SESSION I MAY 25th, 2010

CHAIRPERSONS: F. Geiss and Y. Vander Heyden

SESSION II MAY 25th, 2010

CHAIRRPERSONS: B. Chankvetadze and J. Polański

SESSION III WEDNESDAY, MAY 26th, 2010

CHAIRPERSONS: D. Agbaba and M. Waksmundzka-Hajnos

SESSION IV WEDNESDAY, MAY 26th, 2010

CHAIRPERSONS: I. Zenkevich and A. Voelkel

SESSION V THURSDAY, MAY 27th, 2010

CHAIRRPERSONS: D. Mangelings and Z. Tešić

1.

Application of Cyclodextrins as Chiral Selectors in Capillary Electrophoresis: Recent Studies on Enantioseparation and Chiral Recognition Mechanisms

B. Chankvetadze

Institute of Physical and Analytical Chemistry and Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia
bezhan_chankvetadze@yahoo.com

Cyclodextrines represent one of the most powerful group of chiral selectors for enantioseparations in capillary electrophoresis (CE) [1, 2]. The properties of cyclodextrines, such as their solubility in aqueous and some non-aqueous solvents, UV-transparency of native cyclodextrines, enantioselective complex-formation ability with many chiral compounds, multivariate possibility of chemical derivatiazation, etc. contributed significantly to the success of these macrocyclic molecules as chiral CE selectors. In addition to above mentioned, being the medium size molecules with more or less defined structure makes cyclodextrins as useful targets for studies aimed to better understanding of fine mechanisms of enantioselective noncovalent intermolecular interactions.

This presentation summarizes our recent studies on enantioseparation of chiral drugs in CE using various native, as well as neutral and charged derivatives of cyclodextrins. The major emphasis is put on the relationships between separation and chiral recognition of cyclodextrins in aqueous and non-aqueous medium [2]. The application of other techniques, such as various nuclear magnetic resonance (NMR) spectroscopy methodologies and mass spectrometry, will be shown for better understanding of enantioselective effects in analyte-cyclodextrin interactions.

The examples of anylite involved in these studies include chiral drugs such as ketoconazole and terconazole [3], propranolol [4, 5], dimethindene, ephedrine, norephedrine, tetrahydroziline and others.

1. Chankvetadze, B.: Enantioseparations by using capillary electrophoretic techniques: The story of 20 and a few more years. J. Chromatogr. A **1168**, 45-70 (2007).
2. Chankvetadze, B.: Combined approach using capillary electrophoresis and NMR spectroscopy for an understanding of enantioselective recognition mechanisms by cyclodextrins. Rev. Chem. Soc. **33**, 337-347 (2004).
3. Lomsadze, K., Martinez-Giron, A. B., Castro-Puyana, M., Chankvetadze, L., Crego, A. L., Salgado, A., Marina, M. L., Chankvetadze, B.: About the role of enantioselective selector-selectand interactions and the mobilities of temporary diastereomeric associates in enantiomer separations using capillary electrophoresis, Electrophoresis **30**, 2803-2811 (2009).
4. Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B.: Separation of propranolol enantiomers by capillary electrophoresis using sulfated β-cyclodextrin derivatives in aqueous and nonaqueus electrolytes: Comparative CE and NMR study. Electrophoresis 2010, in press.
5. Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B.: Separation of propranolol enantiomers using capillary electrophoresis with various cyclodextrins: Comparative CE and NMR studies. J. Sep. Sci. 2010, in press.

2.

Use of chlorinated polysaccharide-based chiral stationary phases to update generic separation strategies for capillary electrochromatography.

Ans Hendrickx [1], Debby Mangelings [1], Bezhan Chankvetadze [2], Yvan Vander Heyden [1] *

[1]*Department of Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research*
(CePhaR), Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium
[2]*Department of Physical and Analytical Chemistry and Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, 380028 Tbilisi, Georgia*
**Corresponding author. Tel.: +32-2-477.47.34; Email: yvanvdh@vub.ac.be*

The separation of chiral molecules is an extensively studied field in pharmaceutical analysis because enantiomers display different pharmacodynamic and pharmacokinetic profiles in living systems [1]. Regulatory instances therefore demand the development of separation methods to determine the enantiomeric purity and stability of chiral drugs. Because the separation of these drug molecules often requires extensive method development, generic separation strategies, applicable on large sets of structurally diverse compounds, can be very useful.
Capillary electrochromatography (CEC) combines the properties of both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), and is characterized by its miniaturized character, a high sample loading capacity, high efficiencies and fast separations [2]. It was earlier found suitable as separation technique to define a chiral separation separation strategy using polysaccharide-based chiral stationary phases [3].
However, new types of chiral stationary phases were commercialized in the meanwhile. Therefore, it is currently investigated whether these new types of CSP display a higher enantioselectivity than the older ones. Then they can replace an older CSP in the strategy, resulting in a higher success rate.
The screening conditions of the existing chiral strategy were tested for their applicability on four chlorine-containing polysaccharide-based stationary phases [4]. A test set of 48 structurally diverse drug compounds was analyzed using the screening conditions of the strategy. The enantioselectivity of these phases was compared with those of the four phases used in the existing strategy. The results led to different possibilities to upgrade the current screening strategy.

References

[1] A. Van Eeckhaut, Y. Michotte, “Pharmacological importance of chiral separations” in A. Van Eeckhaut, Y.
Michotte (Eds.) “Chiral separations by capillary electrophoresis”, Taylor and Francis, Florida, 2010, pp. 1-22
[2] I.S. Krull, R.L. Stevenson, K. Mistry, M.E. Swartz, Capillary electrochromatography and pressurized flow
capillary electrochromatography: An introduction, HNB Publishing, New York, 2001
[3] D. Mangelings, M. Maftouh, D.L. Massart, Y.Vander Heyden, LC-GC Europe 19 (2006) 40-47
[4] S. Fanali, G. D’Orazio, K. Lomsadze, B. Chankvetadze, J. Chromatogr. B 875 (2008) 296-303

3.

