



Column Watch

HPLC 2005 was held in Stockholm, Sweden, June 26–30. In this month's installment of "Column Watch," Ron Majors covers this year's hot topics, including proteomics, column technology — especially monoliths — microfluidics, and multidimensional and high-throughput separations. MS and MS-MS dominated HPLC detection.

Ronald E. Majors
Column Watch Editor

Highlights of HPLC 2005

More affectionately known as HPLC 2005, the 29th International Symposium on High Performance Liquid Phase Separations and Related Techniques is the premier scientific event for bringing together the myriad techniques related to separations in liquid and supercritical fluid media. Organized by Professor Douglas Westerlund of Uppsala University, Sweden, who also chaired the HPLC 1989 Symposium, HPLC 2005 assembled over 1200 scientists from a total of 48 countries. This number included vendor representatives from 78 exhibitors for the three-day instrument, software, and consumables exhibition, and conferees, one-day registrants, exhibit-only registrants and press.

The four-day-plus event had a total of 784 symposium papers and 124 oral presentations, many presented during simultaneous sessions, 650 posters in sessions with 26 themes and 10 vendor seminars. Thus, attendees had their hands full deciding how to allocate their time. With an ample social event schedule and seven short courses held during the previous weekend, most attendees who stayed for the week were ready for some relaxation. The short course topics reflected areas of current interest and included peak shape and retention in liquid chromatography (LC), mass spectrometry (MS) detection, preparative LC, FDA method development and validation, enantiomeric separations, monolith columns, and bioanalytical sample preparation.

This year's event featured a special program for young scientists who had completed their highest degree within the past 10 years. Of the 24 lectures presented, surprisingly, 38% of them were female scientists. At the opening ceremony, a number of Halasz Foundation scholarships were awarded to aspiring graduate students. It is satisfying to know that the future of liquid chromatography is in good hands. The late Prof. Halasz of Saarbruecken University in Germany was an early pioneer in gas chromatography (GC) and high performance liquid chromatography (HPLC) technology.

Obviously, HPLC was the predominant technology used by presenters in the tech-

nical sessions at the symposium. From a perusal of the poster and oral presentation abstracts, Table I provides a rough breakdown of the coverage of liquid phase technology and techniques in the separation sciences. Table II is a similar breakdown for application areas. This year, I also tabulated the detection principles (Table III) that were used. Not every abstract indicated the detector used, so only those abstracts that provided this information were counted. The category assignments were based on the main emphasis of a particular scientific paper as well as separation and detection techniques used.

In this installment of "Column Watch," I will present some of the scientific highlights of HPLC 2005. Because it was virtually impossible for one person to adequately cover all oral and poster papers, my coverage will somewhat reflect a personal bias, although some of the presenters provided summaries.

Poster Sessions

The mainstay of HPLC 2005 was the poster sessions, in which more detailed applications and methodology studies were reported, often in very specific areas, and face-to-face discussions with the authors were conducted. Fortunately, many of the poster authors were kind enough to provide small reproductions of their poster papers that could be taken for later perusal. Some authors collected business cards and addresses for sending poster reprints by mail or e-mail. This year, posters were up for all four days, allowing participants to adequately cover these sessions.

This year, I once again had the privilege of leading the Poster Committee that chooses the top three posters at the symposium. With over 650 posters to cover, poster review was a challenging task. Fortunately, I had the able assistance of 32 members of the Poster Committee, who worked very hard to narrow down the huge collection of posters and helped select the three winners by the end of the symposium's fourth day. I want to individually recognize them by providing their names and affiliations in Table IV. Overall, the committee felt that the quality of posters was out-



Figure 1: (Top) Ron Majors presenting first prize to Hideko Kanazawa of the Kyoritsu University of Pharmacy, Tokyo, Japan; (middle) Ron Majors presenting second prize to Lane Sander, NIST, Gaithersburg, Maryland; and (bottom) Ron Majors presenting third prize to Kazuki Nakanishi of Kyoto University, Kyoto, Japan.

standing this year and showed a continued improvement over previous symposia in this series.

The Best Poster Awards, sponsored by Agilent Technologies (Waldbronn, Germany) were announced at the closing session. The winners received Amazon.com gift checks. This year's number one winning poster was titled "Separation of Probe Substrates of Cytochrome P450 Using Temperature Responsive Chromatographic System," written by H. Kanazawa, Y. Okada, E. Ayano, Y. Matsushima of the Kyoritsu University of Pharmacy, Tokyo, Japan, and T. Okano, Tokyo Women's Medical University, Tokyo, Japan. Their novel paper demonstrated the development and application of a temperature-responsive polymer grafted to a microparticulate silica substrate. The "green" chromatographic system used only water as a mobile phase. By changing the temperature only, the column's characteristics could be changed from a hydrophilic to a hydrophobic surface and allowed chromatographic resolution of a number of probe drugs under isocratic conditions.

The second place poster was "Molecular Dynamic Simulations of Chromatographic Surfaces" by K.A. Lipka, L.Sander, and R.D. Mountain of the National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, and was a last-minute poster that was not even in the final program. Their poster covered their work on molecular dynamic simulation models that describe the structural and dynamics of shape selectivity processes in reversed-phase LC when employing polymeric and monomeric phases with alkyl chain lengths, surface coverages, bonding chemistry, and temperature conditions that are typical of actual materials prepared in the laboratory. These resultant bulk structural features were related to the observed shape selectivity properties and were consistent with experimental observations. Among the findings were: alkyl chain segments proximate to the surface are disordered and chain segments at the distal end are more ordered; alkyl chain ordering is enhanced at lower temperatures; and models representing shape-selective phases have a significant region ($\sim 11 \text{ \AA}$) of alkyl chain order and an increase in cavity order whose depths increased for polymeric C30 models, and with lower density monomeric C30 models, highly ordered chain "walls" are observed. The group is further modeling solute retention of solvated phases in acetonitrile–water systems as well as the retention relationships of constrained shaped solutes.

The third place poster, titled "Anisotropic Siloxane-Based Monolith Prepared in Confined Spaces," was written by Y. Suzumura, K. Kanamori, K. Nakanishi, and K. Hirao of the Graduate School of Engineering, Kyoto University, Kyoto, Japan, and J. Yamamichi of Canon, Inc., Kanagawa, Japan. This same group was the first place winner at HPLC 2004. These authors extended their earlier work in this paper. Using sol-gel chemistry, the workers successfully prepared monolithic columns in situ with an oriented pillar structure in a variety of 2-D confined spaces. Due to wetting of the siloxane phase onto the interface of the mold and the effect of gravity, in this confined space, the gel skeletons in the vicinity of the interface are elongated perpendicular to the interface. Using scanning electron microscopy and laser scanning confocal microscopy, geometrical information on gel morphologies was derived. The long range implications are that these tech-

nologies should allow more efficient separations in chip-based separation systems.

Photographs of all three winners receiving their prizes are shown in Figure 1.

