

# SYMPOSIUM N

## Materials for Separations in Analytical Chemistry

April 24, 2000

### Chairs

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**Symposium Support**  
Army Research Office

\* Invited paper

SESSION N1:  
Chair: Deon Anex  
Monday Morning, April 24, 2000  
Franciscan II (Argent)

**8:30 AM \*N1.1**

**ARTIFICIAL ANTIBODIES IN FORM OF SYNTHETIC GELS FOR SELECTIVE RECOGNITION OF PROTEINS.** Stellan Hjerten, Daixin Tong, Department of Biochemistry, Biomedical Center, Uppsala University, Uppsala, SWEDEN.

The conventional imprinting technique is based on functional monomers that interact strongly (often electrostatically) 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 synthesis method by changing the experimental conditions for the preparation of the gels. A simple practical method to select monomers, which, upon polymerization, give a high-specificity gel, will be discussed. In standard electrophoresis in 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 classification 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 explained by a separation mechanism differing from that based on imprinting. The possibility to design analogous capillary electrophoresis and electrochromatography experiments will be discussed.

**9:00 AM \*N1.2**

**DEVELOPMENT OF MATERIALS THAT EXHIBIT AND METHODS THAT HARNESS MOLECULAR RECOGNITION.** Martha Degen, Mohammad Khasawneh, Patrick Vallano, Gabriela Chirica and Vincent Remcho, 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, immunoassays and chromatography. In this work, the potential utility of MIP-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 MIP material was then employed as a sorbent for chromatographic screening of the library. The elution profiles of the library compounds on the MIP 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 sharing key structural features of the template were better 'recognized' by the MIP, 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 AM N1.3**

**THE USE OF MESOPOROUS SILICA IN LIQUID CHROMATOGRAPHY.** Karl W. Gallis, Andrew G. Eklund, Sara T. Jull, James T. Araujo, Joseph G. Moore and Christopher C. Landry, Dept of Chemistry, Univ of Vermont, Burlington, VT.

Porous silica is commonly used as a matrix material for chromatographic separations. The surface areas of commercially available chromatographic grade silicas are generally less than 400 m<sup>2</sup>/g. Mesoporous silica, which can have a surface area in excess of 1500 m<sup>2</sup>/g, is expected to provide superior separating ability as a

chromatographic matrix. The latter type of silica is prepared by a micelle-templated sol-gel polymerization method which produces pore diameters adjustable between 20 and 300 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 silicas 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 AM N1.4**

**UMBRELLA SYNTHESSES: A NEW APPROACH TO SYNTHESIZING REVERSED PHASE MATERIALS ON ZIRCONIA.** Brian Trammell, Marc Hillmyer, Peter W. Carr, 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 sequesterant will be formed in vacuo on the surface. Both steps act to completely block the surface Lewis acid sites. Additionally, this technique will ensure that the polymerization is confined to a very thin layer by anchoring the network to the surface through the chemisorbed sequesterant, 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 99% Lewis acid site coverage and stability. In comparison, carboxylate 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 as the Lewis acid sequesterant for preliminary "umbrella" syntheses.

**10:30 AM \*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 as separation media. Two approaches have been taken to achieve selectivity in these materials. Highly cross-linked organic network polymers have been prepared by molecular imprinting, a general technique for the creation of binding sites for targeted organic molecules. Molecularly imprinted polymers (MIP's) have been fabricated into thick 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 polysilsesquioxanes 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 AM N1.6**

**NEW INORGANIC-ORGANIC HYBRID MATERIALS TAILORED FOR USE IN HIGH PERFORMANCE LIQUID SEPARATIONS.** Bonnie Alden, Yung-Fong Cheng, Raymond Fisk, Christina Gendreau, Pamela Iraneta, Uwe Neue, John O'Gara, Dan Walsh, Thomas Walter, Waters Corp, Milford, MA; Zhiping Jiang, W.R. Grace Corp, Lexington, MA.

