SYMPOSIUM N
Materials for Separations in Analytical Chemistry
April 24, 2000

Chairs

Deon Anex
Sandia National Labs
MS 9671
Livermore, CA 94551-0969
925-294-3644

Frantisek Svec
Chemistry Dept
Univ of California-Berkeley
Latimer Hall
Berkeley, CA 94720
510-643-3117

Rajeev Dadoo
Genentech Inc
South San Francisco, CA 94080-4990
650-225-7865

Symposium Support
Army Research Office

*Invited paper
SESSION N1
Chair: Deon Anex
Monday Morning, 8:30-11:00
Franciscan II (Argent, OR)

8:30 A.M. *N1.1
ARTIFICIAL ANTIBODIES IN FORM OF SYNTHETIC GELS FOR
SELECTIVE DETECTION OF SPOTLINES
Stilianos Hjerten, Daxin Tong, Department of Biochemistry, Biomedical Center, Upplands University, Upplands, SWEDEN.

The conventional imprinting technique is based on functional monomers that interact strongly (often covalently) with the molecule of interest. The method works well for low-molecular-weight compounds like proteins, but the selectivity is relatively low. By using monomers, which interact very weakly with proteins, such as acrylamide, we can prepare gels with a high degree of selectivity. For instance, a gel synthesized to adsorb specifically myoglobin from horse adsorbed this protein but not myoglobin from whale (although the 3-dimensional structure is very similar to that from horse), nor ribonuclease or cytochrome C. Several other proteins have also been adsorbed selectively. When the sample consisted of a mixture of three proteins all three proteins were adsorbed. The protein capacity of the gels has been increased three-fold compared to that obtained by the original imprinting technique by changing the experimental conditions for the preparation of the gel. A simple practical method to select monomers, which, upon polymerization, give a high-specificity gel, will be discussed. In standard electrophoresis, polyacrylamide gels the sharp protein zones migrate without tailing, indicating that the proteins do not interact with the gel matrix. To explain the very strong selective adsorption of proteins to polyacrylamide we have introduced a new chromatographic fractionation system based on the number of bonds between the solute and the matrix and the strength of each bond. In this system the selective recognition is characterized by a large number of weak bonds. We will also discuss whether this recognition can be improved by a separation mechanism different from that based on imprinting. The possibility to design analogous capillary electrophoresis and electrophotography experiments will be discussed.

9:00 A.M. *N1.2
DEVELOPMENT OF MATERIALS THAT EXHIBIT AND
METHODS THAT HARNESS MOLECULAR RECOGNITION
Martha Degerman, Ryszard Borkowski, Valvanova, Gheorghe Chiriac and Vincent Remiddio, Oregon State Univ, Dept of Chemistry, Corvallis, OR.

Molecular imprinting is a technique in which synthesis in the presence of a template molecule results in the formation of specific recognition sites within a polymer. In noncovalent imprinting, the template molecule associates with monomers in free solution through various intermolecular forces (e.g., hydrogen bonding, pi-pi, and ionic interactions) prior to polymerization. The positions of the associated monomers are fixed during polymerization, creating cavities complementary to the template molecule in shape and chemical functionality. After template removal, these cavities function as specific binding sites that have the ability to ‘recognize’ the template molecule. The high degree of molecular recognition attainable with MIPS has rendered these materials useful in several areas of analytical chemistry, including sensors, immunogens, and chromatography. In this work, the potential utility of MIPS-based chromatographic sorbents for combinatorial library screening was investigated. A group of structurally similar pharmaceuticals and related compounds were used to simulate a combinatorial library. MIPS were synthesized using one of the therapeutic agents as the template molecule. The MIPS material was then employed as a sorbent for chromatographic screening of the library. The elution profiles of the library compounds on the MIPS versus a blank polymer synthesized in the absence of template were used to evaluate the degree of selective interaction (i.e., the goodness of fit of each into the surrogate binding pocket). The results of the study revealed that library compounds showing key structural features of the template were better recognized by the MIPS, while the most structurally dissimilar compounds exhibited the least selective interaction. Further studies yielded information on the mechanism of retention of analytes on these MIPS.