Opening Session

Topics of opening sessions usually are chosen to reflect current research areas in which separation science has played a key role. In this year's opening session on Sunday afternoon, Peter Schoenmakers of the University of Amsterdam, The Netherlands, a member of the Permanent Scientific Committee, discussed the state-of-the-art in LC-LC, comprehensive two-dimensional separations. However, as next month's column will be devoted to a review of this technology, I will review his plenary lecture at that time.

Two other plenary lectures were by notable life scientists Ulf Landegren of the University of Uppsala and John Yates of the Scripps Research Institute, La Jolla, California. Landegren's lecture discussed the analysis of proteins at the single-molecule level using molecular tools that represent detected DNA, RNA, or protein molecules as short, circular rings of DNA. His approach allows highly specific analysis of large sets of molecules in solution or for in situ analysis. Using a technique called proximity ligation, antibodies or other suitable reagents that bind a target protein are equipped with these DNA strands that can be joined by ligation when pairs of reagents bind the same target protein molecule. The process effectively reverse translates target proteins into linear or circular signature DNA molecules that can be amplified and identified for highly sensitive, precise protein detection in homogenous or solid-phase assays. In the future, the process might be used for single-molecule detection inside of a cell. Interestingly, Landegren's techniques can be applied to any molecule that binds — not just proteins. Yates reviewed some of his early shotgun proteomics work in which a protein mix undergoes proteolysis to form complex peptide mixtures that are then analyzed by a combination of micro LC combined with tandem MS. The resulting massive amounts of data are then interrogated to hopefully come up with some useful information about the original proteins. The data analysis still represents the biggest challenge. The original multidimensional protein identification technology (MUD-PIT) approach was described, in which

Table I: HPLC 2005 papers presented by technology or technique

Technology/ Technique	Papers Presented (%)
HPLC column technology, phases and design (Monoliths: 40%) (Sub-2 μm : 10%)	23.8
CE and related (that is, MEKC, CZE, IEF)	15.5
Sample Preparation	14.1
Theory/retention mechanisms/ chemometrics	9.0
Multidimensional/column switching	6.0
Instrumentation/software	4.2
High-throughput/fast LC	3.4
Microfluidics, Lab-on-a-Chip	3.4
Preparative LC	3.1
Method development/method transfer	3.1
High temperature	3.1
CEC	3.1
Regulatory/Validation	2.1
UHPLC/UPLC	2.1
SFC	2.0
Other (FFF, GE, PAT, and so forth)	2.0

cation-exchange and reversed-phase columns are connected in series to a 5- μm picospray nozzle into the MS ionization source. He further elaborated on work involving more targeted identification of specific proteins such as phosphorylated proteins. The identification of low stoichiometric amounts of phosphorylated proteins represents a challenging problem, so an additional reversed-phase enrichment column added downstream permits focusing of the analytes. Aside from the common use of trypsin, the use of multienzyme digestion using more specific enzymes also allows the determination of multiphosphorylation sites. The development of new MS detection tools with high mass accuracy, high sensitivity, and higher resolution will further aid the entire field of proteomics.

New Column Technology Highlights

As seen in Table I and similar to the past five years of HPLC Symposia coverage (1–5), the development and study of columns and stationary phases still dominates the new technologies. If one combines all papers pertaining to column technology, including monoliths, about a quarter of the presentations at HPLC 2005 covered this topic. Despite all the advances made in column technology to date, investigations on further developments in academia as well as the commercial side still are taking place.

Monolith column technology: At HPLC 2005, monolithic phases dominated column technology and applications papers with 40% of the presentations devoted to this technology. Overall, more emphasis was devoted to the preparation of monoliths in capillaries and in microchannel chip-based systems than to columns of conventional dimensions.

Monolith columns exhibit high permeability (due to bed porosity), absence of frits to confine the packing material, good separation efficiency, and ease of fabrication. Although this technology has been around for several years, as a routine tool it has yet to see widespread acceptance, but things are changing. Frantisek Svec of the University of California, Berkeley, California, gave a keynote lecture that presented an excellent overview of the development, state-of-the-art, and future directions of both polymeric and silica monolith technology. He discussed some of the newer or emerging synthesis techniques such as atom transfer radical polymerization (ATRP) and ring opening metathesis polymerization (ROMP). Ionic polymerization and polycondensation are other techniques that might also prove useful in the production of new types of monoliths. New inorganic materials such as hafnia and zirconia have been investigated to provide monoliths that can stand up in an extreme pH environment.

Monoliths are starting to see a variety of applications. Monoliths in the capillary format with advanced detection techniques such as matrix-assisted laser desorption ionization (MALDI)-time-of-flight (TOF)-MS and nuclear magnetic resonance (NMR) have been applied to solving problems in proteomics, metabolomics, and other biorelated disciplines. Monoliths have been used as solid-phase extraction (SPE) and immunoaffinity phases, as sensors, and for ion-selective electrodes. Monolithic columns as the second dimension provide an answer to the speed mismatch problems with 2-D systems. Monoliths also provide a solution to some of the problems encountered in capillary electrochromatography (CEC) such as the lack of need for frit formation. Bilayer chemistry can provide protection for basic peptides and proteins by preventing them from touching the underlying ionized surface.

In the future, Svec sees monoliths employed for even smaller devices such as micro- and nanofluidic chip-based systems.

Table II: Presented by application area

Application Area	Papers Presented (%)
Proteomics/proteins/peptides	18
Biological fluids	16.1
Drugs: 63%	
Endogeneous compounds: 37%	
Environmental/industrial hygiene/toxins	11.6
Pharmaceuticals/drug discovery	11.4
Food/food safety*	10.5
Natural products/neutraceuticals/ TCM†	8.9
Chiral	8.6
Carbohydrates/sugars/ oligosaccharides	2.5
DNA/oligonucleotides	1.6
Tissues/cells	1.6
Other‡	9.3

* Pesticides/fungicides/preservatives/chemicals.

† Traditional Chinese medicines.

‡ Includes forensic/drugs of abuse; polymers/organic compounds; inorganic compounds; clinical; cosmetics; amino acids; hydrocarbons/fuel

They can be fabricated easily inside narrow channels, and such approaches already have been shown to provide useful applications. Monoliths might prove useful as a support to immobilize enzymes such as trypsin that should allow rapid proteolysis and on-line systems for proteomics research. Monoliths also can be prepared in the orifice of MS electrospray probes and functionalized using photografting procedures. Such procedures enable miniscule amounts of orthogonal stationary phases to be placed inside of nanochannels, allowing for separation and concentration to occur in a single operation.