This presentation will provide an overview of the synthesis and physical properties of newly developed inorganic-organic hybrid 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 AM N1.7**

**THERMALLY-RESPONSIVE, HYDROPHOBICALLY-MODIFIED POLYACRYLAMIDES FOR MICROCHANNEL DNA ELECTRO-**

PHORESIS. Brett A. Buchholz, Annelise E. Barron, 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 'replaceable' separation media for electrophoresis microchannels. While optimal DNA separations are achieved in concentrated, highly-entangled solutions of high molar mass polymers, such high-viscosity matrices are difficult to load into electrophoresis microchannels. Our group is working to develop 'thermo-reversible' polymeric sol-gel materials that will enable practical application of microfabricated electrophoresis devices for automated, high-throughput DNA sequencing and genetic analysis. This research has involved the synthesis and characterization of high molar mass N,N-dialkyl acrylamide co-polymers that exhibit a reversible, volume phase transition in aqueous solution at a given Lower Critical Solution Temperature (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 microchannels. LCST-exhibiting co-polymers were synthesized under constant-temperature, oxygen-free conditions and their molar mass distributions determined by tandem gel permeation chromatography-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 400 bases in under 45 minutes. The viscosity of the matrix at sequencing conditions (7% (w/v) co-polymer solution of 1X Tris-TAPS-EDTA buffer and 7M urea) is greater than 800 cP, but decreases to just 40 cP at 72°C. Hence, these materials have a thermally-controlled 'viscosity switch' that enables decoupling of the loading and sieving properties of the matrix, simultaneously providing the DNA analysis typically expected from high-viscosity solutions and the rapid, low-pressure loading typically obtained with low-viscosity solutions.

#### 11:30 AM N1.8

NEW THERMOTHICKENING MATRICES FOR HIGH-THROUGHPUT CAPILLARY ELECTROPHORESIS. Jean-Louis Viovy, Jan Sudor, Valessa Barbier, Institute Curie, UMR CNRS 168, Paris, FRANCE; Dominique Hourdet, ESPCI, Lab. Physicochimie Macromoléculaire, Paris, FRANCE.

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 narrow-bore capillaries. We developed a new family of separation matrices, based on the thermothickening 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 N1.9

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 microstructure 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. Microdevices with micro and nano size features, in diverse geometry and pattern layouts have been demonstrated. For microfluidics channel widths between 0.8  $\mu\text{m}$  and 100  $\mu\text{m}$  have been produced yielding a very good structural replication and short production times. The miniaturization of chemical and biochemical instrumentation has made enormous progress over the last years since the pioneering work of Manz[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 like on-chip flow through-PCR[3], microreaction technology[4] and highly parallel electrophoretic separation devices[5]. In the past however, microfluidic devices for all these miniaturized chemical analysis systems ( $\mu\text{TAS}$ ) have been fabricated almost exclusively in silicon, glass or quartz. The main reason for this choice of materials can be found in the fact that microfabrication methods for these materials have been extensively developed for the microelectronics industry over the last four decades. The alternative use of plastic as the chip material addresses cost

issues in product commercialization and biocompatibility of surfaces. Thus an increasing number of devices have been reported recently in the literature, fabricated on a variety of polymer substrates and using different fabrication methods such as laser ablation[6], injection molding[7], silicone rubber casting[8] or embossing[9] for the microfabrication of microchannels. Hot embossing has demonstrated potential to fabricate devices in polymers like polymethylmethacrylate (PMMA), polycarbonate (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 suited 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  $>100\mu\text{m}$  to the lithographic methods in micron and submicron feature sizes. These technologies 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 etched silicon wafer can be utilized directly as a master. The metal or silicon master is mounted in the embossing machine together with the planar polymer substrate and heated separately in a vacuum chamber to a temperature just above the glass transition temperature  $T_g$  of the polymer material. The tool is then brought into 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  $T_g$ . After cooling, the embossing tool is mechanically separated from the substrate that now contains the desired features. Drilling holes and cavities, sealing substrates or aligning in the z-direction with other embossed structures may be further processing steps added to the embossed planar microfabricated plastic substrate. Mastering and hot embossing results for different fields of application would be presented comparing the possibilities of silicon and plastic microfabrication for bioanalytical applications and devices. References: [1] A. Manz, W. Graber, H.N. Widner, Sensors and Actuators B1 (1990), 244-248 [2] D.J. Harrison, A. Manz, Z. Fan, H. Lüdi, H.N. Widner, Anal. Chem. 64 (1992), 1926-1932. [3] M.U. Kopp, A.J. deMello, A. Manz, Science 280 (1998), 1046-1048. [4] W. Ehrfelds (ed.) Proceedings of the 1st International Conference on Microreplication technology, Springer Heidelberg, 1998. [5] P.C. Simpson, D. Roach, A.T. Woolley, T. Thorsen, R. Johnston, G.F. Sensabaugh, R.A. Mathies, Proc. Natl. Acad. Sci. USA 95 (1998), 2256-2261 [6] M.A. Roberts, J.S. Rossier, P. Bercier, H. Girault, Anal. Chem. 69 (11) (1997), 2035-2042. [7] R.M. McCormick, R.J. Nelson, M.G. Alonso-Amigo, D.J. Benvegna, H.H. Hooper, Anal. Chem. 69 (1997), 2626-2630. [8] C.S. Effenhauser, G.J.M. Bruin, A. Paulus, M.Ehrat, Proceedings  $\mu\text{TAS}$  '96, Anal. Methods Instrum. Special Issue (1996), 124-125. [9] L. Martynova, L.E. Locasico, M. Gaitan, G.W. Kramer, R.G. Christensen, W.A. MacCrehan, Anal. Chem. 69 (1997), 4783-4789.