Comparison of different chromatographic supports for liquid chromatography applied to the separation of josamycin and erythromycin

L. Van den Bossche, A. Van Schepdael, J. Hoogmartens, E. Adams

K. U. Leuven, Laboratory for Pharmaceutical Analysis, O&N 2, Herestraat 49, PB 923, B-3000 Leuven (Belgium)

Chromatographic supports for liquid chromatography (LC) should give, fast and highly efficient separations at low cost.

For this purpose, several strategies were developed, such as the use of silica based monolithic columns, sub-2 μm porous particles and fused core technology particles. However, most of the analytical methods reported in the European Pharmacopoeia (Ph. Eur.) use conventional porous silica particle columns, which are characterized by relatively long analysis times and high solvent costs. Therefore, this study investigates whether the conventional silica columns can be replaced easily by monolithic supports or newer types of particle columns, in order to achieve faster analyses with similar/better resolution power. Hence, two Ph. Eur. methods for the separation of the macrolide antibiotics, josamycin and erythromycin, were examined. Potential benefits and problems for the transfer of a method from a conventional porous silica particle column to monolithic columns or small particle columns will be commented in terms of stability, sensitivity, efficiency, resolving power and analysis time.

For the monolithic supports, easy method transfer with minor adaptations of chromatographic parameters was observed. In addition, the monoliths showed good peak capacity, low backpressure and short analysis time. The use of small particle columns requires special LC instrumentation to cope with the high backpressures (up to 1000 bar). Also, the method transfer involves altering of several chromatographic parameters, such as injection volume, flow rate… Furthermore, this research demonstrates that with small particle columns the best analytical results were obtained when the same brand of stationary phase was used as in the conventional silica column on which the method was developed.

4.

Separation of high polar glycosidic compounds from *Verbascum* sp. flower extracts by means of hydrophilic interaction thin-layer chromatography

[1]Łukasz Cieśla, [2]Michał Ł. Hajnos and [1]Monika Waksmundzka-Hajnos

[1]Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6 St, 20-081 Lublin, Poland.
[2]Department of Pharmacognosy, Medical University of Lublin, Chodźki 1 St, 20-093 Lublin, Poland.

Verbascum is a genus of the *Scrophulariaceae* family, including approximately 250 species, native to Europe and Asia. Various mullein species have been commonly used as emollients, expectorants, and for treating infections of the respiratory system. According to previous investigations iridoid glycosides as well as oleanane type triterpenes are widespread in the family *Scrophulariaceae* and constitute a group of compounds with chemotaxonomic significance for the *Verbascum* genus. Unfortunately detection and identification of compounds of these groups encounter certain difficulties. Iridoids are nonvolatile compounds what hinders their analysis by gas chromatography. The application of HPLC with UV detection is also difficult due to the fact this compounds possess rather weak chromophores. What also makes the chromatographic analysis difficult, is iridoid glycosides' strongly hydrophilic character. For the sake of example aucubin and catalpol are characterized by the following logP values: -3.0 and -3.2, respectively. The analysis of triterpene saponins is also challenging, as triterpenoids occur in plant material as multicomponent mixtures of glycosidic forms. A need to develop a new simple method for the resolution and detection of these compounds, in complex mixtures, is steadily growing. However the resolution of high polar compounds, both in normal and revered-phase systems, is somewhat difficult. In such case hydrophilic interaction chromatography (HILIC), known also as aqueous normal phase chromatography, may be a method of choice. It is believed that in HILIC the mobile phase forms a water rich layer on the surface of polar stationary phase, creating a liquid/liquid extraction system.

In this presentation a concept of using two perpendicular HILIC TLC systems for the resolution of high polar glycosidic compounds, from the flower extracts of selected *Verbascum* species, is presented. The obtained results are further used for the comparative studies of the investigated species.

5.

Drug impurity profiling. A methodology for method development

M. Dumarey, Y. Vander Heyden

Department of Analytical Chemistry and Pharmaceutical Technology
Center for Pharmaceutical Research (CePhaR)
Vrije Universiteit Brussel – VUB
Laarbeeklaan 103, 1090 Brussels, Belgium

In drug impurity profiling an HPLC method is optimized that allows separating a drug compound from its impurities. At the moment the method is developed neither the number of compounds is known nor their structures. When method development is performed by means of an experimental design approach then first a screening is performed followed by a response surface design. However, here in HPLC method development we can skip the screening step, because we know for the different factors which are affecting the selectivity of the method most. It are in order of importance, the stationary phase manufacturer and the mobile phase pH, the organic modifier composition and to a lesser extent the slope profile and -time and the temperature. A sequential methodology will be presented to optimize drug impurity profiles. It contains three steps: first a stationary phase selection combined with pH optimization; secondly the organic modifier composition optimization, and finally an optimization of the gradient profile and the temperature. The stationary phase is selected among dissimilar phases and the separation modelled as a function of the pH. Organic modifier is optimized based on Snyders solvents triangle, which in fact is a mixture design, and finally the slope and temperature are optimized using a factorial design.

6.

Thin-layer chromatography for fingerprinting and screening the biological activity of plant extracts

Monika Waksmundzka-Hajnos and Łukasz Cieśla

Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6 St, 20-081 Lublin, Poland.

Chromatographic fingerprint profiling is a very convenient and effective method for quality assessment of herbal materials. Several chromatographic techniques have been extensively applied for constructing chromatographic profiles, e.g.: HPLC, GC, HPTLC or HSCCC. HPTLC offers a number of unique features that can outperform the other separation techniques used for the fingerprinting. Great advantage of HPTLC is the speed of method development and also its flexibility. What is more, TLC is often a method of choice for the screening of plant extracts for the presence of biologically active compounds. It is particularly well suited for the direct biological detection, since the separation result is immobilized prior to the detection and moreover, the open solid bed layer allows direct access to the sample.
In this presentation application of different thin-layer chromatographic techniques are presented for the fingerprinting of the variety of plant samples. The use of special modes of chromatogram development, applied for the fingerprinting, are described and their application for the purposes of the plant chemotaxonomy, are discussed. The quality control of pharmaceutical preparations, by means of TLC, are also presented. Advantages and disadvantages of TLC are widely discussed. The application of TLC for screening the biological activity of plant extracts is also addressed. The main focus is on the use of planar chromatography for detection and identification of free radical scavengers in plant extracts and botanical preparations. The possibilities of future studies and perspectives of method development are also outlined.