In a tutorial, Christian Huber of the University of Saarland, Saarbrücken, Germany gave a more basic overview that was useful for nonspecialists. His tutorial was divided into three sections. The first section contained the facts about monoliths. He covered the preparation and functionalization of silica and polymeric monoliths and showed examples of high-resolution separations on polystyrene-divinylbenzene (PS-DVB) monoliths and showed via scanning electron micrographs how the morphology of the monolith surface can have great impact on chromatographic performance. He then covered the challenges and dispelled the myth that monoliths cannot be manufactured reproducibly by showing comparative data on silica and PS-DVB monoliths and microparticulate columns. The challenges remaining for monoliths are improving their peak symmetries, retention

Table III: Types of detection techniques used at HPLC 2005

Detection Technique Used	Use (%)
LC-MS, incl. LC-MALDI-TOF, ESI-TOF	34.9
LC-MS-MS	22.8
UV/diode array detector (DAD)	20.6
Fluorescence/laser induced fluorescence	8.1
Evaporative light scattering and related	5.9
Electrochemistry	1.8
NMR	3.2
Others*	4.4

*Includes light scattering, refractive index, FT-IR, biosensors, chemiluminescence.

factors, and loadabilities through higher surface coverage with C18 bonding. Finally, he demonstrated the achievements so far with monolith technology and showed a variety of applications ranging from preservatives to phosphorylated and glycosylated peptides to ion chromatography to nucleic acid analysis to preparative separations on monolithic tubes to MS interfacing to multidimensional separations of peptides in proteomics.

Because they possess high permeability, monolith columns can be run at relatively high flow rates and still show good chromatographic efficiency. Pavel Nesterenko and co-workers from Moscow State University, Moscow, Russia, and the Dublin City University, Dublin, Ireland used flow programming to elute polyaromatic hydrocarbons (PAHs) and phthalates from reversed-phase monolith columns without the use of gradient elution. In addition, monolith columns under extremely high flow rate conditions give a solute zone profile that resembles that observed in turbulent flow chromatography. These workers were able to demonstrate that H - v curves at these high flow rates could not be described by the conventional Van Deemter equation.

New monolith stationary phases were the subject of several papers at HPLC 2005. Cabral and co-workers from Concordia University, Montreal, Quebec, Canada developed a tunable stationary phase for CEC based upon a photopolymerized methacrylate-based stationary phase with grafted chemical moieties. This phase with various alkyl side chains permitted the separation of biomolecules. Using similar strategies, the authors were able to develop reactive monoliths that permitted the immobilization of trypsin and other enzymes by chemical reaction. These

immobilized enzyme phases could be used for on-line protein digestion.

Brett Paull and co-workers from Dublin City University, Ireland, and Lomonosov Moscow State University, Moscow, Russia presented work on the application of new zwitterionic silica-based monolithic columns for the rapid separation of inorganic and organic anions. The new short columns (2.5 cm length) were based upon an immobilized phase of a long chain carboxybetaine type surfactant, which exhibited both unusual selectivity toward inorganic anions compared with standard anion exchangers, and a strong pH dependent capacity effect. The new column was shown to be highly stable, with 400 repeated injections of a mixture of inorganic anions showing < 2% variation in retention times, and could be applied directly to rapid isocratic anion separations (< 2 min) without the use of excessively high flow rates. However, the real novelty of this work was the application of a so-called "double gradient" approach to the separation of diverse mixtures of anionic species. The combination of an eluent pH gradient with an eluent flow gradient, meant analysis times with the column for complex mixtures of common inorganic, organic and polarizable anions could be reduced from > 50 min to under 4 min, while still maintaining both efficiency and resolution. Current work is underway to exploit the new double-gradient approach for the rapid analysis of even more challenging mixtures of anions.

Violaine Augustin and co-workers from the ESPCI in Paris, France presented an interesting application of monoliths. They studied the in situ synthesis of polymethacrylate monoliths in capillaries for use in CEC. Efficiency and monolith uniformity were influenced strongly by the various pretreatment procedures. The energy of photopolymerization was important in the formation of a successful monolithic phase.

Flux that was too strong caused polymer degradation and a decrease in performance. Once they had developed a successful in situ procedure, they transposed the methodology to a microdevice format, resulting in a monolithic bed showing 200,000 plates/m efficiency. In addition, they were able to use the monolith to perform a preconcentration step that allowed a sensitivity increase of 140 for a series of PAHs.

Other New Stationary Phases

Joseph Pesek and coworker Maria Matyska (California State University, San Jose, California) described a stationary phase in which hydride groups (Si-H) have replaced most of the silanols (Si-OH). One interesting property of the hydride material is that, in aqueous solution, the retention of acidic and basic compounds increases as the amount of organic solvent increases similar to hydrophilic interaction chromatography (HILIC). Termed aqueous normal phase chromatography, at neutral to slightly basic pH values, acids display aqueous normal phase behavior but at low pH, only reversed-phase LC behavior was noted. For basic compounds, they appear to have both aqueous normal phase and reversed-phase LC behavior depending upon the composition of the mobile phase. As the percentage of acetonitrile increased, Pesek noted that retention goes through a minimum so there is a dual mechanism occurring. Elution order reversals can occur that can be used to the chromatographer's advantage.

To enhance the high pH stability of silica-based phases, César Silva, Carol Collins, and co-workers from the State University of Campinas, Sao Paulo, Brazil have explored the modification of silica supports using titanium alkoxide, followed by silanization with C₁₈ and endcapping. During an accelerated aging test using aqueous phosphate at pH 10 and 50 °C, column failure was observed at 3700 column volumes, while for a similarly prepared C18 phase without titanization, column failure was observed at 2800 column volumes. In spite of better stability, the presence of titanium oxide increases surface acidity and the separation of bases requires the use of buffered mobile phases. To minimize this effect, a C₁₈ urea alkoxysilane was used for silanization of titanized silica. Better peak shapes for bases were observed in nonbuffered mobile phases. However, the stability of the C18 urea titanized phase was somewhat lower than for the C18 titanized phase without embedded urea. The influence of the titanium oxide concentration also was investigated by performing two successive titanization reactions. The double titanized surface did not change the overall performance nor significantly improve stability at high pH, possibly due to the continued presence of residual silanols, which are the points responsible for silica dissolution in high pH. To obtain more stable phases, alternative procedures still must be investi-

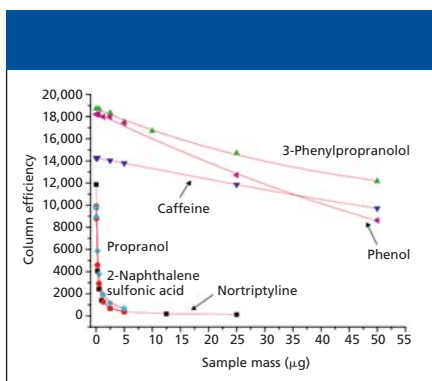


Figure 2: Comparison of overload behavior of three neutral compounds (caffeine, phenol, 3-phenylpropranolol) and three ionized compounds on Xterra MS (Waters, Milford, Massachusetts). Mobile phase ACN / 0.02M formic acid pH 2.7 (6).

gated. In poster presentations, a simple, clean (less solvent), and efficient procedure to prepare reversed phases with different functionalities (C1, C8, C14, and phenyl) was explored using deposition and immobilization of polyalkylmethylsiloxanes onto silica, as an alternative to the conventional silanization reaction that requires the use of reactive chloro- or alkoxy silanes under reflux in organic solvents.

