, 1999)
Karásek, Pavel: Trapping of analytes in supercritical fluid extraction (PhD. thesis, 2002)
Matoušek, Tomáš: Free analyte atom distribution, reactions and analyte reatomization in quartz tube hydride atomizers for atomic absorption spectrometry (PhD. thesis, 2000)
Planeta, Josef: Instrumentation and methodology in supercritical fluid chromatography (PhD. thesis, 2003)
Pól, Jaroslav: Pretreatment of aqueous samples by supercritical fluid extraction (PhD. thesis, 2003)
Šíma, Jan: Electrochemical generation of volatile compounds in atomic absorption spectrometry (PhD. thesis, 2002)

Teaching activities:

Courses, seminars, and laboratory practice, led by the Institute‘s researchers in 1999-2003:

1999	125 hours/year
2000	243 hours/year
2001	218 hours/year
2002	59 hours/year
2003	253 hours/year

Internet sources of additional topical information about the Institute:

http://www.iach.cz/uiach – website of the Institute with links to individual departments as well as to other R&D institutions

http://www.cas.cz – website of the Academy of Sciences of the Czech Republic with links to individual institutes of the Academy

http://www.lib.cas.cz – website of the Library of the Academy of Sciences of the Czech Republic with publication lists of the individual institutes (“ASEP”)

http://www.vyzkum.cz – website of the R&D administration of the Czech Republic with links to nationwide data bases of projects, results, and research intentions

http://www.gacr.cz – website of the Grant Agency of the Czech Republic with lists of the projects funded by the Agency

http://gaav.kav.cas.cz – website of the Grant Agency of the Academy of Sciences of the Czech Republic with lists of the projects funded by the Agency