
USF Patents

May 2011

Sol-gel monolithic column with optical window and method of making

Abdul Malik

James D. Hayes

Follow this and additional works at: https://digitalcommons.usf.edu/usf_patents

Recommended Citation

Malik, Abdul and Hayes, James D., "Sol-gel monolithic column with optical window and method of making" (2011). *USF Patents*. 399.

https://digitalcommons.usf.edu/usf_patents/399

This Patent is brought to you for free and open access by Digital Commons @ University of South Florida. It has been accepted for inclusion in USF Patents by an authorized administrator of Digital Commons @ University of South Florida. For more information, please contact digitalcommons@usf.edu.



US007947174B2

(12) **United States Patent**
Malik et al.

(10) **Patent No.:** **US 7,947,174 B2**
(45) **Date of Patent:** ***May 24, 2011**

(54) **SOL-GEL MONOLITHIC COLUMN WITH OPTICAL WINDOW AND METHOD OF MAKING**

(75) Inventors: **Abdul Malik**, Tampa, FL (US); **James D. Hayes**, Tampa, FL (US)

(73) Assignee: **University of South Florida**, Tampa, FL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/203,586**

(22) PCT Filed: **Feb. 9, 2001**

(86) PCT No.: **PCT/US01/04271**

§ 371 (c)(1),

(2), (4) Date: **Oct. 16, 2002**

(87) PCT Pub. No.: **WO01/58562**

PCT Pub. Date: **Aug. 16, 2001**

(65) **Prior Publication Data**

US 2003/0213732 A1 Nov. 20, 2003

Related U.S. Application Data

(60) Provisional application No. 60/181,371, filed on Feb. 9, 2000, provisional application No. 60/181,642, filed on Feb. 10, 2000.

(51) **Int. Cl.**

B01D 15/08 (2006.01)

(52) **U.S. Cl.** **210/198.2; 210/502.1; 210/635; 210/656**

(58) **Field of Classification Search** 210/635, 210/656, 659, 198.2, 502.1; 204/450, 600; 422/70; 436/161, 178

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,509,964 A	4/1985	Hubball et al.	
4,675,300 A *	6/1987	Zare et al.	204/452
4,966,785 A	10/1990	Springston	
5,128,291 A	7/1992	Wax et al.	
5,192,406 A	3/1993	Woolley	
5,240,577 A *	8/1993	Jorgenson et al.	210/656
5,262,052 A	11/1993	Rossiter et al.	
5,270,027 A	12/1993	Balducci et al.	

(Continued)

FOREIGN PATENT DOCUMENTS

WO WO-00-11463 3/2000

(Continued)

OTHER PUBLICATIONS

Merriam-Webster's Collegiate Dictionary, Tenth Edition, Merriam-Webster, Incorporated, Springfield Massachusetts 1998, p. 555.*

(Continued)

Primary Examiner — Ernest G Therkorn

(74) *Attorney, Agent, or Firm* — Saliwanchik, Lloyd & Eisenschenk

(57)

ABSTRACT

A method of preparing a sol-gel monolithic column includes the step of forming a separation bed (14) from a sol-gel solution in a single process step. This column has improved characteristics for CEC based on its incorporated surface charge and ease of operation due to a lack of or need for end frits. Also, a second type of column includes an optical window (30) for on-column detection.

23 Claims, 7 Drawing Sheets



U.S. PATENT DOCUMENTS

5,308,495	A	5/1994	Avnir et al.	
5,522,994	A *	6/1996	Frechet et al.	210/635
5,589,396	A	12/1996	Frye et al.	
5,624,875	A *	4/1997	Nakanishi et al.	501/39
5,637,135	A	6/1997	Ottenstein et al.	
5,691,206	A	11/1997	Pawliszyn	
5,869,152	A	2/1999	Colon	
6,136,187	A *	10/2000	Zare et al.	210/198.2
6,344,242	B1	2/2002	Stolk et al.	
6,613,234	B2	9/2003	Voute et al.	
6,759,126	B1	7/2004	Malik et al.	
6,783,680	B2	8/2004	Malik	
6,998,040	B2 *	2/2006	Malik et al.	210/198.2
2003/0075447	A1	4/2003	Malik et al.	
2004/0129141	A1	7/2004	Malik et al.	
2005/0106068	A1	5/2005	Malik et al.	
2006/0013981	A1	1/2006	Malik et al.	
2006/0013982	A1	1/2006	Malik et al.	
2006/0113231	A1	6/2006	Malik	
2007/0062874	A1	3/2007	Malik et al.	
2007/0095736	A1	5/2007	Malik et al.	
2007/0172960	A1	7/2007	Malik et al.	