#### SESSION N2: IN-ROOM POSTER SESSION

Chair: Frantisek Svec  
Monday Morning, April 24, 2000  
10:00 AM  
Franciscan III (Argent)

#### N2.1

THE SURFACE PROPERTIES OF TETRADECYLTRI-METHYLAMMONIUM BROMIDE OBSERVED BY CAPILLARY ELECTROPHORESIS. D.L. Cocks, R. Schennach and Z. Yu, Gill Chair of Chemistry and Chemical Engineering, Lamar University, Beaumont, TX.

Capillary electrophoresis has been used to follow the electroosmotic flow as a function of tetradecyltrimethylammonium bromide (TTAB) concentration and shown to have distinct structural zones that depend on the pH. The data is discussed in terms of previously 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 non-coulombic in nature. Although the structural nature of the various adsorbate states remains an open question, the observations in this work and the possible corresponding surface structures have very broad implications in the science and technology of surfactant adsorption.

#### N2.2

MOLECULAR IMPRINTING WITHOUT TEMPLATE? Ken Hosoya, Masashi Teramachi, Tohru Ikegami, Nobuo Tanaka, Kyoto Institute of Technology, Dept. of Polymer Science, Kyoto, JAPAN.

Cross-linked polymer should remember the preparation conditions including polymerization temperature, porogen, et al, and this

memory affects molecular recognition ability if the polymer is used separation medium in high performance liquid chromatography (HPLC). Therefore, if we choose the preparation conditions very carefully, we can determine and control the nature of 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 also recognize to result in shape recognition. If 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-ICP-MS WITH DIFFERENT CHROMATOGRAPHIC CONDITIONS.** Amit Chatterjee, Yasuyuki Shibata, Jun Yoshinaga and Masatoshi Morita, National Institute for Environmental Studies, Environmental Chemistry Division, Ibaraki, Tsukuba, JAPAN.

The silica-based cation-exchange (LC-SCX, 20 mM pyridine pH-2.6); styrene-divinylbenzene copolymer-based PRP-X100 anion exchange (30 mM phosphate at pH 6.0); the ODS reversed-phase (10 mM tetraethylammonium hydroxide + 4 mM malonic acid in 0.05% methanol, pH 6.8) and the gel-permeating GS-220 (25 mM tetramethylammonium hydroxide + 25 mM malonic acid pH 6.8) columns, which are connected with the HPLC-ICP-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), methylarsonic acid (MA) and arsenic acid. The concentration of AB, estimated by standard addition method, found in the LC-SCX, and GS-220 columns are  $71.5 \pm 7.7$  (n=27) and  $72.6 \pm 8.13$  (n=9) mg l<sup>-1</sup>. The arsenous acid is not detected in the urine and is verified by using the LC-SAX1 anion exchange column. The high concentration of chloride that co-elutes with the arsenic acid from the LC-SCX and with the AB from the GS-220 columns is 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 exacting the chloride interference ( $Cl^{37}:Cl^{35} = 1:3.1271$ ) by subsequently measuring the Ar<sup>40</sup>Cl<sup>37</sup>. 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-SCX, the AB has been baseline separated from the other arsenic compounds and also from the chloride with the 20 mM pyridine at pH-2.60. So, the LC-SCX and GS-220 are recommended and used for the determination of AB in human urine.

### **N2.4**

**SEPARATION OF SIXTEEN POLYCYCLIC AROMATIC HYDROCARBONS BY CAPILLARY ELECTROCHROMATOGRAPHY USING A PHOTO-INITIATED MICROPOROUS POLYMER MONOLITH.** Wen-Yee Choi, Stanford University, Stanford, CA; Sarah Ngola, Timothy J. Sheppard, Deon S. Anex, Yolanda Fintschenko, Sandia National Laboratories, Livermore, CA.