7.

On alternative fingerprinting solutions in phytochemical research

T. Kowalska[1], M. Waksmundzka-Hajnos[2], M. Sajewicz[1]

[1] *Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland*
[2] *Department of Inorganic Chemistry, Medical University of Lublin, 6 Staszica Street, 20-081 Lublin*, Poland

Kingdom of plants is an inexhaustible reservoir of compounds which have not yet been detected, isolated, and their respective structures properly identified, and moreover, many of those have not yet been synthesized either. Over the centuries, kingdom of plants has also proved a practically inexhaustible reservoir of curative compounds and pharmaceutical concepts, although until now, far from being sufficiently explored.

Presently, several critical issues in phytochemical research are on top of the agenda and the crucial one is application of flexible and efficient separation, isolation and identification tools, adequate to a given research task. The second crucial issue is availability of phytochemical standards, generally characterizing with relatively high costs.

This paper focuses on a discussion of selected novel fingerprinting strategies. First part of this presentation is devoted to the fingerprinting potential and flexibility of the TLC-MS interface device, able to transform thin-layer chromatography into an essential part of a multidimensional fingerprinting system. Second part of this presentation is devoted to the multidimensional fingerprinting strategy with use of the HPLC separation unit and a selection of the employed detecting systems in a parallel or consecutive set-up. Attention of the audience will be drawn to the MS, DAD, ELSD, and polarimetric detectors as those truly important for an efficient multidimensional fingerprinting of the natural products. Last not least, videoscanning of planar chromatograms as an additive means to generate digital fingerprints will also be mentioned.

8.

Approaching the Quality Assurance of Artesunate

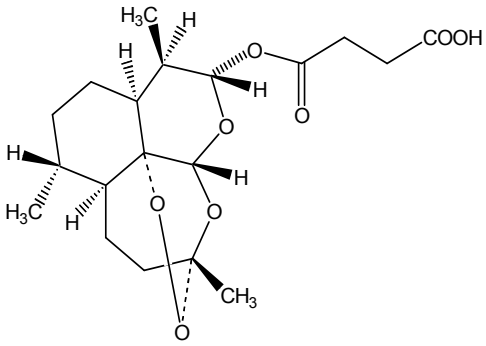
Angelika Koch ,

Frohme-Apotheke, Frohmestr. 14, D-22457 Hamburg

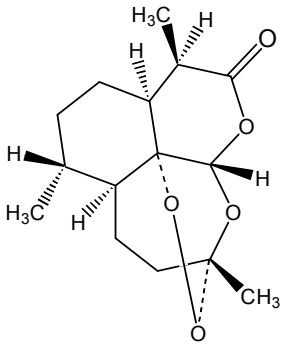
Artemisinin (**2**) is a sesquiterpene lactone isolated from *Artemisia annua*, a herb that has traditionally been used in China for the treatment of malaria. Due to its insolubility in water Artemisinin is not recommended for intravenously administration. Therefore chemically modified derivatives with appropriate properties have been developed (fig.1).
Artesunate (**1**) is a slightly water-soluble hemisuccinate derivative of artemisinin (**2**) but it is unstable in neutral solutions and might be degraded to dihydroartemisinin/artenimol (**3**) or artemisinic acid (**4**).
Artesunate and its active metabolite dihydroartemisinin (3) reveal remarkable activity against otherwise multidrug-resistant *Plasmodium falciparum* and *P. vivax* malaria.
The functional group responsible for antimalarial activity of artesunate is the endoperoxide bond. Orphan designation of intravenously administered artesunate had been granted in the United States for immediate treatment of malaria. In addition to this application
Artesunate has now been analyzed for its anti-cancer activity against leukemia and colon cancer cell lines. Hence it makes a market in non-malaria countries, too.
The demand and supply are increasing but there is a lack of defined specifications and purity test methods.

The International Pharmacopoeia offers drafts of the appropriate monographs. IR-spectrometry is used for identity tests and TLC and HPLC for purity tests.
The TLC test uses ethyl acetate and toluene (5+95) as the mobile phase with a very low separation power It is proposed that the TLC method be omitted,whereas we think it would make sense to change the polarity of the mobile phase. We demonstrate the usefulness of an appropriate TLC method which is suitable to determine the identity of the raw substances and the related substances of artemisinin derivatives.
Furthermore we checked FTIR- spectra (regarding the striking differences across the range of interest (e.g. stretching of –OH, =O and –O) for suitability of a rapid purity test method (fig.2).

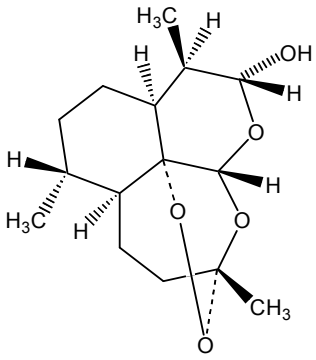
Figure 1. Chemical structures of artesunate and some of its derivatives