FOREIGN PATENT DOCUMENTS

WO	WO 00/11463	3/2000
WO	WO-02-072225	9/2002
WO	WO-02-094410	11/2002

OTHER PUBLICATIONS

- Zhang (Journal of Liquid Chromatography, 18(17), 3373-3396 (1995).*
- Behnke et al., "Pressurized gradient electro-high-performance liquid chromatography," J. Chromatogr. A. 680:93-98, 1994.
- Behnke et al., "Evaluation of the parameters determining the performance of electrochromatography in packed capillary columns," J. Chromatogr. A. 716:207-213, 1995.
- Colon et al., "Ninth International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques," (HPCE '97). Anaheim, California 1997. Abstract.
- Dittmann et al., "Capillary electrochromatography—a high-efficiency micro-separation technique," J. Chromatogr. A. 744:63-74, 1996.
- Dulay et al., "Automated capillary electrochromatography: reliability and reproducibility studies," J. Chromatogr. A. 725:361-366, 1996.
- Ericson et al., "Preparation of continuous beds for electrochromatography and reversed-phase liquid chromatography of low-molecular-mass compounds," J. Chromatogr. A. 767:33-41, 1997.
- Ericson et al., "Reversed-Phase Electrochromatography of Proteins on Modified Continuous Beds Using Normal-Flow and Counterflow Gradients. Theoretical and Practical Considerations," Anal. Chem. 71:1621-1627, 1999.
- Frame et al., "Simplification of capillary electrochromatography procedure's," J. Chromatogr. A. 798:243-249, 1998.
- Fujimoto et al., "Charged Polyacrylamide Gels for Capillary Electrochromatographic Separations of Uncharged, Low Molecular Weight Compounds," Anal. Chem. 67:2050-2053, 1995.
- Fujimoto et al., "Fritless Packed Columns for Capillary Electrochromatography: Separation of Uncharged Compounds on Hydrophobic Hydrogels," Anal. Chem. 68:2753-2757, 1996.
- Hayes et al., "Sol-Gel Monolithic Columns with Reversed Electroosmotic Flow for Capillary Electrochromatography," Anal. Chem. 72:4090-4099, 2000.
- Hileman et al., "In Situ Preparation and Evaluation of Open Pore Polyurethane Chromatographic Columns," Anal. Chem. 45:1126-1130, 1973.
- Hjerten et al., "High-performance liquid chromatography on continuous polymer beds," J. Chromatogr. 473:273-275, 1989.
- Hjerten et al., "Continuous beds: high-resolving, cost-effective chromatographic matrices," Nature 356:810-811, 1992.
- Ishizuka et al., "Designing monolithic double-pore silica for high-speed liquid chromatography," J. Chromatogr. A. 797:133-137, 1998.
- Jorgenson et al., "High-Resolution Separations Based on Electrophoresis and Electroosmosis," J. Chromatogr. A. 218:209-216, 1981.
- Knox et al., "Miniaturisation in Pressure and Electroosmotically Driven Liquid Chromatography: Some Theoretical Considerations," Chromatographia 24:135-143, 1987.
- Li et al., "Continuous Beds for Microchromatography: Cation-Exchange Chromatography," Anal. Biochem. 223:153-158, 1994.
- Ludtke et al., "Application of 0.5- μ m porous silanized silica beads in electrochromatography," J. Chromatogr. A. 786:229-235, 1997.
- Minakuchi et al., "Effect of domain size on the performance of octadecylsilylated continuous porous silica columns in reversed-phase liquid chromatography," J. Chromatogr. A. 797:121-131, 1998.