Kenneth Markus and co-workers from Clemson University, Clemson, South Carolina presented a talk on capillary-channeled polymer fibers as a packing material for HPLC. These fibers display high wicking action and a higher surface area than traditional fibers. They are about 25–60 μm in diameter with eight capillary channels running on their periphery. In this study, polypropylene, polyester, and nylon fibers were studied. In their approach, a fiber bundle is pulled through a conventional column (about 20,000 fibers in a 4.6-mm column). These packed columns show 70% less pressure than commercial packed columns. They appear to work best with large molecules like proteins and can be operated at a higher flow rate than typical protein columns. The use of a single fiber for the separation of dyes was demonstrated with the wicking action being the only driving force. Sample preparation and process scale separations could be other potential areas of applications. Column efficiencies have a way to go before these columns are comparable to typical packed reversed-phase columns.

Theory and Retention Mechanisms

Two oral sessions and one poster session were devoted to theoretical presentations. Many of the theoretical presentations had

practical consequences. The first day, Georges Guiochon of the University of Tennessee, Knoxville, Tennessee presented a keynote titled “Retention Mechanisms in RPLC Are Still More Complex Than We Thought,” in which he discussed the behavior of the retention of polar basic and acidic analytes on reversed-phase packings in methanol and acetonitrile. For a typical C18 reversed-phase packing, there are two types of sites: high energy and low energy. The high-energy sites, yet unidentified, mainly control retention since they are where polar analyte molecules go first. Unfortunately, their density is low, hence the saturation capacity is small compared to the surface area of the packing. In the 0–60% methanol range, the energy and density of the high-energy sites are sensitive to the organic solvent content, while the properties of the low-energy sites are not. The organic modifier influences these sites, forming an adsorbed layer of solvent at the interface between the packing surface and the bulk mobile phase. Methanol, which mixes fully with water, gives a monolayer, swelling the bonded alkyl chains. Acetonitrile is merely a hydrogen bond acceptor, not both a donor and an acceptor as methanol. Its molecules tend to associate in aqueous solution, forming a thick ($\sim 14 \text{ \AA}$) adsorbed layer separating the bonded alkyl-chain layer from the acetonitrile–water mobile phase. The presence of buffer also influences adsorption isotherms. Because most reversed-phase LC packings are heterogeneous with both high- and low-energy sites, retention mechanisms usually are mixed, which makes it difficult to compare different packings.

David McCalley of the University of the West of England, Bristol, U.K., followed up with a talk on overloading of basic compounds in reversed-phase LC as a function of pH in the range of 2.7–10. He studied not only silica-based packings but also inorganic–organic hybrids and pure polymeric packings. At low pH for 4.6-mm i.d. columns using phosphate buffers, he observed overloading problems for ionized solutes, even at the 0.1- μg level. Overloading was especially severe for weak acid buffers such as formic acid, a volatile buffer system popular in LC–MS. Figure 2 provides some overloading data for a series of neutral and ionizable compounds on a hybrid reversed-phase column. As the pH of formic acid was raised from 2.7 to 3.7, overloading was reduced, possibly due to

higher ionic strength at the higher pH, or to increased ion pair effects. Using phosphate buffers on pH stable columns, high pH (up to 10) decreases solute ionization and thus reduces overloading, in the absence of interaction with ionized silanols. Many of the results were in agreement with theories from the earlier presentation of Guiochon.

Torgny Fornstedt and co-workers of Uppsala University, Sweden, presented a lecture entitled “Why Are the Injected Solutes Not Always Found in the Peaks?” An equilibrated system consisting of an eluent and a solute establishes a concentration plateau. If a small excess of solute is injected into this system, a single peak will appear in the chromatogram. This peak is known as a system peak or perturbation peak and consists of displaced plateau molecules whereas the injected molecule is coeluted later with a deficiency of plateau molecules and is therefore not normally detected. This present study investigated the multicomponent case of this phenomenon. For this study, both the theory and methodology were extended for the *n*-component case. The researchers cleverly designed a new experimental approach using enantiomers of the two solutes to be studied and a binary reversed-phase LC system. Fractions were collected from the eluate from the nonchiral system and then reinjected into a chiral column. In this way, the molar ratios of the nonchiral eluate could be measured and retention times for the two tracer peaks be determined. It was shown that the retention times of the binary tracer peaks allowed the straightforward calculation of the real isotherm data points without any isotherm model assumptions. The 2-D isotherm surfaces could easily be plotted directly from the retention times. The results of this study should be important for large scale process design as well as for characterizations of new LC columns. The difficulty in this experiment is to selectively detect only the injected molecules among a population of identical ones already present in the system. The next steps will involve the use of different solute isotopes and MS detection to perform these more difficult experiments.

Attila Felinger, now of the University of Veszprém, Veszprém, Hungary, presented a lecture on combining linear and nonlinear chromatography to investigate the adsorption behavior in HPLC. The goal of his presentation was to show (through several

examples) that not only in nonlinear but also in linear chromatography, heterogeneity of the adsorption–desorption kinetics could be characterized. Although the information furnished is more limited than the one obtained with the measurement of nonlinear equilibrium isotherms, peak shape analysis can reveal several details of the chromatographic process without the need for lengthy isotherm determinations. With the mostly ignored stochastic model of chromatography, he determined the number of adsorption–desorption events and the kinetic rate constants through peak-shape analysis. With this approach, the presence of adsorption sites on which desorption is slow was recognized. Among other things, he studied the retention behavior of phenol in reversed-phase LC and found, in agreement with Guiochon, that phenol is retained on two different types of sites with interaction energies that differ roughly by 10 kJ/mol. Furthermore, he characterized how the number of adsorption events and the kinetic rate constants depend upon temperature, mobile phase composition, and solute concentration. With a simple peak-shape analysis, he

determined the contribution of the individual sites to the retention of the analyte.

Piotr Gzil and co-workers from the Vrije Universiteit Brussel, Brussels, Belgium challenged the audience to think outside the box and represented plate height and flow resistance data in a kinetic plot instead of in the classical Van Deemter plot format. Circumventing the need for a characteristic mass transfer length that would be common to all possible support shapes, the kinetic plot method provides a universal, morphology-independent basis to compare the separation efficiency and speed of different support types directly. To further promote the kinetic plot method, it was shown that it requires no rigorous mathematics, only the multiplication of a few data columns in a spread-sheet program like MS Excel. The audience was referred to a freely downloadable MS Excel template file (<http://www.ir.vub.ac.be/chis/download/>) providing an automatic readout of all possible curve characteristics and kinetic performance data. Applying the method to an extensive set of silica monolith and packed-bed literature data showed at a glance that the open-porous structure of

the monolithic type of supports is more advantageous for separations requiring large plate numbers, while the more compact structure of a packed bed column is better suited for easy, high-speed separations. The obtained plot also revealed the existence of a currently “forbidden region,” which can only be invaded by preparing homogeneous open-porous monoliths with smaller domain sizes than currently achievable or by working under ultrahigh pressure LC conditions in densely packed systems, provided the frictional heating problem can be controlled.

