

**CEEPUS Summer School in Pardubice, Sept. 6 – 17, 2005.**  
**Advances in HPLC method development – separation selectivity, detection methods and hyphenated techniques.**

**PROGRAM:**

**Tuesday Sept. 6: Arrival, registration, accommodation of participants, get-together, introduction to the course.**

**Sept. 7 – 10: Lectures, laboratory experiments**

**Sept. 11 – 14: Participation at the 11<sup>th</sup> International Symposium on Separation Sciences, Pardubice.**

**Sept. 15: One-day course on polymer separations.**

**Sept. 16: Lectures, discussion of practical separation problems, participant comments, closing remarks (Prof. B. Buszewski, Prof. M. Hutta, Prof. P. Jandera, Assoc. Prof. M. Holčápek et al.).**

**Sept. 17: End of Summer School, departure of participants.**

**TOPICS:**

**Lectures:**

**Wednesday Sept. 7: Programmed HPLC techniques (Prof. P. Jandera, University of Pardubice)**

- 1) General elution problem and its solution: Temperature, flow and mobile phase programming, column switching, two-dimensional separations.
- 2) Principles and theory of gradient elution.
- 3) Instrumental aspects – pitfalls and solution: Instrumental gradient dwell volume, detection, miniaturization. Micro-gradient LC.
- 4) Prediction of retention data and optimization of binary and multi-solvent gradients.

**Thursday Sept. 8: LC/MS (Assoc. Prof. M. Holčápek, University of Pardubice)**

- 1) Basics of coupling of liquid separation techniques to mass spectrometry. (ion formation process, ionization techniques, mobile phase compatibility, instrumental arrangement, tandem mass spectrometry).
- 2) Overview of current instrumentation in mass spectrometry. (quadropole analysers, spherical and linear ion traps, triple quadrupoles, TOF, QqTOF, magnetic sector instruments, FTICR).
- 3) Interpretation of mass spectra. (molecular weight determination, differences between electron ionization and soft ionization techniques, basic rules for the fragmentation, effect of functional groups on the fragmentation behaviour, typical spectra of organic compounds and biopolymers).

**Friday Sept. 9: Optimization of HPLC separations using Dry-Lab software (K. Novotná, University of Pardubice)**

Principles, strategies, practical examples – optimization of isocratic and gradient HPLC.

**Saturday Sept. 10: Optimization of rapid HPLC separations on monolithic columns using ChromSword software (Prof. M. Hutta, Comenius University, Bratislava)**

Principles, application examples.

**Sept. 7 - 9 afternoon:**

**Laboratory course:** (approximately 3 hours per day), 3 groups

**(M. Holčapek, M. Lísa, T. Hájek, V. Staněk, P. Česla, J. Fischer, A. Bačák)**

- 1) **HPLC/MS** Basic mass spectrometric experiment using direct infusion of sample, multistage tandem mass spectra ( $MS^n$ ), HPLC/MS, HPLC/MS/MS, ESI and APCI ionization in both polarity modes, total ion current (TIC) vs. reconstructed ion current (RIC) chromatograms, basic spectra interpretation.
- 2) **HPLC/multichannel electrochemical detection.** Principles of coulometric detection, instrumentation, peak tracking/identification using dominant – predominant – postdominant peaks, optimum detection conditions. Experiment: separation of natural antioxidants in beverages.
- 3) **Capillary electrophoresis.** Basic principles, instrumentation, effects of the composition of working buffer, background electrolyte additives to enhance the selectivity of separation, practical separation example.

**Sept. 11 – 14: 11<sup>th</sup> International Symposium on Separation Sciences (ISSS 2005)**

Organized by the Group for Chromatography and Electrophoresis, Czech Chemical Society under the auspices of Central European Group for Separation Science.

**Thursday Sept. 15: Polymer Separations – One-day Short Course**

**Lecturer: Prof. Dr. Peter Schoenmakers , University of Amsterdam, NL and Dutch Polymer Institute, Eindhoven, The Netherlands**

**Course objectives**

The objectives of this course are to make the participants familiar with various methods for determining molecular distributions in synthetic and natural polymers and to create a good understanding of the strengths and weaknesses, the practical applicability, and the potential pitfalls of the techniques.

The course mainly focuses on (soluble) synthetic polymers, but the techniques discussed are equally relevant for a number of natural polymers, such as polysaccharides.