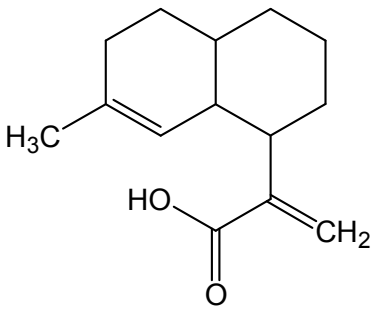
1: Artesunate



2: Artemisinin

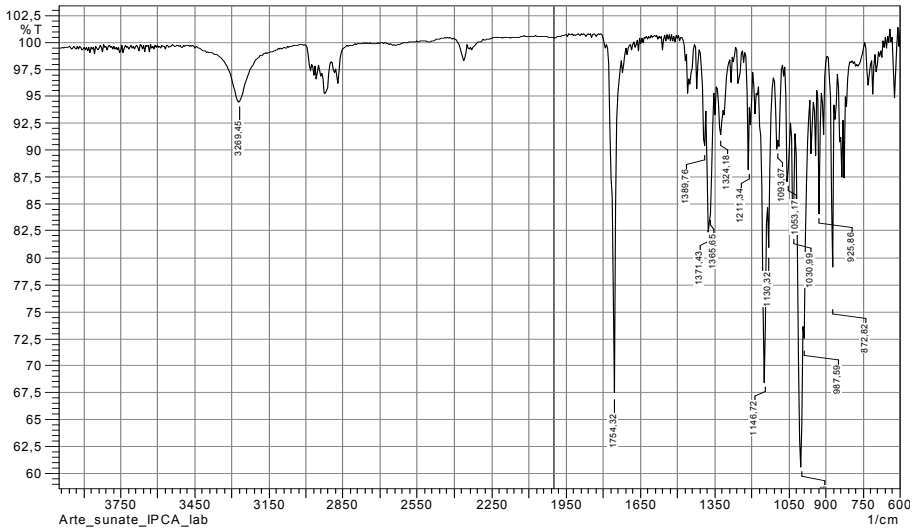


3:Dihydroartemisinin, Artenimol

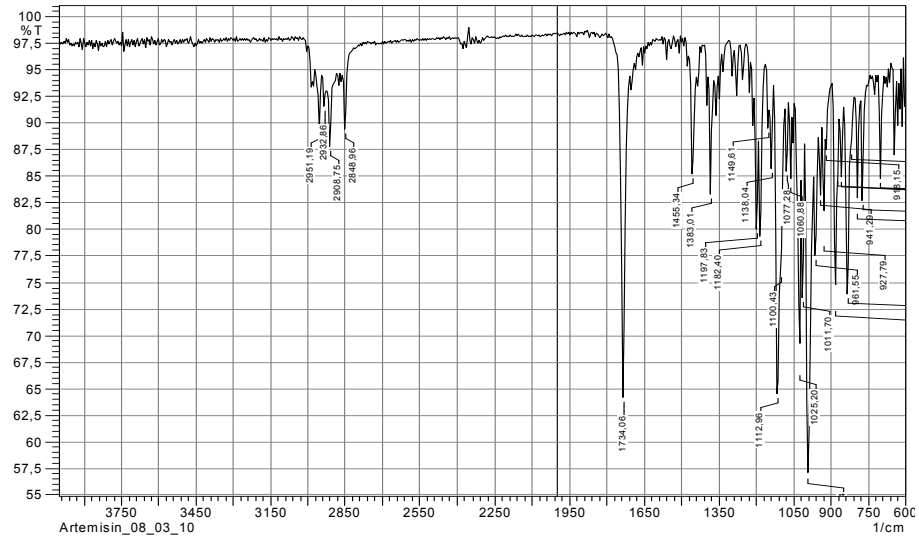


4: Artemisinic acid

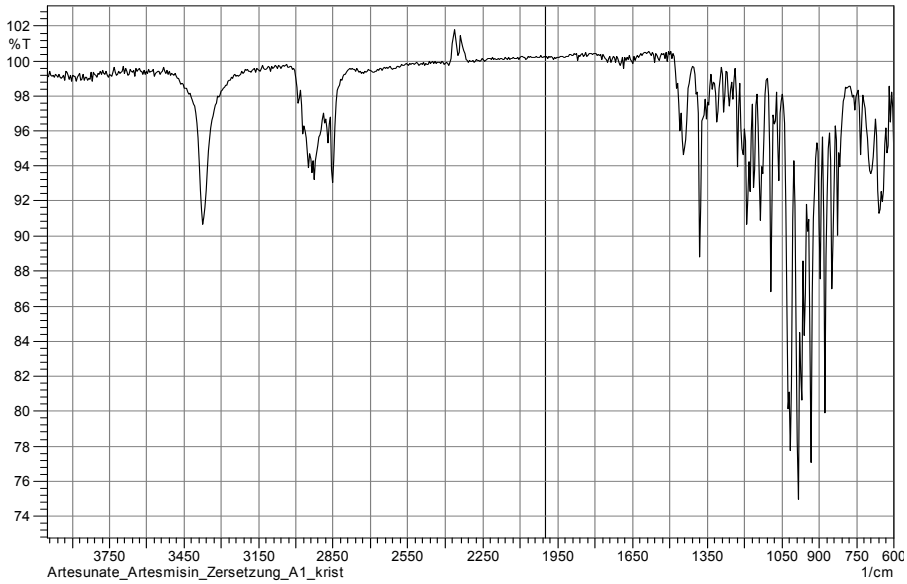
Figure 2: FTIR-spectra of Artesunate, Artemisinin and Dihydroartemisin/Artenimol



Artesunate (**1**)



Artemisinin (**2**)



Dihydroartemisin, Artenimol (**3**)

9.

Teaching computers chemistry: on-line technologies in chemoinformatics

Jarosław Polański

Department of Organic Chemistry, Institute of Chemistry, University of Silesia, PL-40-006 Katowice, Poland, e-mail: polanski@us.edu.pl

A first concept of social interaction by computer networking has been suggested in sixties and a first cable connection has been constructed in 1969 in the USA. Nobody at that time could have predicted the importance of this idea and its impact on the economy and science. Currently computers are more and more dependent on the web technologies. Accordingly, a number of chemical resources available in the web steadily increases. A term chemoinformatics has been coined recently to describe a discipline organizing and coordinating the increasing application of computers in chemistry, in particular also by networking chemical *in silico technologies*.