M.C. Garcia-Alvarez-Coque with coworker J.R. Torres-Lapasio of the University of Valencia, Valencia, Spain presented a tutorial lecture on the search for the best conditions in chromatography: A Magical Mystery Tour. Some new tools for interpretive optimization supported by different models were presented. The use of peak purity as a resolution measurement that expresses the area fraction free of interferences was outlined. The most important peak purity concept was that of the limiting purity, which represents the maximum separation capability for a given compound in

the chosen sample and chromatographic system. Limiting purities were shown to allow establishing the resolution threshold of the chromatographic system, or the maximal practical complexity in a gradient program.

Optimization levels were classified as total, partial, and specific strategies. Total strategies try to resolve all compounds in a sample within a single run. Partial strategies resolve only certain target compounds. All compounds in the mixture can be resolved using more than one run with wisely chosen chromatographic conditions in the so-called complementary optimizations. Conventional criteria have the pitfall of offering noninformative resolution maps in low-resolution situations. A straightforward criterion accounting for the success in the separation, instead of the fail level (that is, the limiting peak count) was shown to discriminate the resolution power in these situations. Difficult coelutions cannot be resolved only chromatographically and require deconvolution. For these situations, a resolution measurement oriented to deconvolution, namely, the multivariate selectivity, was presented.

Applications Highlights

As can be seen in Table II, protein separations and proteomics again dominated liquid-phase applications. The analysis of drugs, drug metabolites, and endogenous compounds in biological samples had a strong showing in the applications arena. This is an area in which sample preparation using protein precipitation and SPE in the 96-well format seems to be catching on.

In proteomics, 2-D gel electrophoresis is still in widespread use but 1-D and 2-D LC coupled to MS is growing rapidly for the investigation of these complex mixtures of proteins and peptides. Hence, a large number of contributions at HPLC 2005 were devoted to such studies (see Detection section). This year saw the emergence of a number of investigations for biomarkers for various disease states. With techniques now available for the removal of high-abundance proteins, trace levels are more readily detectable.

Biomarkers are often small polar molecules present in the mammalian body at micromolar-to-millimolar levels. Sara Bergstrom and co-workers from Uppsala University, Uppsala, Sweden, and Karolin-

ska Institute, Stockholm, Sweden investigated the use of microdialysis for fast monitoring of biomarkers in tissue samples. Small biomarkers like glucose, glycerol, and pyruvate readily pass through a membrane much faster than larger biomolecules. In their experiments, microdialysis probes were implanted into rat livers and chemical stimulations were performed by the addition of potassium chloride or paracetamol to the perfusion solution. Samples were taken before and after stimulation. After microdialysis, C18 columns are used for preconcentration and desalting. Finally, the samples were directed online to a mass spectrometer for detection and identification. After analysis, the average mass spectra of the elution plug were compared by principle component analysis to look for sample trends. Using this approach, they were able to investigate potential biomarkers in the 200–1100 Da molecular weight range.

Monoclonal antibodies (MAbs) have been studied widely as potential diagnostic tools or for clinical application. MAbs are complex glycoproteins that inherently contain multiple forms of heterogeneity resulting from production and degradation pathways. Analytical characterization of MAbs has been hindered by the lack of chromatographic methods to be used in conjunction with high-resolution MS. Thomas Dillion and co-workers from Amgen, Thousand Oaks, California reported on a rapid reversed-phase HPLC method that can be connected in tandem to a high-resolution mass spectrometer for the analysis of intact and reduced MAbs.

The reversed-phase HPLC method parameters that were found to be critical for good recovery of antibodies and efficient separation included higher column temperatures (70–80 °C), stronger organic solvents with higher eluotropic values (isopropanol and *n*-propanol), and stable Zorbax Stable Bond (SB) (Agilent Technologies, Wilmington, Delaware) functionality. The deconvoluted mass spectra of intact antibodies detected glycosylation profiles, which typically included three to five peaks (grouped around a molecular weight approximately equal to 150 kDa) that were equally spaced by the mass of a galactose residue (162 Da). Additional carbohydrate heterogeneity such as the loss of core fucose, high mannose forms, and aglycosylated heavy chains were detected at lower levels. Other modifications that were moni-

Table IV: Poster committee

Name	Affiliation
Dr. Anant Vailaya	Merck, Rahway, NJ
Dr. David Lloyd	Bristol Myers Squibb, New Brunswick, NJ
Dr. Denise Wallworth	Astec Ltd., Manchester, U.K.
Dr. Dieter Lubda	Merck, Darmstadt, Germany
Dr. Gerard Rozing	Agilent Technologies, Waldbronn, Germany
Dr. Imre Molnar	Molnar Institute, Berlin, Germany
Dr. Jennifer Van Anda	Berger Instruments, Mettler-Toledo, Newark, DE
Dr. Michel Girard	Health Canada, Ottawa, Ontario, Canada
Dr. Robert Stevenson	Abacus Group and American Laboratory, Lafayette, CA
Dr. Ronald E. Majors (Chair)	Agilent Technologies, Wilmington, DE
Dr. Roy Eksteen	Tosoh Biosep, Montgomeryville, PA
Dr. Steve Cohen	Waters, Milford, MA
Dr. Uwe Neue	Waters, Milford, MA
Dr. William Barber	Agilent Technologies, Wilmington, DE
Prof. Attila Felinger	Univ. of Veszprém, Hungary
Prof. Christian Huber	Univ. Saarland, Germany
Prof. Coral Barbas	San Pablo-CEU University, Spain
Prof. David McCalley	Univ. of Western England, Bristol, UK
Prof. Frank Steiner	Univ. Saarland, Germany
Prof. Frank Svec	Univ. of California, Berkeley, CA
Prof. Gert Desmet	Vrije Universiteit Brussel, Brussels, Belgium
Prof. Joseph Pesek	California State University, San Jose, CA
Prof. Jun Haginaka	Mukogawa Womens University, Japan
Prof. Maria Matyska	California State University, San Jose, CA
Prof. Peter Schoenmakers	Univ. of Amsterdam, Amsterdam, The Netherlands
Prof. Reg Cross	Swinburne University of Technology, Australia
Prof. Robert Kennedy	University of Michigan, Ann Arbor, MI
Prof. Ulrich Tallarek	Otto-von-Guericke-Universität Magdeburg, Germany
Prof. Vicki McGuffin	Michigan State University, East Lansing, MI
Prof. Carol Collins	State University of Campinas, Brazil
Dr. Cesar Silvas	State University of Campinas, Brazil
Dr. David Brennen	E.I. DuPont, Wilmington, DE
Dr. John Frenz	Genetech, So. San Francisco, CA

tored and characterized by reversed-phase LC–MS include: glycation, cleavage at the hinge region, N-terminal pyroglutamate formation, and C-terminal lysine variants. The development of a rugged reversed-phase HPLC method with enhanced MS sensitivity represents a major advancement for in-depth analytical characterization of antibody heterogeneity and stability.