- Moffatt et al., "Capillary Electrochromatography. Abnormally High Efficiencies for Neutral-Anionic Compounds under Reversed-Phase Conditions," Anal. Chem. 71:1119-1124, 1999.
- Nakanishi et al., "Double Pore Silica Gel Monolith Applied to Liquid Chromatography," J. Sol-Gel Sci. and Tech. 8:547-558, 1997.
- Palm et al., "Macroporous Polyacrylamide/Poly(ethylene glycol) Matrixes as Stationary Phases in Capillary Electrochromatography," Anal. Chem. 69:4499-4507, 1997.
- Peters et al., "Molded Rigid Polymer Monoliths as Separation Media for Capillary Electrochromatography," Anal. Chem. 69:3646-3649, 1997.
- Peters et al., "Molded Rigid Polymer Monoliths as Separation Media for Capillary Electrochromatography. 1. Fine Control of Porous Properties and Surface Chemistry," Anal. Chem. 70:2288-2295, 1998.
- Pietrzyk et al., "Ion Exchangers," Packings and Stationary Phases in Chromatographic Techniques; Unger, K.K., vol. 47, Marcel Dekker, Inc.: New York and Basel, 1990, Chapter 10.
- Pretorius et al., "Electro-Osmosis a New Concept for High-Speed Liquid Chromatography," J. Chromatogr. 99:23-30, 1974.
- Seifar et al., "Capillary electrochromatography with 1.8- μ m ODS-modified porous silica particles," J. Chromatogr. A. 808:71-77, 1998.
- Smith et al., "Capillary zone electrophoresis in pharmaceutical and biomedical analysis," Journal of Pharmaceutical & Biomedical Analysis 12:579-611, 1994.
- Svec et al., "Continuous Rods of Macroporous Polymer as High-Performance Liquid Chromatography Separation Media," Anal. Chem. 64:820-822, 1992.
- Svec et al., "Modified poly(glycidyl methacrylate-co-ethylene dimethacrylate) continuous rod columns for preparative-scale ion-exchange chromatography of proteins," J. Chromatogr. A. 702:89-95, 1995.
- Tang et al., "Continuous Bed Columns Containing Sol-Gel Bonded Large-Pore Octadecylsilica for Capillary Electrochromatography," J. Microcol. Sep. 11:550-561, 1999.
- Tsuda, T., "Electrochromatography Using High Applied Voltage," Anal. Chem. 59:521-523, 1987.
- Van Den Bosch, "Experiences with packed capillary electrochromatography at ambient pressure," J. Chromatogr. A. 755:165-177, 1996.
- Wang et al., "Macroporous Polymeric Stationary-Phase Rod as Continuous Separation Medium for Reversed-Phase Chromatography," Anal. Chem. 65:2243-2248, 1993.
- Brinker, C.J.; Scherer, G.W., *Sol-gel Science: The Physics and Chemistry of Sol-gel Processing*, Academic Press, San Diego, CA 1990.
- Chong, S.; Wang, D.; Hayes, J.D.; Wilhite, B.W.; Malik, A., *Sol-Gel Coating Technology for the Preparation of Solid-Phase Microextraction Fibers of Enhanced Thermal Stability*, Anal. Chem., 1997, 69, 3889-3898.
- Cortes, H.J.; Pfeiffer, C.D.; Richter, B.E.; Stevens, T.S., *Porous Ceramic Bed Supports for Fused Silica Packed Capillary Columns Used in Liquid Chromatography*, J. High Resolut. Chromatogr. And Chromatogr. Commun., 1987, 10, 446-448.
- Dulay, M.T.; Kulkarni, R.P.; Zare, R.N., *Preparation and Characterization of Monolithic Porous Capillary Columns Loaded with Chromatographic Particles*, Anal. Chem., 1998, 70, 23, 5103-5107.
- Fields, S.N., *Silica Xerogel as a Continuous Column Support for High-Performance Liquid Chromatography*, Anal. Chem., 1996, 68, 2709-2712.