With the greater and greater potential of informatics *in silico chemistry* has significantly increased the scope of interest and the available field of investigations. Chemoinformatics focuses on drug design, molecular engineering and organic chemistry. The application of computer assisted methods for molecular manipulation and prediction, synthesis design and property oriented synthesis are illustrative examples. On-line chemical technologies collect the sites offering an access to chemical data, educational resources, free or commercial software, e-commerce, on-line chemistry journals and many others. In this lecture we will focus on the interactive molecular data resources which recently have significantly increase in usability and functionality. We will show illustrative examples in various of chemical branches. The advantages and problems will be discussed, including the data exchange standards, molecular codes and editors.

10.

The renaissance of additive schemes for evaluation of retention indices in gas chromatography

Igor G. Zenkevich

St. Petersburg State University, Chemical Department
Universitetskii prosp., 26, St. Petersburg 198504, St. Petersburg, Russia
E-mail: izenkevich@mail15.com

The evaluation of GC retention indices (RI) using "classical" additive schemes (2) is perceived sometimes like obsolete and primitive procedure comparing with contemporary QSPR approaches based on multi-parameter linear regressions (1):

$\mathbf{RI \approx \Sigma k_i A_i}$ (1) where A_i are the descriptors of different origin, k_i – coefficients.	$\mathbf{RI \approx RI_0 + \Sigma \Delta RI_i}$ (2) where RI_0 – the RI value for basic structure containing no substituents characterized by increments ΔRI_i.

The case $RI_0 = 0$ (only atomic increments are involved) is known [1]. However, precalculation and following summarizing of increments is resulted in a large uncertainty of results.

The alternative approach includes no RI increments at all, because it is based on the direct superposition of chemical structures. If we characterize the target molecule **ABCD**, we can combine it from the simpler precursors **ABC** and **BCD** subtracting the duplicated fragment **BC** by the following way:

ABC + BCD - BC = ABCD

The arithmetical operations with reference RI values directly correspond to this equality:

RI(ABCD) ≈ RI(ABC) + RI(BCD) - RI(BC)

This kind of additive scheme was shown to be effective in precalculation of RIs of 839 polychlorinated hydroxybiphenyls [2], 211 structural isomers of 4-nonylphenols [3], products of free-radical chlorination of cyclohexane [4], ionic chlorination of aliphatic carbonyl compounds [5], bromosubstituted anilines [6], etc. Its features and not numerous restrictions are discussed.

References:

1. *Stein S.E., Babushok V.I., Brown R.L., Linstrom P.J.* J. Chem. Inf. Comput. Model. **47** (2007) 975.
2. *Zenkevich I.G., Moeder M., Koeller G., Schrader S.* J. Chromatogr. A. **1025** (2004) 227.
3. *Zenkevich I.G., Makarov A.A., Schrader S., Moeder M.* J. Chromatogr. A. **1216** (2009) 4097.
4. *Zenkevich I.G., Eliseenkov E.V., Kasatochkin A.N.* Chromatographia. **70** (2009) 839.
5. *Zenkevich I.G., Eliseenkov E.V., Kasatochkin A.N., Zhakovskaya Z.A., Khoroshko L.O.* Rus. J. Anal. Chem. 2010 (in press).
6. *Gruzdev I.V., Alferova M.V., Kondratenok B.M., Zenkevich I.G.* Rus. J. Anal. Chem. 2010 (in press).

11.

Hansen Solubility Parameters – Their Determination by IGC and Applications

A. Voelkel, K. Adamska, B. Strzemiecka

Poznań University of Technology, Institute of Chemical Technology and Engineering, pl. M. Skłodowskie-Curie 2, 60-965 Poznan, Poland, Adam.Voelkel@put.poznan.pl

The solubility parameter concept found an application in many industries for explanation different properties of the components forming a formulation. Knowledge of the solubility parameter data for different components is important to predict the magnitude of interaction between the components of formulation (miscibility, compatibility or adsorption) and further stability of the product.

Solubility parameter called *Hildebrand solubility parameter* or *Hildebrand parameter* is applied only for regular solution. So-called *Hansen solubility parameter* (HSP) is extension of the Hildebrand solubility parameter to polar and hydrogen bonding systems. Hansen assumed, that cohesive energy can be considered as a sum of contributions from dispersive (E_d), polar (E_p) and hydrogen bonding (E_h) interactions:

$$-E_{coh} = -E_d - E_p - E_h \qquad (1)$$

and the total solubility parameter (δ_T) is expressed as

$$\delta_T^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \qquad (2)$$

where: δ_p, δ_p, δ_h denote dispersive, polar and hydrogen bonding contribution, respectively. The estimation of HSP for the group of nanomaterials and modified nanomaterials from Inverse Gas Chromatographic (IGC) data is presented and discussed.

12.

Modelling of the chromatographic retention from organic modifier content by different equations- theoretical comparison and practical aspects

Łukasz Komsta

Chair and Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland, lukasz.komsta@umlub.pl

Binary mixtures of weak diluent and strong modifier are commonly used mobile phases in HPLC and TLC, giving a possibility to control the retention in wide range. An estimation of retention from the content of modifier is widely elaborated topic and many theoretical and empirical equations were proposed till now [1]:

1. Equations modelling ln *k*: semilogarithmic model of Soczewiński-Wachtmeister, log-log model of Snyder-Soczewiński, quadratic and square-root models of Schoemakers, two models of Nikitas, a variable-power model of Zapała, recursive model of Zenkevich and recent Box-Cox transform model by Komsta
2. Equations modelling *k* or 1/*k* proposed by: Row, McCann, Kaczmarski and Zapała
3. Two equations modelling R_F proposed by Kowalska.

After fitting the data to an equation for particular component and chromatographic system, its retention can be then interpolated (to find modifier concentration bringing best separation) or extrapolated to pure diluent (concentration equal to zero). The extrapolation in reversed chromatography is an important approach and common method for determination of solute lipophilicity.