High-Throughput Analytical and Preparative LC

Again this year, high throughput was a major theme permeating many presentations. Both lecture sessions and a poster session were devoted to ways to improve throughput. The application of high-temperature HPLC (up to 100 °C) combined with multiplexed electrospray MS and UV detection was the subject of a lecture by Nebojsa Djordjevic and co-workers of Cytokinetics, So., San Francisco, California. Using parallel LC–MS analysis, four multiplexed eluent streams can be fed into a single mass spectrometer. The use of high-temperature further shortens analysis time, narrows peaks, and decreases detection limits. Column-to-column temperature repro-

ducibility was good because relative standard deviations were less than 1%. Using this approach, up to 300 samples/h could be analyzed.

Chemists from Arqule, Woburn, Massachusetts have developed a high-throughput LC–MS purification system for drug discovery. Even though there is a trend for smaller compound libraries with focus on higher value compounds, high-throughput screening still requires high-purity compounds. Arqule's Djamel Cherrak presented a paper in the high-throughput session that combined a preparative LC with split-flow (1:1000) MS monitoring and a 24-well sample collection device that provides evaporation and weighing of collected fractions. Because turnaround time is of the essence, faster columns also are needed. They investigated monoliths as well as packed columns for injections up to 2.5-mg of compound. Under similar conditions, monoliths displayed about 20% lower pressure than packed particles but also showed a lower sample capacity. They showed an interesting lifetime plot of the number of injections obtained for a series of 150 packed columns. Average number of injec-

tions ranged from a low of 400 up to 1600 per column.

Frank Steiner and coworkers from Saarland University and Dionex, Germering, Germany have developed an MS-triggered semipreparative HPLC system for automated purification and fractionation of complex mixtures. Unlike most systems reported in literature, their system was not based upon a passive splitter. The tubing and the size of a make-up flow to enhance MS detection were optimized by theoretical modeling and experimental results. It turned out that the crucial system delay time (time between band arrival at MS and at the fraction collector) was independent of the make-up flow rate over a wide range. However, the delay time was sensitive to variations in the prep column flow rate. This delay time was of the utmost concern when using a high-permeability silica monolith column for speeding up the preparative fractionation. In such a case, the tubing design was adopted to generate the required delay time.

The optimized system was applied to target-oriented fractionation of complex natural products. Unlike a "one sample, one

fraction" strategy as primarily pursued with MS-triggered automated purification in combinatorial synthesis, this mode of application was not yet found in literature. They reported the preparative fractionation of St. John's Wort extracts obtained from accelerated solvent extraction (ASE) using the automated purification system. The obvious advantage of a MS trigger over a nonselective UV trigger, namely, to reduce the number of fractions to those that are desired, is of crucial importance when the system is applied to samples of high complexity.

Karin Cabrera of Merck, Darmstadt, Germany, and Japanese co-workers from Kyoto University and Kyoto Monolith further elaborated on their commercial Chromolith monolithic columns and contrary to the Arquele presentation, her feeling was that large library screening is still required, so her talk was poised around screening with 200,000 compound libraries. By using monolith columns with multiplexed LC-MS systems, they could achieve around 1560 samples/day throughput. She also reported in their experience, the reproducibility of monolith columns was better than particle-packed columns and that they were twice as stable.

Zeki Altun and co-workers from Astra Zeneca, Sodertalje, Sweden, and Karlstad University, Kalsdad, Sweden improved sample preparation throughput by using monolith polymer plugs packed in 96-well tips rather than SPE packed beds. The increased flow rates through the monolith allowed them to run a single 96-well plate of samples in about 2 min. They were able to validate a method for a model drug compound in plasma much faster than previously.

Microfluidics and HPLC-on-a-Chip

One of the more interesting areas of separation science is the movement towards miniaturization driven by the need for the analysis of smaller, complex samples, cost reduction, decrease in use of solvents and chemical waste, smaller instrument footprints, more compatible interfacing to MS, simple automation, and increased sensitivity.

Work towards improving performance of microfluidic devices was the subject of several presentations. In the Closing Plenary Session, Mike Ramsey and co-workers from the University of North Carolina, Chapel Hill, North Carolina gave an update on micro- and nanofabricated devices for

chemical separations. He covered some of the fabrication approaches used to generate chip-based devices, compared microchip-based electrokinetic separation systems, and surveyed a few of the commercial products. He then proceeded to talk about work focused on hydraulic-driven chip-based separation systems rather than electrodriven systems. To date, most pressure-driven chip-based systems have provided poor performance compared to conventional HPLC. There have been several demonstrations of generating hydraulic pressure using electroosmotic forces in capillaries and a few on microfabricated fluidic devices. Electrokinetic-based pumps allow a relatively straightforward approach to integrating both pumps and LC columns on a single microchip and decoupling mobile-phase velocity from the electrically driven electrokinetic phenomenon. Ramsey's group has been investigating designs to accomplish this decoupling. By using appropriately designed microfluidic architectures (channel widths, depths, and lengths) with spatially selective control of surface chemistry, they have been able to generate pressure-generated flow, which is linear with applied electric field. The electrokinetic-based pumps have been able to generate sufficient pressure to perform chromatography in open-channel microchip devices. In addition, the group has been investigating the synthesis of silica-based monolithic stationary phases within the microchannel structures. Because organic monoliths show swelling within the channels, the silica-based monoliths should provide better performance.

Gerard Rozing of Agilent Technologies, Waldbronn, Germany has continued studies on zone dispersion in nanoflow HPLC columns, especially with respect to the shape of the container holding the packed bed. Microfluidic HPLC column designs have nonconventional shapes. Rather than a cylindrical shape, they have rectangular, square, or other shapes that are more easily fabricated by laser ablation or other microconstruction techniques. Because most of these columns are interfaced with MS, minimizing extra band dispersion at the interface is very important.

In their latest study, Rozing and co-workers found that chip-based nanoflow HPLC columns could be packed with a similar interstitial porosity as conventional cylindrical nanoflow HPLC columns. By careful design of the HPLC chips, overall

dead volume and band broadening by, for example, end filters, was reduced substantially. For example, the total in-chip dead volume was approximately 1 nL, while a typical nanoflow electrospray ionization (ESI)-spray tip has approximately 12.5 nL. For nanoflow HPLC columns, the dead volume in connections and spray needle amounted to 20% of the column volume, which is definitively too much.