- Hayes, J.D.; Malik, A., *Sol-gel chemistry-based Ucon-coated columns for capillary electrophoresis*, *J. Chromatogr. B.*, 1997, 695, 3-13.
- Malik, A.; Chong, S. in Pawliszyn, J. (ed.), *Sol-Gel Technology for Thermally Stable Coatings in SPME, Applications of Solid Phase Microextraction*, Royal Society of Chemistry, 1999, United Kingdom, Ch. 6, pp. 73-91.
- Aichholz, R., "Preparation of Glass Capillary Columns Coated with OH-Terminated (3,3,3-Trifluoropropyl) Methyl Polysiloxane (PS 184.5)", *Journal of High Resolution Chromatography*, 13, 71-73 (1990).
- Alltech, *Chromatography Catalog*, 172 (1997).
- Altgelt, K. et al., *Chromatography in Petroleum Analysis*, Marcel Dekker, Inc., New York and Basel (1979).
- Albin, M. et al. "Sensitivity Enhancement for Capillary Electrophoresis", *Analytical Chemistry*, 65, 489-497A (1993).
- Belardi, R. et al., "The Application of Chemically Modified Fused Silica Fibers in the Extraction of Organics from Water Matrix Samples and their Rapid Transfer to Capillary Columns", *Water Pollut. Res. J. Can.*, 24, 179-191 (1989).
- Berezkin, V.G. et al., "Capillary Columns with Several Layers of Different Immobilized Stationary Phases", *J. Anal. Chem.—USSR*, 47, 600-604 (1992).
- Berezkin, V.G. et al., *Gas Chromatography in Air Pollution Analysis*, Elsevier, Amsterdam—Oxford—New York—Tokyo, Chapter 8, 165-207 (1991).
- Bigham, S. et al. "Sol-Gel Capillary Microextraction", *Anal. Chem.* 74, 752-761 (2002).
- Blau, K. et al., eds., *Handbook of Derivatives for Chromatography*, 2nd ed., John Wiley & Sons, Chichester—New York—Brisbane—Toronto—Singapore, 1-30 (1993).
- Blomberg, L. et al., "Modification of Glass Capillary Columns by Cyclic (3,3,3-Trifluoropropyl)methylsiloxanes", *Journal of HRC & CC*, 3, 527-528 (1980).
- Brinker, C.J. et al., *Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing*, Academic Press, San Diego—New York—Boston—London—Sydney—Tokyo—Toronto, 97-233 (1990).
- Britz-McKibbin, P. et al. "Selective Focusing of Catecholamines and Weakly Acidic Compounds by Capillary Electrophoresis Using a Dynamic pH Junction", *Anal. Chem.*, 72, 1242-1252 (2000).
- Britz-McKibbin, P. et al. "On-Line Focusing of Flavin Derivative Using Dynamic pH Junction-Sweeping Capillary Electrophoresis with Laser-Induced Fluorescence Detection", *Anal. Chem.*, 74, 3736-3743 (2002).
- Brown, P. B. et al. "The Separation and the Characterization of Long Chain Fatty Acids and Their Derivatives by Reversed Phase High Performance Liquid Chromatography", *Analytical Chemistry*, 21, 193-208, (1989).
- Cao, C.-X. et al. "Stacking Ionizable Analytes in a Sample Matrix with High Salt by a Transient Moving Chemical Reaction Boundary Method in Capillary Zone Electrophoresis", *Anal. Chem.* 74, 4167-4174 (2002).
- Chen, Z. et al. "Chemically Modified chiral monolithic Silica Column Prepared by a Sol-Gel Process for Enantiomeric Separation by Micro High-Performance Liquid Chromatography", *J. Chromatogr.*, 942, 83-91 (2002).
- Chien, R.-L. et al. "Sample Stacking of an Extremely Large Injection Volume in High-Performance Capillary Electrophoresis", *Anal. Chem.*, 64, 1046-1050 (1992).
- Chien, R.-L. et al. "On-Column Sample Concentration Using Field Amplification in CZE", *Anal. Chem.*, 64, 489A-496A (1992).
- Chun, M.-S. et al. "Protein Analysis with Large Volume Sample Stacking with an Electroosmotic Flow Pump: A Potential Approach for Proteomics", *Microchem. J.*, 70, 247-253 (2001).
- Church, M. N. et al. "Transient Isotachophoretic-Electrophoretic Separations of Lanthanides with Indirect Laser-Induced Fluorescence Detection", *Anal. Chem.*, 70, 2475-2480 (1998).
- Cifuentes, A. et al. "Capillary Isoelectric Focusing of Erythropoietin Glycoforms and its Comparison with Flat-bed Isoelectric Focusing and Capillary Zone Electrophoresis", *J. Chromatogr.*, 830, 453-463 (1999).
- Clarke, N. J. et al. "Capillary Isoelectric Focusing-Mass Spectrometry: Analysis of Protein Mixtures from Human Body Fluids", *Biomed. Chromatogr.*, 16, 287-297 (2002).