9:30 A.M. *N1.3
THE USE OF MESOPOROUS SILICA IN LIQUID CHROMATO-

Mesoporous silica is commonly used as a matrix material for chromatographic separations. The surface area of commercially available chromatographic grade silica is generally less than 400 m²/g. Mesoporous silica, which can have a surface area in excess of 1500 m²/g, is expected to provide superior separation ability as a chromatographic matrix. The latter type of silica is prepared by a microsized templated sol-gel polymerization method which produces pore dimensions adjustable between 2 and 50 Å, with narrow pore size distributions. It has been used for selective separation of heavy ions from solution, and its use in gas chromatography and high performance liquid chromatography (HPLC) has been explored briefly. The dramatically higher surface area of mesoporous silica enhances resolution by increasing capacity factors to allow effective separations of analytes. In this study, we compare the physical properties of several types of mesoporous silica to commercially available chromatographic grade silicas. We also compare the separating abilities of these types of silica in a variety of liquid chromatographic (LC) techniques, including normal phase LC, reverse phase LC, HPLC, chiral HPLC, and thin layer LC (TLC).

9:45 A.M. *N1.4
UMBRELLA SYNTHESSES: A NEW APPROACH TO
SYNTHESIS REVERSIBLE PHASE MODIFICATION ZIRCONIA,
Brian Trammell, Marc Billmeyer, Peter W. Cser, Department of Chemistry, University of Minnesota, Minneapolis, MN.

To overcome the limitations involved in preparing HPLC phases by the normal route of depositing a polymer from the liquid phase, zirconia will be coated by a novel ‘umbrella’ technique. First, the Lewis acid sites on zirconia’s surface will be sequestered by exhaustively chemisorbing a crosslinkable, polymerizable, hard Lewis base monomer. Second, a crosslinked polymer network or ‘umbrella’ which incorporates the sequestrate will be formed in vacuo on the surface. Both steps act to completely block the surface Lewis acid sites. Additionally, this technique with careful control of chemistry is conducted confined to a very thin layer by anchoring the network to the surface through the chemisorbed sequestrate, thus improving stationary phase mass transfer. We are currently investigating four methods for synthesizing the polymer umbrella. The methods are different, but the goal is the same, an anchored polymer network that completely sequesters the Lewis acidity.

Various hard Lewis base monomers were investigated to determine their effectiveness in sequestering Lewis acid sites on zirconia’s surface. The residual Lewis acid site coverage was determined by evaluating the percent recovery and k’ of benzoic acid under reversed phase conditions. Phosphonate monomer phases show greater than 90% Lewis acid site coverage and stability. In comparison, cyclohexylamine monomers did not block access to Lewis acid sites. Chemisorbed phosphonic acids provide excellent Lewis acid site coverage and good stability under typical reversed phase conditions; therefore, vinylphosphonic acid is being employed on the Lewis acid site sequesterate for preliminary ‘umbrella’ syntheses.

10:30 A.M. *N1.5
NEW POROUS ORGANIC AND HYBRID ORGANIC/INORGANIC
NETWORK MATERIALS FOR SEPARATION TECHNOLOGY
Kenneth J. Shea, Dept of Chemistry, University of California, Irvine, CA.

The talk will focus on recent developments in the synthesis and evaluation of new porous materials for use in separation media. Two approaches have been taken to achieve selectivity in these materials. High-quality-linked organo-networks have been prepared by molecular imprinting, a general technique for the creation of binding sites for targeted organic molecules. Molecularly imprinted polymers (MIPs) have been fabricated into thin films for selective transport of targeted molecules. Imprinted polymers incorporating receptor sites for benzodiazepines have also been prepared and evaluated. In the second approach, bridged polyisocyanurates are prepared by sol-gel polymerization. These porous hybrid materials contain a variable organic component that can be used to optimize affinity for targeted molecules.

11:00 A.M. *N1.6
NEW INORGANIC-ORGANIC HYBRID MATERIALS TAILORED
FOR USE IN HIGH PERFORMANCE LIQUID SEPARATIONS
Bennie Alken, Yang-Fong Cheng, Raymond Fisk, Christina Gendreau, Pamela Fritschi, Uwe Neue, John O’Gara, Dan Walsh, Thomas Wieder, Waters Corp, Milford, MA; Zhuping Jiang, W.R. Grace Corp, Lexington, MA.