The purpose of the presentation is to collect all existing retention equations and compare them theoretically (against their mathematical properties) and practically (on two TLC retention datasets) according to both interpolation and extrapolation abilities.

A particular attention will be pointed at uncertainty (confidence of prediction) during extrapolation and the resulting usefulness in lipophilicity estimation [2].

[1] Komsta, Ł. Acta Chromatogr. 2/2010 (in press)

[2] Unpublished results.

13.

Mapping Drug Architecture by MoStBioDat - Rapid Screening of Catechol Motifs

Andrzej Bak, Tomasz Magdziarz, Agata Kurczyk, Jaroslaw Polanki

Department of Organic Chemistry, Institute of Chemistry, University of Silesia, PL-40-006 Katowice, Poland
e-mail: abak@us.edu.pl

Computer-assisted simulations are the most progressive component of the present day chemical investigations, producing enormous amount of data. The constraint of processing and sharing such data is thought as a major impediment in the drug discovery process. Furthermore, among the steepest barrier to overcome in the high-throughput screening (HTS) studies is the restricted amount of a reliable, publicly available repositories combining the detailed drug data with the comprehensive drug target information. Only the proper dataset aggregation and unified standards of data organization enable massive *in silico* knowledge mining. By offering a uniform data storage and retrieval mechanism various data might be compared and exchanged easily.

Structure-based database screening is a rapidly growing and an efficient technique in the early stages of the drug development process, gaining considerably from the current progress in the computer technology. Particularly, the subsequent sampling of a virtually infinite chemical space (VCS) in order to optimize the ligand diversity of chemical libraries (VLS) with appropriate binding affinity places emphasis more on the probability field with accidentally developed drugs than on traditional principles of the rational drug discovery. In consequence, the tools and techniques for organizing and intelligently mining this information are highly desirable.

In an effort to make the virtual screening more accessible the Molecular and Structural Bioinformatics Database (MoStBioDat) project has been established as a management platform for an efficient storage, access and exchange of the biomolecular data with an extensive array of software tools for the structural similarity measures and pattern matching. It could potentially serve as a dual purpose storage environment integrated with database management system (DBMS) to explore 3D drug-target interactions or compare and measure the structural similarities between chemical structures.

In the current studies we have investigated the application of MoStBioDat software platform for the massive analysis of the spatial arrangements and conformational examinations of hydroxyl groups in the catechol-containing compounds, widely regarded as the main substructure block in many antiviral inhibitors. The geometrical orientation of the hydroxyl groups seems to determinate the ability of catechol derivatives to recognize the surrounding environment by forming the inter- and intra-molecular hydrogen bonds. The detailed analysis of the torsion angles, taking into account the spatial coordinates of the hydroxyl groups and the adjacent aromatic carbon atoms has been conducted using 3D structures taken from the freely accessible repositories.

14.

Drug metabolism development concepts and its bioanalytical technics

Imre Klebovich

Semmelweis University, Department of Pharmaceutics,
H-1092 Budapest, Hőgyes E. St. 7., Hungary

Drug-metabolism research relies on multidisciplinary approaches comprising receptor biology, enzymology, recombinant DNA technology, biochemical toxicology, and drug disposition into study design and conduct balanced *in vitro* and *in vivo* experiments.
Successful drug-metabolism research must integrate to allow a full understanding of the mechanisms of individual variability in drug therapy and drug safety.
The present lecture intends to give an overview of the process and up-to-date bionalytical tools of the *in vitro* and *in vivo* drug metabolism. Several examples illustrate the possibilities of the quick fingerprint radio-bioanalytical examination of drug molecule in the comparison of species.

For *in vitro* or *in vivo* biotransformation investigations drugs labeled radioactively with ^{3}H- and/or ^{14}C- isotopes provide the possibility to track and quantitatively analyze the metabolites in complex biological matrices using separation techniques coupled to radioactivity detection methods. Nowadays the radiochemical detection of different on-line hyphenated techniques (GC-RD, HPLC-RD, OPLC-RD) are of great impact in the complex study of the pharmacokinetics and metabolite kinetics of the parent compound and its metabolites, while the hyphenated off-line techniques (OPLC-DAR/PIT – Digital Autoradiography; Phosphor Imaging Technology) are essential in the metabolite isolation and purification. The different types of radioactive detection enables high selectivity (only the ^{3}H-, ^{14}C- labeled compound and its metabolites are detectable) with extremely good sensitivity.
In vitro metabolism studies bring important decisional elements for the selection of the best candidate(s) entering clinical development and represent valuable tools to optimize future clinical studies. Pharmacokinetic and metabolism information of different species, contributing to registration, are also summarized.

The application of the new, flexible and rapid high-performance complex solution and their possible combinations, including single- and multi-step separation and isolation in metabolism research will also be presented.

15.

Evaluation of methacrylate-based monolithic stationary phases for the analysis of drug molecules in the capillary format.

Indiana Tanret, Debby Mangelings, Yvan Vander Heyden *

Department of Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research (CePhaR), Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium
**Corresponding author. Tel.: +32-2-477.47.34; Email: yvanvdh@vub.ac.be*

The separation of pharmaceutical samples with efficient methods is relevant in several fields, like drug development, drug safety assessment, quality control and toxicity studies. Monolithic stationary phases offer many advantages, of which the most important are easy column preparation and the possibility to tailor their morphology. They are prepared via polymerization of a so-called polymerization mixture that consists of monomers, poreforming solvents and an initiator of the polymerization reaction. The shape and functionalities of monolithic columns can easily be adapted by varying the composition of the polymerization mixture [1]. In our study, the potential use of methacrylate-based monoliths for the analysis of small molecules with basic or acidic properties, i.e. drug molecules, was evaluated. In a first stage, central-composite design-based experiments were performed to find a polymerization mixture from which columns with good chromatographic properties could be synthesized [2,3]. This polymerization mixture was then used for the analysis of
drug molecules in capillary electrochromatography (CEC) [4] and pressure-assisted CEC.
CEC combines the properties of capillary electrophoresis (CE) and liquid chromatography (LC). As in CE, the mobile phase is driven by the electro-osmotic flow (EOF), while the presence of a stationary phase reminds of liquid chromatography (LC). Pressurized CEC (pCEC) is based on CEC, but the flow is controlled both electrophoretically and by pressure [5].