As a step towards photodefinable materials for on-chip analytical separations, we have developed photoinitiated microporous polymers as monolithic stationary phases for capillary electrochromatography. These polymers are acrylate-based and contain 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS) to give the monoliths the surface charge required for electroosmotic flow. The synthesis is designed so that the polymerization reagents can be replaced with the required separation mobile phase using electroosmotic flow without an intervening pressure-driven rinse. Monoliths were polymerized in Teflon AF (TradeMark)-coated capillaries (360 micron o.d., 100 micron i.d.) using 365 nm for photoinitiation. These columns were coupled to laser-induced fluorescence detection using a 257-nm excitation beam from a frequency-doubled argon ion laser. Detection was off-column. Separation efficiencies greater than 150 000 plates/m were demonstrated using a standard test mixture containing sixteen polycyclic aromatic hydrocarbons (PAHs). Fifteen of the sixteen PAHs were resolved in 30 minutes using a 75:25 acetonitrile/Tris (pH 8) mixture as the mobile phase. Benz[a]anthracene and chrysene coeluted under these conditions. The relative standard deviation (n=3) in the retention time for the last peak, indeno[1,2,3-cd]pyrene, was 1.4%. The mobile phase could be changed rapidly and easily with full recovery of chromatographic capabilities. By changing the mobile

phase to 70:10:20 methanol/acetonitrile/Tris (pH 8), all 16 PAHs were resolved in 2.5 hours.

### **N2.5**

**MONODISPERSE, SPHERICAL AND NONPOROUS ZIRCONIA PARTICLES DESIGNED FOR ULTRAFAST LIQUID CHROMATOGRAPHY.** Bingwen Yan<sup>1</sup>, Clayton V. McNeff<sup>3</sup>, Fang Chen<sup>1</sup>, Peter W. Carr<sup>2</sup> and Alon V. McCormick<sup>1</sup>. <sup>1</sup>Department of Chemical Engineering, <sup>2</sup>Department of Chemistry, University of Minnesota, Minneapolis, MN, <sup>3</sup>ZirChrom Separations, Inc., Anoka, MN.

Monodisperse, spherical, nonporous particles, to be used for ultrafast chromatographic stationary phase, were synthesized by hydrolyzing alcohol solutions of zirconium tetra-alkoxides in the presence of long-chain carboxylic acids as first described by Lerot and co-workers. The critical steps for the production of highly monodisperse, well shaped particles have been identified and optimized to achieve a high yield of 1.8 micron particles. Scanning electron microscopy, confocal fluorescence microscopy and fluoride adsorption uptake measurements show that the final zirconia particles are highly monodisperse, spherical and nonporous. The surface area of the nonporous particles is only 1.7m<sup>2</sup>/g as inferred from the fluoride adsorption capacity. The work is consistent with Ogihara's suggestion which indicates that the particles grow via a surface reaction mechanism. The particles were then coated with carbon to form a novel reversed-phase type liquid chromatographic stationary phase which is used for environmental, pharmaceutical and biological analyses under ultrafast high temperature liquid chromatographic conditions.

### **SESSION N3:**

Chair: Rajeew Dadoo

Monday Afternoon, April 24, 2000

Franciscan II (Argent)

### **1:30 PM \*N3.1**

**CAPILLARY GEL ELECTROCHROMATOGRAPHY WITH REPLACEABLE MEDIA.** Mark R. Schure, 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 electroosmosis. Recently, the electroosmotic 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 electroosmotic flow from charged acid groups. In this way when a column is clogged, one simply renews 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 this work will be discussed including sample 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 Peterson, Ting Chen, Keith Tellman, 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 groups. 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 studied because of the range of chemistries that could be developed based on these backbones, and because successful development of silicone phases would make it possible to employ much of the stationary phase chemistry developed in the past thirty years. The challenges with silicone-based chemistries are to develop sufficiently water-soluble polymers with controlled chemistry and structure. Copolymers of 2-acrylamido-2-methyl-1-propane

sulfonic acid and hydrophobic monomers have also been developed. The various copolymers have been studied with respect to their chromatographic performance and selectivity.

### 2:30 PM N3.3

THE PREPARATION OF MONOLITHIC MEDIA WITH CONTROLLED POROUS PROPERTIES AND SURFACE FUNCTIONALITIES BY PHOTOINITIATED IN SITU POLYMERIZATION. A SUITABLE APPROACH TOWARDS STATIONARY PHASES FOR ELECTROCHROMATOGRAPHY ON A MICROCHIP. Cong Yu, Frantisek Svec, Jean M. J. Frechet, University of California, Dept of Chemistry, Berkeley, CA.