This presentation will provide an overview of the synthesis and physical properties of newly developed inorganic-nanohybrid materials for use in HPLC and related separation sciences. Performance attributes such as extended pH stability, and improved chromatographic peak shape will be used to illustrate advances over conventional separation materials. New application areas, enabled by these novel materials will also be highlighted.

11:15 A.M. *N1.7
THERMALALLY-RESPONSIVE, HYDROPHOBICALLY-MODIFIED
POLYCRYLAMIDES FOR MICROCHANNEL DNA ELECTRO-

PHORESIS. Brett A. Buchholz, Annemie E. Barrow. Northwestern University, Dept of Chemical Engineering, Evanston, IL.

Microfabricated genetic analysis devices are currently the best hope of the Genome Project for continued increase in the speed and efficiency of DNA sequencing. Yet, along with the many advantages of miniaturization (including increased DNA separation efficiency, reduced sample requirement, and short analysis times) come significant challenges for the design and engineering of appropriate 'repealable' separation media for electrophoresis microchannels.

While optimization is needed, highly-entangled solutions of high molar mass polymers, such high-viscosity media are difficult to load into electrophoresis microchannels. Our group is working to develop 'thermo-reversible' polymer systems that will enable practical application of microfabricated electrophoresis devices for automated, high-throughput DNA sequencing and genetic analysis. This research involves the synthesis and characterization of high molar mass N,N'-dialkylated polyamidoamines that exhibit a reversible temperature-sensitive phase transition in aqueous solution (LCST). The LCST phase transition results in a precipitous drop in the viscosity of the polymer solution, and can be exploited to allow facile loading into microchips. LCST-exhibiting copolymers were synthesized under constant-temperature, oxygen-free conditions and their molar mass distributions determined by tandem gel permeation chromatography using multiple-angle laser light scattering. Our preliminary polymer formulations, having LCST transitions at 70°C in sequencing buffer and weight-average molar masses greater than 10 million g/mol, yield DNA sequencing read lengths of nearly 1000 bases under our current conditions. We are currently investigating the use of these systems in highly-entangled solutions of high molar mass polymers, such as polyethylene glycol, for microfluidic devices with applications in high-throughput DNA sequencing and genetic analysis.

11:30 AM N18

The explosive development of large-scale genome projects puts a strong demand on DNA sequencing. The new generation of sequencers uses the electrophoretic separation of labeled DNA fragments inside large arrays of nanowire capillaries. We developed a new family of separation matrices, based on the thermo-switching properties of specially designed and synthesized polymers. Thanks to this property, the separation medium can be easily replaced between each separation in the fluid state, and present optimal sieving properties at the temperature at which separation occurs. The correlation between structural, rheological and sieving properties will be discussed.

11:45 AM N19
MICRODEVICES FABRICATED BY POLYMER HOT EMBOSsing FOR BIOANALYTICAL APPLICATIONS. M. Goretty Alonso-Amigo, Jenoptik Mikrotechnik, West Coast Office, Hayward, CA; Holger Becker, Jenoptik Mikrotechnik, Jena, GERMANY.