Finally, the synthesized monolithic capillary columns were used within a so-called lab-on-achip set-up, where the injection, separation and detection all happen on one small surface. The separation on a chip can be performed in either electrophoretic, electrochromatographic or pressurized mode, depending on the needs of the analysis. The chips used in our experimental work were prepared in-house by etching into a polydimethylsiloxane layer.

[1] E.F. Hilder, F. Svec, J.M.J. Fréchet, Electrophoresis, 23 (2002) 3934-3953.
[2] I. Tanret, D.Mangelings, Y. Vander Heyden, Electrophoresis, 29 (2008) 4463-4474.
[3] I. Tanret, D.Mangelings, Y. Vander Heyden, J Pharm Biomed, 48 (2008) 264-277.
[4] I. Tanret, D. Mangelings, Y. Vander Heyden, Optimizing the separation of acetylsalicylic acid from some of its impurities using monolithic capillary columns in CEC, in preparation.
[5] A. Ivanov, C. Horváth, B. Karger, Electrophoresis, 24 (2003) 3663-3673.

16.

Multidimensional SHIMADZU chromatography

Marek Szklarczyk
Shim-Pol, ul. Lubomirskiego 5, 05-080 Izabelin.

Chromatographic analysis has become the fundamental technique for the exceptional and ultimate identification of a sample of interest. The presentation will enlight different techniques developed by Shimadzu .

The technical design of the newest instruments and outstanding GC×GC, GC×GC-MS, LC×LC, LC×LC-MS and LC×GC-MS, techniques will be presented. These new state of art chromatographic techniques are called 2D and comprehensive solutions.

The benefits and advantages of these techniques over standard techniques will be discussed. The basis of these techniques are multi column chromatographic analysis which lead to the perfect separation of the studied sample, and straight identification of analytes not detected or separated with the standard GC and LC techniques. The key role of MS detection will be presented.

A wide range of application of these techniques, in different analytical fields will be presented.

17.

A comparative study of lipid composition of the brain of chicken and rat during myelination; A chromatographic-densitometric analysis.

F. Helmy, Morris

Delaware State University, Dover, DE

The lipid profile of brain from 10 day and 18 day old chick embryo, one day old chick and adult chicken (10 weeks old), as well as full-term fetus rat, 21 day old male rat, young adult male rat, and pregnant female rat brain; were analyzed by thin layer chromatography and densitometry. The emphasis was on the major glycolipids of brain during myelination (i.e. galactocyl diglyceride (GDG), normal fatty acid and hydroxyl fatty acid ceramide monohexosides (n-CMH and h-CMH respectively), and ceramide monohexide sulfatides (CMS) as well as the choline lipids sphingomyelin (SM), and phosphatidyl choline (PC), and the species of phosphatidyl ethanolamine plasmalogen (PE_1 and PE_2).

10-day old chick embryo brain revealed a low concentration of GDG, n-CMH, h-CMH, and CMH-S. The concentration of these glycolipids increased gradually as the chick embryo advanced in development, indicating age relatedness, and reached the highest level in the brain of adult chicken. Rat brain did not begin to show the presence of these lipids until at least 21 days old, possibly indicating that the nervous system of the bird model develops faster than that of mammals. In addition, phosphatidyl ethanolamine plasmalogen (PE), was shown to be the only alkenyl phospholipid in all samples analyzed. Two molecular species have been identified (PE_1 and PE_2), and PE_2 has been shown to correlate with the myelination process. PE_2 is not seen until after hatching in chick brain, while it is seen at the 21 day old stage rat brain. Phosphatidyl choline (PC), and sphingomyelin (SM), were also present. SM concentration increased gradually during development, and reached its highest level in adult chicken brain, as in rat brain.

The correlation of myelination, brain development and the level of these glycolipids, indicate the important role of these glycolipids in both brain maturation and function.

18.

Two dimensional separation of amino acids with thin-layer chromatography and pressurized planar electrochromatography in normal and reversed phase systems

Adam Chomicki, Kinga Kloc, Ewa Materna-Witkowska, Tadeusz H. Dzido

Department of Physical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland

Combination of different chromatographic modes is very attractive tool for separation of complicated sample mixtures, especially of biological origin such as peptides or amino acids. High efficiency of two dimensional separation (2-D) is achieved with respect to different selectivity of both modes involved in this process.

Thin layer chromatography (TLC) is very popular method in biomedical, pharmaceutical and environmental protection analyses. It has advantages such as low analysis cost, simple sample preparation, various methods of detection, chromatographic plates with sample bands on it can be stored after separation process and many other.

Pressurized planar electrochromatography (PPEC) is a relatively new separation mode in which the mobile phase is driven into movement by electric field (electroosmotic effect). The mode is characterized by few advantages in comparison to thin-layer chromatography such as high performance, short time of separation process and different selectivity. The last attribute is concerned with electrophoretic effect, which is involved in separation process when solute molecules undergo dissociation.

The attributes of TLC and PPEC mentioned above are very advantageous for combination of both methods into two dimensional separation process (2D TLC/PPEC). Such combination leads to considerable increase of separation efficiency. This feature of 2D TLC/PPEC mode will be demonstrated in our presentation for separation of some dye and amino acid mixtures applying normal and reversed phase systems.

19.

The Use of Technology in-needle Extraction in the Determination of Organic Compounds

M. Pietrzyńska, K. Bielicka-Daszkiewicz, A. Voelkel

Poznań University of Technology, Institute of Chemical Technology and Engineering, Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, e-mail: monikapietrzynska@gmail.com

A new method of in-needle samples preparation has been used for the determination of samples containing organic compounds in water and gaseous samples. Specially designed needle is packed with sorbent on which the analytes are retained.