Gert Desmet and coworkers from the Vrije Universiteit Brussel, Brussels, Belgium, and Twente University, Enschede, The Netherlands reported on the challenges and opportunities of the miniaturization of analytical separations using the shear-driven flow principle. This approach uses shear forces in 1-D nanochannels as an alternative universal flow driving principle to overcome the limitations of pressure-driven and electrically driven LC. The talk was conceived as a call to the microfluidics community to join them in their effort to go from the current stage of proof-of-principle stage to a practically useful chromatographic device. After having shown some ultrafast nanochannel separations of coumarin dye mixtures, rhodamine dye mixtures, and mixtures of fluorescently labeled polypeptides, all within the distance of a single millimeter and in less than 1 s, the talk addressed the many challenges that still must be overcome such as increasing the column length, increasing the mass loadability via the application of thicker stationary phase layers, improving the mechanical stability of the devices, and so on. The talk continued by showing some experimental results obtained with solutions that they are currently developing to address these shortcomings. For example, they demonstrated that a transversally oriented detection groove can be incorporated into the nanochannels to enhance the detection sensitivity with at least a factor of five without significantly disturbing the separation, and that a layer of porous silicon can be deposited to drastically increase the retention capacity of under reversed-phase LC conditions. Whereas all of their work up to now has been performed in linear channels, they also showed CCD camera video recordings of the flow of a tracer plug moving through a circular channel with submicrometer depth without any notable band broadening. This work might open the road to rotating channel variants with columns that are tens of centimeters long on a wafer that is only 2–4 in. in diameter.

Capillary Electrophoresis and Capillary Electrochromatography

The number of papers using capillary electrophoresis (CE) and related techniques was quite strong again this year (Table I). This technique, which has slowed commercially, has been used in solving many problems in the life sciences, environmental science, metal analysis, and many other complex problems. Ed Yeung from Iowa State University, Ames, Iowa provided a tutorial lecture in which he traced the history of CE, especially in the context of how the technology was used to sequence the human genome in record time. The various technologies in crosslinked and non-crosslinked polymers, the latest developments in CE in free solutions, and on focusing effects on increasing sensitivity were discussed. Finally, detector developments specific to CE along with some pertinent applications were shown to the audience.

Ulrich Tallarek of Otto-von-Guericke-Universität Magdeburg, Germany presented a theoretical paper on investigations of electrical field-assisted transport of charged species in hierarchically structured electrochromatographic media containing dis-

crete ion-permselective regions (like the mesoporous skeleton of a monolith or the mesoporous particles of a fixed bed), in addition to the quasielectroneutral macropore space. Concentration polarization induced by the applied electrical field is manifested by extended regions; that is, diffusion boundary layers of reduced and enriched electrolyte concentrations along the anodic and cathodic interfaces of a cation-selective porous particle or monolith skeleton, respectively. This phenomenon and its effect on retention, migration, and dispersion of co- and counterionic analytes depends upon applied field strength, surface charge density, mesopore size, and mobile phase ionic strength. A secondary, electrical field-induced electrical double layer and instability of the resulting induced-charge electroosmosis represent extreme scenarios of that phenomenon.

Relevant transport phenomena (electroosmosis, electrophoresis, concentration polarisation) were analyzed locally in monoliths and spherical packings with respect to macroscopic transport behavior of electroneutral and charged analytes. It appears that effects related to the actual

intensity of concentration polarisation in electrochromatographic media are the key to understanding retention, migration, and dispersion behavior, which has been (and is still) puzzling people working both theoretically and experimentally in that field.

CEC, still looking for that "killer application," commands a great deal of attention and several strong papers were presented at HPLC 2005.

Along the lines of using materials to fill the capillary, Peter Spiegel and co-workers from Lund University, Lund, Sweden discussed work on nanoparticle CEC. To prevent contamination or modification of the packing by sample matrix, their work showed that the stationary phase can be exchanged after every separation. They used a partial filling capillary technique using polymeric nanoparticles. A plug of nanoparticle slurry is injected into the capillary before the sample. As the voltage is applied, the sample starts to migrate through the nanoparticle plug and will eventually reach the UV detector before the nanoparticle plug and hence, the nanoparticles will not interfere with the detection. The particles are prepared so that they will interact with the analytes and have an electrophoretic mobility that is different from that of the analytes. There are several advantages of the technique, aside from that of having a new "stationary phase" in every separation. The selectivity of the column can be drastically changed without having to exchange the capillary. As the particles are not packed, very small particles can be used without backpressure problems. The small sizes of the particles promote fast mass transfer and thus excellent chromatography. The latest step in nanoparticle CEC has been the coupling of the technique to MS. Using a novel continuous full-filling CEC technique with an orthogonal ESI source, analytes coeluted with nanoparticles can be detected, which allows for the continuous introduction of nanoparticles into the separation capillary.

Two CEC applications papers were of interest. The first, by Debby Mangelings and co-workers from Vrije Universiteit, and Sanofi-Aventis, Toulouse, France, had a goal to define a generic separation strategy for 15 acidic and 48 basic bifunctional or neutral chiral molecules by CEC. Because of their broad enantioselectivity, four polysaccharide chiral stationary phases were chosen to develop the strategies: Chiralcel OD-RH and OJ-RH, Chiralpak AD-RH,

and AS-RH. Separate strategies were defined for acidic compounds on the one hand and for basic, bifunctional, and neutral substances on the other. Both strategies use a different pH due to the necessity of the compounds to be uncharged. They are constructed as decision trees: first a general screening experiment is executed on the different stationary phases and according to a specified column testing order. Depending upon the obtained separation, occasionally an optimization is performed. The conditions for the screening experiment were carefully worked out. The generic approach handles cases to optimize resolution or decrease time when a partial separation occurs, a full separation occurs, or no separation occurs under the screening conditions by adjusting method parameters such as applied voltage, organic solvent content, and temperature.

For their generic method, enantioselectivity was observed for 82.5% of the compounds studied and 49.2% could be baseline separated. The group plans to investigate the use of monolith phases, which might solve some of the fragility problems with present chiral columns.

In a second applications paper, Marja-Liisa Riekkola and coworkers from the University of Helsinki and Wihuri Research Institute, Helsinki, Finland presented a keynote lecture. It has been suggested that the oxidation of lipids and proteins in lipoproteins and cell membranes leads to impairment of lipid transport and to cell injury, and thereby contributes to the development of various diseases. In her lecture, Riekkola introduced the results where the fused-silica capillary and microchip walls have been coated with 30–80 nm very-low-density and 22 nm low-density lipoprotein particles isolated from human plasma. Riekkola showed that open tubular electrochromatography offers an excellent novel approach for oxidation and interaction studies under widely different conditions, shedding light also on the role of low-density proteins as a carrier of drugs in the mammalian body. Miniaturized asymmetrical field flow fractionation, scanning electron microscopy, and MS have been implemented in their studies as well.

Detection and Hyphenated Techniques

As can be seen in Table III, LC–MS and LC–MS–MS were the dominant detection methods used in the oral and poster presen-

tations at HPLC 2005. Almost 60% of all papers that provided information on their detection technique used some form of MS.

As might be expected, UV detection, especially diode-array spectrophotometers, was the second favored detection technique, albeit a distance behind MS.