Polymer microfabrication methods are becoming increasingly important as low-cost alternatives to the silicon or glass-based MEMS technologies. Polymer hot embossing is a replication method for planar microstructures applicable to a diversity of polymer substrates. Hot embossing has been demonstrated in the replication of chips containing microchannels for capillary electrophoresis (CE), and for microfluidics devices in biotechnology and biomedical applications. Microfluidics devices and nanostructures of high aspect ratio and pattern layouts have been demonstrated. For microfluidics channel widths between 0.8 μm and 100 μm have been produced yielding a very good structural replication and short production times. The miniaturization of chemical and biological microanalysis has made enormous progress over the last few years since the pioneering work of Muru[1] and Harrison[2]. Nowadays almost any technology known to work in the macroscopic world in these fields has been successfully miniaturized, with recent developments on-on-chip flow through-PCR[3], microreaction technology[4] and highly parallel electrophoretic separation devices[5]. In the past however, microfluidic devices of all kinds have been fabricated on silicon wafers by micromachining techniques such as DRIE[6], ICP[7], silicon wafer bonding[8] or embossing[9] for the microfabrication of microchannels. Hot embossing has demonstrated potential to fabricate devices like polymer microfluidic chips (PMMA), polyurethane (PC) and several hydrocarbon based polymers. The technology offers the advantage of relatively low costs for embossing tools, a short-cycle replication process with few variable parameters and high structural accuracy and is therefore suitable for a wide range of microfabrication applications from rapid prototyping to high-volume mass fabrication. In the first step of the hot embossing process, a mask with the desired structures for the fabrication of the embossing master has to be designed. The embossing master fabrication can be realized in a variety of techniques, from the traditional CNC-machining of materials like stainless steel in structures >10μm to the lithographic methods in micron and submicron feature sizes. These techniques can yield a metal tool, however for structures with low aspect ratio or for rapid prototyping where the lifetimes of the master is less crucial, an ejected silicon wafer can be utilized directly as a master. The metal or silicon master is mounted in the embossing machine together with the polymer substrate and heated separately in a vacuum chamber to a temperature just above the glass transition temperature Tg of the polymer material. The tool is then brought in contact with the substrate and embossed with a controlled force, typically of the order of several kN for several seconds. While applying the embossing force, the tool-substrate sandwich is then cooled to just below Tg. After cooling, the embossing force is removed from the substrate that now contains the desired features. Drilling holes and cavities, sealing substrates or aligning in the x-direction with other embossed structures may be further processing steps added to the embossed planar microfluidic chips described above. Mastering and hot embossing results for different fields of application would be presented comparing the possibilities of silicon and plastic microfabrication for biocatalytic applications and devices. References: [1] A Muru, W. Grote, H.N. Widner, Sensors and Actuators B 90 (2003), 244-248 [2] D.J. Harrison, A. Muru, Z. Fan, H. Lüdi, H.N. Widner, Anal. Chem. 64 (1992), 1925-1932 [3] M.U. Kopp, A.J. deMello, A. Muru, Science 280 (1998), 1046-1048 [4] W. Ehrfeld, ed. Proceedings of the 1st International Conference on Micromachined Sensor, Actuator Technology, Springer, Heidelberg, 1998. [5] P.C. Simpson, D. Ronch, A.T. Wooley, T. Thomen, R. Johnston, G.F. Seabright, R.A. Murchie, Proc. Natl. Acad. Sci. USA 95 (1998), 2356-2361 [6] M.A. Roberts, J.S. Rossier, P. Gieg, P. Jarbeau, H. Girault, Anal. Chem. 69 (11) (1997), 2007-2013 [7] R.M. McCormick, R.J. Nekon, M.G. Alonso-Amigo, D.J. Bennewitz, H.H. Hooper, Anal. Chem. 69 (1997), 2626-2630. [8] C.E. Eissenhauer, G.M. Bruin, A. Puchan, M. Ehren, Proceedings of JTN 96, Anal. Methods Instrum. Special Issue (1996), 124-125. [9] L. Mazzorana, L.E. Lencisco, M. Guinon, G.W. Kramer, R.G. Christensen, W.A. MacCrehan, Anal. Chem. 69 (1997), 4783-4789.

SESSION N2 IN-ROOM POSTER SESSION
Chair: Franksek Seve, Monday, April 24, 2000 10:00 AM

N2.1 THE SURFACE PROPERTIES OF TETRADECYLMETHYLMONONIUM BROMIDE OBSERVED BY CAPILLARY ELECTROPHORESIS. D.L. Coke, R. Schnitzler and Z. Yu, Gilr Chair of Chemistry and Chemical Engineering, Lamar University, Beaumont, TX.

Capillary electrophoresis has been used to follow the electrophoretic flow as a function of tetradecyltrimethylammonium bromide (TTAB) concentration and shown to have distinct structural zones that depend on the pH. The presence of the TTAB species in the electrophoretic zone was confirmed by proposed surface structures ranging from unimolecular adsorption to hemimicelles and micelles of TTAB adsorbed on the hydrated fused silica. In addition, the data indicates a form of TTAB adsorbed that is not surfactable in nature. Although the adsorption of the various adsorbed states remains an open question, the observations in this work and the possible corresponding surface structures have widespread implications in the science and technology of surfactant adsorption.