- 1) Properties of polymers and their relevance to the participants.  
Polymer distributions (molecular-weight distribution – MWD, chemical-composition distribution – CCD, functionality-type distribution – FTD, *etc.*)
- 2) Size-exclusion chromatography, including dedicated detectors (light scattering, viscometry).  
The principles (mechanism, columns, calibration) will be briefly reviewed. Contemporary developments, such as fast SEC, will be addressed. Emphasis will be on the quality of SEC data.
- 3) Mass spectrometry of polymers has taken giant strides forward, thanks to the development of Electrospray-ionization and matrix-assisted laser-desorption/ionization (MALDI) techniques. The possibilities and limitations of these new methods will be discussed.
- 4) Isocratic (critical) and gradient-elution liquid chromatography of polymers may be used to characterize various distributions (MWD, but especially CCD or FTD).
- 5) Two-dimensional polymer separations allow several mutually dependent distributions, such as an MWD and a CCD or FTD, to be measured simultaneously. This offer exciting possibilities for the characterization of complex polymers.

Possible applications to participants' problems. Throughout the course, it will be attempted to connect with the specific needs and applications of the participants.

#### **Friday Sept. 16:**

**Morning – Lectures: Direct chiral separations by HPLC. Prof. Jozef Lehotay and Prof. Ján Krupčík, Department of Analytical Chemistry, Faculty of Chemical and Food Technology, STU in Bratislava**

- 1) Mechanistic aspects of the direct chiral separations. Interaction mechanisms influencing direct HPLC separation of enantiomers.
- 2) Characterization of selectors for the direct HPLC separation of enantiomers, with the stress to antibiotic chiral selectors. Thermodynamic characterization of chiral recognition of enantiomers.
- 3) Study of the interconversion of enantiomers of thermally labile chiral compounds during the HPLC separation.
- 4) Optimization of two coupled chiral column selectivity for the HPLC separation of multicomponent mixture of enantiomers.

**Afternoon – Discussion of practical separation problems, participant comments, closing remarks (Prof. B. Buszewski, Prof. M. Hutta, prof. J. Lehotay, Prof. J. Krupčík, Prof. P. Jandera, Assoc. Prof. M. Holčapek et al.).**

## **LIST OF PARTICIPANTS:**

### **PhD. Students:**

**Marianna Mamrošová**  
(from Comenius University, Bratislava)

**Andrea Fedurcová**  
**Demián Meričko**  
**Janka Mydlová**  
(all from Slovak Technical University, Bratislava)

**Ewa Szymanska**  
(from Medical University of Gdansk)

**Ewa Klodzinska**  
**Sylwia Kowalska**  
**Katarzyna Krupczynska**  
**Elzbieta Plewa**  
**Tomasz Welerowicz**  
**Wojciech Zebrowski**  
(all from Nicolaus Copernicus University, Torun)

**Ivona Jasprica**  
**Ana Mornar**  
(all from Faculty of Pharmacy, University of Zagreb)

**Petr Česla**  
**Tomáš Hájek**  
**Miroslav Lída**  
**Dana Moravcová**  
**Kateřina Novotná**  
**Jiří Urban**  
(all from University of Pardubice, Czech Republic)

### **Teachers:**

**Prof. Milan Hutta**  
(from Comenius University, Bratislava, Slovakia)

**Prof. Jozef Lehotay**  
**Prof. Ján Krupčík**  
(both from Slovak Technical University, Bratislava, Slovakia)

**Prof. Boguslaw Buszewski**  
**Dr. Renata Gazdala-Kopciuch**  
(both from Nicolaus Copernicus University, Torun, Poland)

**Prof. Pavel Jandera**  
**Assoc. Prof. Michal Holčapek**  
**Assoc. Prof. Jan Fischer**  
**(all from University of Pardubice, Czech Republic)**

**Guest Ph.D. students:**  
**Jan Grafnetter**  
**Vlastimil Hruška**  
**Jana Lokajová**  
**Jan Srbek**  
**(all from Charles University, Prague, Czech Republic)**

**Guest lecturer:**  
**Prof. Peter Schoenmakers**  
**(from University of Amsterdam, The Netherlands)**

**Organization of Summer School:**

<b>Program:</b>	<b>Prof. Pavel Jandera</b>	<b>Pavel.Jandera@upce.cz</b>
<b>Accommodation, contact person:</b>	<b>Dr. Václav Staněk</b>	<b>Vaclav.Stanek@upce.cz</b>