Electrochromatography is a technique in which the liquid mobile phase is driven through a separation device by electroosmotic flow 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 phase since it is difficult to pack particles efficiently 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 PM N3.4

NOVEL MACROPOROUS CONTINUOUS CHIRAL STATIONARY PHASES FOR ENANTIOSELECTIVE CAPILLARY ELECTROCHROMATOGRAPHY (CEC). M. Lämmerhofer, F. Svec, J.M.J. Fréchet, Department of Chemistry, University of California, Berkeley, CA; W. Lindner, Institute of Analytical Chemistry, University of Vienna, AUSTRIA.

Novel macroporous continuous chiral stationary phases for enantioselective capillary electrochromatography (CEC) have been prepared by in situ copolymerization of chiral monomer 1, O-[(2-methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine, 2-hydroxyethyl methacrylate (HEMA) or glycidyl methacrylate (GMA) as co-monomer, and ethylene dimethacrylate (EDMA) as cross-linker in the presence of 1-dodecanol/cyclohexanol as porogenic solvents within untreated fused silica capillaries. The polymerization is initiated 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 1-dodecanol/cyclohexanol proportion in the polymerization mixture. Further, it turned out that poly(1-co-HEMA-co-EDMA) monoliths exhibited much higher enantioselectivity and also higher efficiency than corresponding poly(1-co-GMA-co-EDMA) monoliths. For enantioselective CEC application, the quinidine carbamate groups play a double role: i.) Due to the basic quinidine the surface of the chiral monoliths is positively charged under CEC conditions yielding an anodic electroosmotic flow (EOF). ii.) It provides the chiral moiety for enantioselective anion exchange as chromatographic selectivity principle. These chiral monoliths showed reasonable enantioselectivity towards N-derivatized amino acids.

### 3:30 PM \*N3.5

OPEN TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY. Joseph Pesek, Maria Matyska, Department of Chemistry, San Jose State University, San Jose, CA.

In capillary electrochromatography 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 silanols present a serious problem for the separation of basic compounds. The use of an etched inner capillary wall followed by chemical modification of this new surface can provide a separation medium that overcomes the problems of packed columns while allowing for improved resolution of many mixtures in comparison to ordinary HPCE. The etching process increases 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 solutes via a plug flow profile similar to HPCE rather than a parabolic flow which is encountered in pressure driven systems. The etched surface is characterized by photoelectron spectroscopy (ESCA) and the modified surface by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. Atomic force microscopy (AFM) 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 biomolecules will be used to illustrate the enhanced resolution 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 PM 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 diameter (75-150 micron) capillaries filled with porous media 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 high back pressures (several hundred bar), 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 nanoporous materials. Microporous materials with high zeta potentials in the mobile phase of choice are required for constructing efficient electrokinetic pumps for the HPLC. Nanoporous salt bridges are used to transfer solvent 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 optimal speed and selectivity for a given separation. In addition to presenting isocratic and gradient separations, we will discuss a variety of materials and approaches we have taken to make our new separation system applicable to a wide variety analytes and separation modes. Recent developments of microfabricated systems with on-chip pumps and optimized connections and injections will also be presented.

### 4:15 PM N3.7

CHEMICAL AND MATERIALS CONTROL IN ULTRASMALL VOLUMES: EXPLOITING ELECTROKINETIC EFFECTS. Mark A. Hayes, Nolan A. Polson, Nanette K. Hartley, Arizona State University, Department of Chemistry and Biochemistry, Tempe, AZ.

Control of ultrasmall volumes (pico- to femtoliters) of fluids 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 functional. 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. However, to truly exploit the possibilities of small volume manipulations these two effects must be independently controlled. 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 electrochemical phenomenon 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 electroosmotic flow can allow for unique manipulation of materials. These include the concentration of chemically specific materials in picoliter 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 micron). 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/ electrophoretic migration phenomenon. Data will be shown in standard fused silica capillaries and on microfabricated devices.

### 4:30 PM N3.8

REPRODUCIBLE GRADIENTS AT ULTRA-LOW FLOW RATES. Susan M. Steinike, Curtis R. Campbell, Shimadzu Scientific Instruments, Inc., Columbia, MD.

A growing number of analysts are using techniques such as microbore HPLC and tandem LC-MS which require flow rates from 5-250  $\mu$ L/minute. Several factors must be considered when working in this range: appropriate back pressure for reliable pump performance, efficient mixing in a very limited volume, and reduction of dead

volume to minimize run time and bandspreading. We are able to generate accurate, reproducible linear and step gradients at flow rates as low as 5  $\mu\text{L}/\text{minute}$ . 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  $\mu\text{L}$  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.