N2.2 MOLECULAR IMPRINTING WITHOUT TEMPLATE. Ken Hosoya, Masashi Taniyama, Takasato Kegami, Nobuo Tanaka, Kyoto Institute of Technology, Dept. of Polymer Science, Kyoto, JAPAN.

Cross-link polymer should remember the preparation conditions including polymerization temperature, porogen, or this...
memory affects molecular recognition ability if the polymer is used in separation medium in high performance liquid chromatography (HPLC). These preparation conditions are very carefully, we can determine and control the nature of the cross-linked polymer to recognize some molecules. This is not real molecular imprinting, but one of easy technique to get specific molecular recognition ability. For example, the molecular information such as shape or size of porogenic solvent utilized can be somehow memorized on polymer network, which afford some preferential recognition towards the porogen molecule. In addition, resemble molecule to the porogen can be imprinted to result in recognition. So we used cross-linking agents having different length between two polymerizable groups, we can get quite different molecular recognition ability due to the difference in the cross-linking agents. This is not so unusual phenomenon. But, even with the same cross-linking agent, polymerization temperature affects the nature of cross-linking structure to afford quite different molecular recognition ability. To control these parameters, we can determine molecular recognition ability on the polymer separation media like molecular imprinting technique.

N2.3 ESTIMATION OF ARSENIC COMPOUNDS IN HUMAN URINE BY HPLC-I CP-MS WITH DIFFERENT CHROMATOGRAPHIC CONDITIONS: Amit Chatterjee, Yuayuki Shibata, Jun Yoshimura and Masahito Mori, National Institute for Environmental Studies, Environment Chemistry Division, Tsukuba, Ibaraki, JAPAN

The silicon-bonded cation-exchange [LC-SX, 20 mm pyridine pH 2.6]; styrene-divinylbenzene copolymer-based PRP-X100 ion exchange [30 mm pyridine pH 2.6]; porous-styrene-divinylbenzene copolymer (100 mesh, tetramethylammonium hydroxide 4 + 4 mmolal malic acid in 0.055% methanol, pH 6.8) and the gelpermutating GS-220 (25 mm tetramethylammonium hydroxide 25 mmolal malic acid pH 6.8) columns, which are connected with the HPLC-I CP-MS, have been used for the separation, identification, and quantification of arsenic compounds, specially arsenobetaine (AB) present in NIES Candidate Human Urine. The AB is the predominant arsenic species followed by dimethylarsenic acid (DMA), monomethylarsenic acid (MA) and arsenic acid. The concentration of AB, estimated by standard addition method, found in the LC-SX, and GS-220 columns are 71.5 ± 7.7 (n = 27) and 72.6 ± 8.13 (n = 9) ng ml⁻¹. The arsenic acid was not detected in the urine and is verified by using the LC-SAX 1 ion exchange column. The high concentration of chloride that co-elutes with the arsenic acid from the LC-SX column and AB from the GS-220 columns are interfered and enhanced the concentrations of arsenic acid and AB in the urine. So, in the GS-220, the concentration of AB has been carefully estimated after excising the chloride interference (Cl⁻/Cl⁵⁺ = 1.3:1271) by subsequently measuring the Ar³⁵/Cl³⁵⁻. In the ODS column, the peak of DMA has been overlapped with the peak of AB with current mobile phase conditions and has perplexed the estimation of AB. But in the LC-SAX, the AB has been baseline separated from the other arsenic compounds and also from the chloride with the 20 mm pyridine at pH 2.6. So, the LC-SX and GS-220 are recommended and used for determination of AB.