- Collinson, M. M. et al. "Sol-gels and Electrochemistry", *Analytical Chemistry*, 72, 702A-709A (2000).
- Coulbaly, K. et al. "An overview of Solid-Phase Extraction of Food Flavor Compounds and Chemical Residues", *Food Rev. Int.*, 12, 131-151 (1996).
- Deng, Y. et al. "Chip-Based Quantitative Capillary Electrophoresis/Mass Spectrometry Determination of Drugs in Human Plasma", *Anal. Chem.*, 73, 1432-1439 (2001).
- Ettre, L.S., "Performance of Open Tubular Columns as a Function of Tube Diameter and Liquid Phase Film Thickness", *Chromatographia*, 18, 477-488 (1984).
- Ettre, L.S. et al, *Basic Relationships of Gas Chromatography*, Advanstar, Cleveland, OH, 1-34 (1993).
- Feroli, V. et al., "High-Performance Liquid Chromatography of Dihydroxyacetone as its bis-2,4-Dinitrophenylhydrazone Derivative", *Chromatographia*, 41, 61-65 (1995).
- Guo, Y. et al. "A Stationary Phase for Open Tubular Liquid Chromatography and Electrochromatography Using Sol-Gel Technology", *Anal. Chem.*, 67, 2511-2516 (1995).
- Hamlet, C. et al., "Novel Sol-Gel Dendrimer Coatings for Ultra-Trace Environmental Analysis by Capillary Microextraction Coupled to Gas Chromatography", *5th International Symposium on Advances in Extraction Technologies*, St. Pete Beach, FL (Mar. 5-7, 2003).
- Hartmann, H. et al., "Trace Determination of Pesticides in Water by Coated Capillary Micro Extraction (CCME) and Reversed-Phase High Performance Liquid Chromatography", *Fresenius Environmental Bulletin*, 7, 96-103 (1998).
- Haruvy, Y. et al., "Sol-Gel Replication of Microoptical Elements and Arrays", *Chem. Mater.*, 9, 2604-2615 (1997).
- Hayes, J.D. et al., "Sol-Gel Monolithic Columns with Reversed Electroosmotic Flow for Capillary Electrochromatography", *Anal. Chem.*, 72, 4090-4099 (2000).
- Hayes, J.D. et al., "Sol-Gel Open Tubular ODS Columns with Reversed Electroosmotic Flow for Capillary Electrochromatography", *Anal. Chem.*, 73, 987-996 (2001).
- Hayes, J.D. et al., "Sol-Gel Chemistry-Based Columns for Capillary Electrophoresis", *J. Chromatogr. B*, 695, 3-13 (1997).
- Hiraoka, A. et al. "One-Step Capillary Isoelectric Focusing of the Proteins in Cerebrospinal Fluid and Serum of Patients with Neurological Disorders", *J. Chromatogr. A*, 961, 147-153 (2002).
- Hjertén, S. et al. "Adaptation of the Equipment for High-Performance Electrophoresis to Isoelectric Focusing", *J. Chromatogr.*, 346, 265-270 (1985).
- Huang, M. et al. "Charged Surface Coatings for Capillary Electrophoresis", *J. Microcol.*, 5, 199-205 (1993).
- Janák, K. et al., "Static Coating of Capillary Columns by Means of Liquefied Gases", *Journal of High Resolution Chromatography & Chromatography Communications*, 8, 843-847 (1985).
- Kameoka, J. et al. "A Polymeric Microfluidic Chip for CE/MS Determination of Small Molecules", *Anal. Chem.*, 73, 1935-1941 (2001).
- Kataoka, H. et al., "Automated In-Tube Solid-Phase Microextraction Coupled with Liquid Chromatography/Electrospray Ionization Mass Spectrometry for the Determination of β -Blockers and Metabolites in Urine and Serum Samples", *Anal. Chem.*, 71, 4237-4244 (1999).
- Kataoka, H. et al. "Simple and Rapid Determination of Amphetamine, Methamphetamine, and Their Methylenedioxy Derivatives in Urine by Automated In-Tube Solid-Phase Microextraction Coupled with Liquid Chromatography-Electrospray Ionization Mass Spectrometry", *Journal of Analytical Toxicology*, 24, 257-265 (2000).
- Kim, J.-B. et al. "On-line Sample Concentration in Micellar Electrokinetic Chromatography Using Cationic Surfactants", *J. Chromatogr. A*, 916, 123-130 (2001).
- Koivusalmi, E. et al., "Quantitative RP-HPLC Determination of Some Aldehydes and Hydroxyaldehydes as Their 2,4-Dinitrophenylhydrazone Derivatives", *Anal. Chem.*, 71, 86-91 (1999).
- Lichtenberg, J. et al. "Sample Preconcentration by Field Amplification Stacking for Microchip-Based Capillary Electrophoresis", *Electrophoresis*, 22, 258-271 (2001).