In-needle method combines the advantages of solid phase extraction (SPE) and stationary phase microextraction (SPME) despite their drawbacks, such as labor expense in the case of the SPE and necessity of careful handling of expensive fiber for SPME. Conventional sample preparation methods still requires large amount of organic solvents. Classic liquid–liquid extraction (LLE) or solid-phase extraction (SPE) are relatively complicated and time-consuming procedures. In-needle extraction compared with these methods is much more economical. The amount of solvent can be reduced to less then one milliliter. In-needle extraction device is cheaper then SPE or SPME. Another advantage is its mobility. In-needle extraction device can be taken to the place where samples will be collected. Analytes can be retained in needles and transported into laboratory.

The extraction was made by pumping the aqueous/gaseous sample into the needle extraction device. The subsequent desorption process was carried out by a flow of desorption solvent through the needle into the gas chromatograph.

Several solutions has been checked to find the best in-needle extraction device. The most important parameters are: needle size, sorbent size, pore diameter. The needle filling should assure satisfactory flow rate with good recovery. Flow velocity is higher for bigger size of grains. Fine-grained fill provides a short path of diffusion of a substance inside the grains. Kinetic properties will be better for the sorbent having thinner active layer.

20.

Chosen strategy of basic drugs analysis in RP-HPLC systems

Jolanta Flieger

Department of Analytical Chemistry, Medical University, Staszica 6, 20-081 Lublin.

Basic compounds constitute quite a big group of separable analytes by reversed-phase high performance chromatography (RP-HPLC). This separation method covers the compounds which are important due to their biomedical applications. The most common strategy of controlling the retention of these compounds in RP systems is special modification of the mobile phase.

In case of weak bases required retention and efficiency of chromatographic system can be achieved by the use of addition of buffer components enabling suppression of analyte ionization. For the stronger bases creation of ion-pairs is required. Due to the presence of hydrophobic chains either cationic or anionic additives tend to be strongly adsorbed by hydrophobic stationary phase and the initial properties of a column are hard to recover again.

Attractive alternative, in context to previously used methods of basic compounds analysis, is application of chaotropic effect in RP-HPLC. It appears that salts possessing anions with chaotropic properties provide not only retention increase of protonated basic molecules in agreement with their order in liotropic Hofmeister series but they also improve efficiency of chromatographic system, peak symmetry and additionally radically perfect separation selectivity. The interest in this chromatographic technique constantly increases because of its application simplicity connected with method of system modification and fast recovery of the column initial properties. Mechanism of retention in this technique is still the subject of speculations. Participation of dynamic ion exchange mechanism as well as creation of ion-pair in the mobile phase is mentioned in context of chaotropic effect.

Recently also short chain perfluorinated acids may replace the need for addition of hydrophobic “ion-pairing” reagents, chaotropic salts or ionic liquids which have to be applied together with additional components of buffering systems. Although in the past, higher concentrations of such acidic modifiers were broadly avoided because silica based stationary phases could undergo degradation under highly acidic environment. Now approach to mobile phase modifiers can be revised owing to the advancement of silica based packings and availability of RP columns with excellent chemical stability.

21.

The LC/MS/MS analysis of the nucleation precursors in the formation of secondary organic aerosols (SOA)

Bartłomiej Witkowski*, Tomasz Gierczak*

**Department of Chemistry, Warsaw University, al. Żwirki i Wigury 101, 02-089 Warsaw, Poland*

Aerosols are very important constituents of the atmosphere. They can act as the cloud-condensation nuclei (CCN), contribute to the light-scattering effect and could be hazardous to humane health. Gas-phase ozonolysis of alkenes is known to produce aerosols. Since aerosols may affect environment it is important to understand the process of particle formation of SOA produced from ozonolysis of alkenes (for instance terpenes; vast amount of α-pinene is introduced every year into the atmosphere from the biogenic sources). Many theories concerning nucleation precursors of aerosols produced from gas-phase ozonolysis of alkenes were developed. It was proposed that the second-generation dicarboxylic acids are responsible for self-nucleation and particle growth. After extensive studies diacids were excluded as nucleation precursors, because they are produced too slowly and they are too volatile to induce self-nucleation. Recently, the theory taking into account the formation of dimers was suggested. Ozone addition to the carbon-carbon double bond initially produces the high-energy primary ozonide. This ozonide rapidly decomposes, producing species called excited Criegee intermediate (ECI). ECI can decompose, producing wide variety of products with various oxygen-containing functional groups, or it can be collisionally stabilized with N_2 or O_2 to become thermally stabilized Criegee intermediate (SCI). Studies show that SCI plays an important role in the particle formation process during gas-phase ozonolysis of alkenes. Scavenging SCI with low molecular weight compounds significantly decreased particle number in comparison with experiments where no scavenger was used. Literature sources report that SCI have strong reactivity towards oxygen-containing functional groups. Reaction with carbonyl-containing compounds and carboxylic acids produces secondary ozonide and hydroperoxide. It was proposed, since SCI is the most reactive with carboxyl containing compounds, that nucleation precursors may be the products of gas-phase reaction between SCI and carbonyl-containing acids.

The primary aim of this study is to prepare the analytical method for the analysis of hydroperoxides formed in the reaction of SCI with carbonyl-containing acids. In our experiments cyclohexene was used as a model compound. Because standards for cyclohexene oxidation products are not available, the hydroperoxides were generated using liquid-phase ozonolysis of cyclohexene in the presence of investigated scavenger. The scavengers used included carbonyl-containing acid (first-generation products) and diacids (second-generation products). Synthesized hydroperoxides were analyzed by high performance liquid chromatography (HPLC) coupled with triple quadrupole mass spectrometer with electrospray ion source (ESI). Tandem mass spectra (MS^2) for structural studies of synthesized compounds were obtained.