N2.4 SEPARATION OF SIXTEEN POLYCYCLIC AROMATIC HYDROCARBONS BY CAPILLARY ELECTROCHROMATOGRAPHY USING A PHOTOCHEMICAL MICROFAST TECHNIQUE TO SYNTHESIZE POLYMERS FOR ELECTROKINETIC CHROMATOGRAPHY: Christopher P. Palmer, Wei Shi, Dominic Petersen, Ting Chen, Kent Tang, New Mexico Institute of Mining and Technology, Department of Chemistry, Socorro, NM

Amphiphilic polymers have been shown to have significant advantages relative to micelles as pseudo-stationary phases for electrokinetic chromatography. The polymers are significantly more stable than micelles, allowing separations to be performed in media where micelles are not stable. Additionally, polymeric phases can provide unique chemical selectivity for electrokinetic chromatography. In this presentation, recent results in the development and characterization of polymeric phases of a variety of chemistries will be described. Amphiphilic copolymers have been shown to have different chemical selectivity depending on the nature of the hydrophobic group. Mixtures of polymeric phases with different chemical selectivity allow separations with unique and intermediate selectivity. More importantly, the separations achieved with mixtures can be predicted with, in most cases, less than 5% error in the analyte mobilities. Silicone materials are being used because of the range of chemistries that could be developed based on these backbones, and because successful development of silica phases would facilitate the easy and rapid method development that is critical to common practice.

SESSION N3

Chair: Rajeev Daloo
Monday Afternoon, April 24, 2000
Franciscan II (Argent)

1:30 PM **N3.1** CAPILLARY GEL ELECTROCHROMATOGRAPHY WITH REPLACABLE MEDIA: Mark B. Schray, Theoretical Separation Science Laboratory, Rohm and Haas Company, Spring House, PA.

A number of years ago it was demonstrated that liquid chromatography could be conducted by allowing an electric field to pump fluid through a packed bed via electrophoresis. Recently, the electrosmotic fluid pump approach has become popular because smaller chromatographic particles can be used allowing for higher efficiency with no pressure drop. Our laboratory has further refined this approach. We have synthesized polymers which can be easily pumped into a capillary and provide hydrophobic retention from grafted hydrophobic groups and electrosmotic flow from charged acidic groups. In this way when a column is clogged, one simply replaces the polymer in the capillary and continues the work, rather than dispose of some expensive column. Performance for non-charged small molecular weight solutes is typically more than 500,000 plates per meter in this configuration. A number of aspects to the work will be discussed including simple loading, mechanism, solvent range, applications, and retentive phase synthesis.

2:00 PM **N3.2** PERFORMANCE AND SELECTIVITY OF POLYMERIC PSEUDO-STATIONARY PHASES IN ELECTROKINETIC CHROMATOGRAPHY: Christopher P. Palmer, Wei Shi, Dominic Petersen, Ting Chen, Kent Tang, New Mexico Institute of Mining and Technology, Department of Chemistry, Socorro, NM.

Amphiphilic polymers have been shown to have significant advantages relative to micelles as pseudo-stationary phases for electrokinetic chromatography. The polymers are significantly more stable than micelles, allowing separations to be performed in media where micelles are not stable. Additionally, polymeric phases can provide unique chemical selectivity for electrokinetic chromatography. In this presentation, recent results in the development and characterization of polymeric phases of a variety of chemistries will be described. Amphiphilic copolymers have been shown to have different chemical selectivity depending on the nature of the hydrophobic group. Mixtures of polymeric phases with different chemical selectivity allow separations with unique and intermediate selectivity. More importantly, the separations achieved with mixtures can be predicted with, in most cases, less than 5% error in the analyte mobilities. Silicone materials are being used because of the range of chemistries that could be developed based on these backbones, and because successful development of silica phases would facilitate the easy and rapid method development that is critical to common practice.
sulfonic acid and hydrophobic monomers have also been developed. The various copolymers have been studied with respect to their chromato- graphic performance and selectivity.

2:30 P.M. N3.3
THE PREPARATION OF MONOLITHIC MEDIA WITH CONTROLLED POROUS PROPERTIES AND SURFACE FUNCTIONALITIES BY PHOTONINITIATED IN SITU POLYMERIZATION. A SUITABLE APPROACH TOWARDS STATIONARY PHASES FOR ELECTROCHROMATOGRAPHY ON A MICROCHIP.