Gerard Hopfgartner and co-workers from the University of Geneva, Switzerland and MDS Sciex, Concord, Ontario, Canada discussed the current status of micro- and nanoseparation sciences with MS detection. LC–MS has an intrinsic problem for qualitative analysis because most LC peaks are eluted too fast to perform all possible MS experiments in one single run. For quantitative analysis LC–MS multiplexing remains challenging and run cycle times of 1–5 min are still too long for very high throughput or on-line analytics. The decoupling of LC and MS offers new opportunities for the analysis of pharmaceutical compounds and metabolites. Two new concepts were presented either based upon fraction collection into a 96-well plate or onto a MALDI plate via a postcolumn split during LC–MS analyses.

For chip-based infusion, microbore columns were used, while for MALDI, spotting capillary or nanobore columns were preferred. In both modes, one of the key advantages is that the LC separation can be archived and the sample can be reanalyzed on various types of MS instrumentations at any time. The triple quadrupole linear ion trap and the quadrupole TOF instruments were found to be particularly attractive for these applications.

In some cases, chromatography can be omitted completely. Quantitative analysis of talinolol in human plasma was demonstrated without any chromatography using MALDI-selected reaction monitoring (MALDI-SRM). An assay was validated in the calibration range from 1 to 1000 ng/mL and the MS analysis time was of 3-s/sample allowing ultrafast throughput analysis. For quantitative analysis sensitivity of one picogram (on-column) for the analysis of talinolol using LC–MALDI/SRM was demonstrated using a 1-min high-pressure gradient separation. Furthermore, integrating LC into the sample preparation should allow easier multiplexing of assays and a more efficient use of MS equipment.

Barry Karger of the Barnett Institute, Northeastern University, Boston, Massachusetts reported on his continuing work on coupling capillary LC to MALDI-MS. The latest work involves fast LC-MALDI, in which complex peptide mixtures can be run with a 10-min gradient. Now multiplex operation is possible in which parallel processing allows multiple samples to be run at the same time. Because parallel processing results in many thousands of spots, increasing throughput is a must to perform this task in a reasonable time. To meet these higher throughput demands, their research group has developed a new instrument with a 10-fold higher repetition rate laser (2 kHz) than commercially available units. With this system, a spectrum can be run in less than a sec with a spectral resolution of 10,000–12,000 and total data acquisition time of a typical MALDI target of around 8 min. Obviously, with such a fast system, a precise scanning pattern must be achieved. Their system has a mechanical and optical tracking. They are now applying this system for the rapid quantitative analysis of selected serum glycoproteins using isotopically labeled peptides. The group is also investigating new MALDI targets, which employ a nanowire surface now known as NALDI, where “N” stands for nanowire. Using this new matrix, sensitivity of 200 fg have been achieved for small molecules.

Although still in its infancy, the coupling of HPLC with nuclear magnetic resonance (NMR) spectroscopy offers additional benefits and supplements other detection techniques. Fiona Fitzpatrick and co-workers from AstraZeneca, Molndal, Sweden and Lund University, Sweden interfaced LC with both NMR and MS to characterize modified celluloses. The compounds often are used as excipient products in the pharmaceutical industry. The number and type of substituent groups on the cellulose chain control their characteristics. By a size-based separation of an enzymatically hydrolyzed sample, a clear relationship between size fraction and level of substitution as determined by NMR.

Evaporative light scattering detection (ELSD) is gaining in popularity. This detector is universal, can be used with gradients, and might be more sensitive than a refractive index for a variety of applications. Chemiluminescence nitrogen detection (CLND) is a specific and sensitive detection method that has found some applica-

tion for environmental and pharmaceutical samples. In their work aimed at establishing a reliable single-calibrant quantification protocol for high-throughput analysis of samples often existing only as a few tens of micrograms in the presence of nonchromophoric contaminants (solvents, water, and so forth), Ian Mutton and the group from GlaxoSmithKline, Stevenage, U.K. rigorously compared gradient HPLC using both ELSD and CLND with a “Gold Standard” technique (ERETIC proton NMR). 5-Fluorocytosine was used as HPLC calibrant and protocols allowed fully for the effects of gradient upon response. A double-blind study with 20 widely different compounds showed both NMR and HPLC-CLND enabled accurate quantification of 1–20 mM solutions in DMSO. HPLC-ELSD, however, consistently underestimated target concentrations. This behavior also was observed when using the three techniques to quantify 117 new chemical entities in the microtiter plate format.

CLND and NMR gave good quantification because they are essentially “atom counters.” Single-calibrant ELSD methods were shown by theory and flow injection analysis to be vulnerable to the algebraic consequences of the response regime and the inherent diversity of ELSD response coefficients, even for closely related materials. Although ELSD is used widely in the industry to provide semiquantitative results when no suitable reference materials exist, the group now prefers to use CLND when greater accuracy is required.

The charged aerosol detector based upon corona discharge might become a supplemental universal detector to ELSD. Asa Darwin of ESA, Chelmsford, Massachusetts presented a lecture on this new detector. From the data presented, the charged aerosol detector appears to be an order of magnitude more sensitive than the ELSD. He showed carbohydrate (for example, fructose, glucose, sucrose, and lactose) on-column detection to be about 10 ng and steroids at the 5-ng level. Other compounds studied were free amino acids, phospholipids, PEG 400, surfactants, and antibiotics. The charged aerosol detector’s response is based upon a quadratic response, while ELSD has a polynomial response that varies with the amount of compound. The dynamic range was reported to be four orders of magnitude.

Although biosensors are not necessarily

chromatographic detectors, their use in biomarker detection could prove useful for early screening for diseases such as cancer. Ed Nice and co-workers from the Ludwig Institute for Cancer Research, The Royal Melbourne Hospital, and the University of Melbourne, all located in Melbourne, Victoria, Australia presented their work on a new automated biosensor based upon amplified luminescence for the sensitive and specific detection of telomerase an enzyme that is undetectable in normal cells but is expressed in greater than 85% of tumors. The successful use of the technology to detect low levels of exfoliated cells in the urine of bladder cancer patients was presented. An additional study in the use of biosensor surfaces in instrumental biosensors as microaffinity platforms to purify low levels of protein complexes from colon cancer extracts was also presented.

HPLC 2006 Is Next

The next major symposium in this series, the 30th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2006), moves back to the United States and will be held in San Francisco, California from June 17–23, 2006. Based upon the highly successful HPLC 1986 and HPLC 1996, this event promises to be the best of the new millennium. The chairman of this upcoming event will be Dr. John Frenz of Genentech, San Francisco, California. For more information, consult the official website at <http://www.HPLCSymposium.org>.

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Ronald E. Majors

“Column Watch” Editor
 Ronald E. Majors is business development manager, Consumables and Accessories Business Unit, Agilent Technologies, Wilmington, Delaware, and is a member of LCGC’s editorial advisory board. Direct correspondence about this column to “Column Watch,” LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lccgcredit@lccgmag.com.