- Liu, Q. et al. "Poly(diallyldimethylammonium chloride) as a Cationic Coating for Capillary Electrophoresis", *J. Chromatogr. Sci.*, 36, 126-130 (1997).
- Locke, S. et al. "Techniques for the Optimization of Proteomic Strategies Based on Head Column Stacking Capillary Electrophoresis", *Anal. Chem.*, 72, 2684-2689 (2000).
- Lee, M. L. et al. "Fused Silica Capillary Column Technology for Gas Chromatography", *J. Chromatogr. Sci.*, 22, 136-142 (1984).
- Lopez-Avila, V. et al. "Evaluation of Soxhlet Extraction Procedure for Extracting Organic Compounds from Soils and Sediments", *J. AOAC International*, 76, 864-880 (1993).
- Mackenzie, J. D. et al. "Hybrid Organic-Inorganic Materials, The Sol-Gel Approach" in *ACS Symposium Series*, 585, 226-236 (1995).
- Majors, R. E. "Liquid Extraction Techniques for Sample Preparation", *LC GC International*, 10, 93-101 (1997).
- Malik, A. et al. "Advanced Sol-gel Column Technology for Condensed-phase Microseparations", 25 Proc. 19th International Symposium on Capillary Chromatography and Electrophoresis, Wintergreen, VA, USA, 54-55 (May 18-22, 1997).
- Martin, A. J. P. et al. "Displacement Electrophoresis", *Proc. Roy. Soc. Lond. A.*, 316, 493-514 (1970).
- Minnich, M. M. et al. "Extraction Methods for Recovery of Volatile Organic Compounds from Fortified Dry Soils", *J. AOAC International*, 79, 1198-1204 (1996).
- Mukherjee, S.P., "Supercritical Drying in Structural and Microstructural Evolution of Gels: A Critical Review", *Ultrastructure Processing of Advanced Ceramics*, J.D. MacKenzie and D.R. Ulrich, eds., John Wiley & Sons, New York—Chichester—Brisbane—Toronto—Singapore, 747-758 (1988).
- Nawrocki, J. "Silica Surface Controversies, Strong Adsorption Sites Their Blockage and Removal. Part I", *Chromatographia*, 31, 177-205 (1991).
- Novak, B. M. "Hybrid Nanocomposite Materials—Between Inorganic Glasses and Organic Polymers", *Advanced Materials*, 5, 422-433 (1993).
- Núñez, O. et al. "Sample Stacking with Matrix Removal for the Determination of Paraquat, Diquat and Diflufenoxuron in Water by Capillary Electrophoresis", *J. Chromatogr. A*, 912, 353-361 (2001).
- Oesterhelt, G. et al. "Analyse von Hydroxypivalaldehyd als Trimethylsilylderivat des Oxims mittels Gas-Chromatographie", *Fresenius Z. Anal. Chem.*, 321, Abstract (1985).
- Palkar, V.R., "Sol-Gel Derived Nanostructured γ -Alumina Porous Spheres as an Adsorbent in Liquid Chromatography", *NanoStructured Materials*, 11, 369-374 (1999).
- Pawliszyn, J., "Theory of Solid-Phase Microextraction", *Journal of Chromatographic Science*, 38, 270-278 (1999).
- Palmer, J. et al. "Stacking Neutral Analytes in Capillary Electrokinetic Chromatography with High-Salt Sample Matrices", *Anal. Chem.*, 72, 1941-1943 (2000).