Y. Fréchet, V. Fréchet, University of California, Dept. of Chemistry, Berkeley, CA.

Electrophoresis is a technique in which the liquid mobile phase is used to separate detection devices by electrophoretic field and the separation process relies on the interaction of dissolved solutes with the functionalities of a stationary phase. Currently, the functionalized surfaces of open channels on a chip are mostly used as the stationary phases. This is difficult and reproducibly into very narrow channels. In contrast, our process involving the filling of channels with a liquid polymerization mixture followed by in situ photopolymerization can afford efficient porous monolithic separation media. Our current model systems show that both the chemistry and the porous properties of the monolithic materials can be controlled within a broad range by the composition of the polymerization mixture, thus enabling the preparation of tailor-made media with properties optimized for specific applications.

2:45 P.M. N3.4
NOVEL MICROPORE CONTINUOUS CHIRAL STATIONARY PHASES FOR ENANTIOSELECTIVE CAPILLARY ELECTROCHROMATOGRAPHY (CEC). M. Lüning, P. Wee, J.M.J. Fréchet, Department of Chemistry, University of California, Berkeley, CA; W. Linder, Institute of Analytical Chemistry, University of Vienna, Austria.

Novel micro-porous chiral stationary phases for enantioselective capillary electrophoresis (CEC) have been prepared by in situ copolymerization of chiral monomer 1, 5-[2-hexylthiophenyl]-10,11-dihydroquinine, 5-hydroxymethyl methacrylate (HEMA) and/or glycidyl methacrylate (GMA) as cosolvent, and ethylene dimethacrylate (EDMA) as crosslinker in the presence of l- and d-2-dodecanol as organic solvents within untreated fused silica capillaries. The polymerization is induced either by temperature (60°C) or UV irradiation at ambient temperature. The porosity, a key parameter determining the efficiency of these separation materials, can be adjusted by the l-dodecanol/2-dodecanol proportion in the polymerization mixture. Further, it turned out that poly[5-HEMA-co-EDMA] monoliths exhibited much higher enantioselectivity and also higher efficiency than corresponding poly[5-GMA-co-EDMA] monoliths. For enantioselective CEC application, the quinidine carbamate groups play a double role: 1.) Due to the basic quinidine the surface of the chiral monolithic column is positively charged under CEC conditions and thus good enantiomeric electrophoretic mobility (EOF). 2.) It provides the chiral moiety for enantioselective union exchange as chromatographic selectivity principle. These chiral monoliths showed reasonable enantioselectivity towards N-octylated amino acids.

3:30 P.M. N3.5
OPEN TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY.

Joseph Pueck, Maria Muricy, Department of Chemistry, San Jose State University, San Jose, CA.

In capillary electrophoresis separation depends on differences in electrophoretic mobility and/or solute/bonded phase interactions. In CEC with packed capillaries, bubble formation is often encountered due to the presence of the silica particles and the frits used to retain the stationary phase. Residual silica presents a serious problem for the separation of compounds, so it is of great interest to find an inner capillary wall followed by chemical modification of this new surface can provide a separation method that overcomes the problems of packed columns while allowing improved resolution of many mixtures in comparison to ordinary CEC. The resulting increase in the surface area by up to 1000-fold and produces radial extensions from the surface that facilitate solute/bonded phase interactions. Application of a potential will drive the solute via a plug flow profile similar to HPLC rather than a parabolic flow which is encountered in pressure driven systems. The electrophoretic surface is characterized by photoelectron spectroscopy (ESCA) and the modified surface by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. ATMS-SE for microscopy can be used to elucidate the topography of the surface along with SEM. AFM can also provide information about surface roughness and chemical forces at the surface. Examples of separations of small molecules and bacteriophage lambda DNA in capillary electrophoresis are presented along with performance obtained by this approach. Good peak shape is obtained for all solutes, even basic molecules. Several approaches are also described for evaluating the solute/bonded phase interactions and their contribution to the separation mechanism.

4:00 P.M. N3.6
DEVELOPMENT OF MICROPOROUS ELECTROKINETIC PUMPS AND THEIR APPLICATION TO MICROSCALE HPLC.

Katherine Bullard Smith, Phillip H. Paul, and David W. Neyer, Sandia National Laboratories, Livermore, CA.

Employing a breakthrough in the design of electrokinetic pumps that deliver high pressures with no moving parts, we have developed and demonstrated a completely microscale high performance liquid chromatography (HPLC) system. The system consists of a group of small (75-150 μm diameter) capillaries filled with gel that are coupled together to perform not only separation, but also the pumping and injection necessary for a complete microscale analysis system. By harnessing electroosmotic flow to produce microliters per minute flow rates into binary solvent mixtures (several hundreds), these stable, high pressure pumps are ideal for separations in capillary chromatography columns. At the heart of this new instrument is a collection of micro and nanoscale materials. Microporous materials with high gas/vapor permeability in the mobile phase of choice are required for constructing efficient electrokinetic pumps for the HPLC. Nanoporous salt bridges are used to transfer solute electrolysis outside the high pressure system and novel injection scheme using additional microporous materials with little or no retention of the sample is also employed. Finally, the key component of a good HPLC system is the separation column which can be filled with a variety of microporous materials tailored to give optimum speed and selectivity for a given separation. In addition to the separation column, we will discuss the variety of columns and approaches we have taken to make our new separation system applicable to a wide variety of analytes and separation modes. Recent developments of microfabricated systems with on-chip pumps and optimized connections and injections will also be presented.

4:15 P.M. N3.7
CHEMICAL AND MATERIALS CONTROL IN ULTRASMAALL VOLUMES: EXPLOITING ELECTROKINETIC EFFECTS.

Mark A. Hayes, Noln A. Polon, Nanette K. Hartley, Arizona State University, Department of Chemistry and Biochemistry, Tempe, AZ.

Control of ultrasmall volumes (~pro- to femtoliters) is important for modern separation techniques on microchip devices. Furthermore, chemically selective movement of species in these small volumes can provide for distinctive abilities for chemical analysis and interactions. Electrokinetic effects (electrophoresis and electroosmosis) are well suited to this task, but present application of this technology is merely fundamental. Much more elegant and useful application of these effects can be envisioned. To realize this fine control one must first recognize that in standard systems all electrokinetic effects are directly related to the potential field strength by definition. To truly exploit the possibilities of small volume manipulations these two effects must be independent of each other. To accomplish the independent control of electrokinetic effects, several obstacles must be overcome. For instance to provide independent control of electroosmosis issues in surface chemistry, materials, electrostatics and electrophysical phenomena must be considered. Systems to non-invasively monitor the flow in these systems also must be developed. The location and magnitude of the potential field must be carefully considered. The successful independent control of electrokinetic flow can allow for unique manipulation of materials. These include the concentration of chemically specific materials in proctor volumes (based on electrophoretic mobilities), the stagnation of specific materials in the presence of flow, and the selective sequestration of materials near intersecting channels (on microchips). These manipulations are carried out without the aid of any moving parts and can be used in channels of very small dimension (< 1 mm). These manipulations, combined with the advantageous properties of electroosmosis, can provide for exquisite control of materials on small and fast systems. This lecture will focus on strategies to independently control electroosmosis, monitor flows in small channels, and exploit flow electroosmotic migration phenomenon. Data will be shown in standard fused silica capillaries and on microfabricated devices.

4:30 P.M. N3.8
REPRODUCIBLE GRADIENTS AT ULTRA-LOW FLOW RATES.

Susan M. Staukus, Curtis R. Campbell, Shimadzu Scientific Instruments, Inc., Columbia, MD.

A growing number of analyses are using techniques such as microbore HPLC and tandem LC-MS which require flow rates from 5-250 μL/minute. Several factors must be considered when working in this range: aggregate back pressure (a dead end pump limited here), efficient mixing in a very limited volume, and reduction of dead
volume to minimize run time and band broadening. We are able to
generate accurate, reproducible linear and step gradients at flow rates
as low as 5 μL/minute. A system plumbed with capillary tubing using
a low volume mixer, micro injection valve, 10 cm x 1 mm column, and
a micro flow cell has a volume of approximately 90 μL from the mixer
inlet to the detector. Systems of this nature are ideal for capillary
chromatography, splitless injection to a mass spectrometer, and
coupling to microfabricated devices.