- Palmer, J. et al. "A Universal Concept for Stacking Neutral Analytes in Micellar Capillary Electrophoresis", *Anal. Chem.*, 71, 1679-1687 (1999).
- Palmer, J. et al. "Electrokinetic Injection for Stacking Neutral Analytes in Capillary and Microchip Electrophoresis", *Anal. Chem.*, 73, 725-731 (2001).
- Quirino, J. P. et al. "Approaching a Million-Fold Sensitivity Increase in Capillary Electrophoresis with Direct Ultraviolet Detection: Cation-Selective Exhaustive Injection and Sweeping", *Anal. Chem.* 72, 1023-1030 (2000).
- Quirino, J. P. et al. "Sweeping of Analyte Zones in Electrokinetic Chromatography", *Anal. Chem.* 71, 1638-1644 (1999).
- Quirino, J. P. et al. "Exceeding 5000-Fold Concentration of Dilute Analytes in Micellar Electrokinetic Chromatography", *Science*, 282, 465-468 (1998).
- Quirino, J. P. et al. "On-line Concentration of Neutral Analytes for Micellar Electrokinetic Chromatography II. Reversed Electrode Polarity Stacking Mode", *J. Chromatogr. A*, 791, 255-267 (1997).
- Quirino, J. P. et al. "Sweeping with an Enhanced Electric Field of Neutral Analyte Zones in Electrokinetic Chromatography", *J. High Resol. Chromatogr.*, 22, 367-372 (1999).
- Quirino, J. P. et al. "On-line Concentration of Neutral Analytes for Micellar electrokinetic Chromatography 5. Field-Enhanced Sample Injection with Reversed Migrating Micelles", *Anal. Chem.*, 70, 1893-1901 (1998).
- Quirino, J. P. et al. "On-line Concentration of Neutral Analytes for Micellar Electrokinetic Chromatography I. Normal Stacking Mode", *J. Chromatogr. A*, 781, 119-128 (1997).
- Righetti, P. G. et al. "Study of haptoglobin-hemoglobin Complexes by Titration Curves, Capillary Electrophoresis and Capillary Isoelectric Focusing", *J. Chromatogr. A*, 767, 255-262 (1997).
- Rosenfeld, J. "Gas Chromatography Profiling in Biomedical Investigations" in *Chemical Analysis: Gas Chromatography*, Clement, R. E. (ed.), 111, 181-215 (1990).
- Shen, Y. et al. "High-Efficiency Capillary Isoelectric Focusing of Peptides", *Anal. Chem.*, 72, 2154-2159, (2002).
- Shende, C. et al. "Sol-Gel Poly(ethylene Glycol) Stationary Phase for High-Resolution Capillary Gas Chromatography", *Anal. Chem.*, 75, 3518-3530 (2003).
- Shihabi, Z. K. "Stacking in Capillary Zone Electrophoresis", *J. Chromatogr. A.*, 902, 107-117 (2000).
- Shihabi, Z. K. "Stacking and Discontinuous Buffers in Capillary Zone Electrophoresis", *Electrophoresis*, 21, 2872-2878 (2000).
- Shihabi, Z. K. "Transient Pseudo-Isotachopheresis for Sample Concentration in Capillary Electrophoresis", *Electrophoresis*, 23, 1612-1617 (2002).
- Shihabi, Z. K. et al. "Insulin Stacking in Capillary Zone Electrophoresis", *J. Chromatogr. A.*, 807, 129-133 (1998).
- Spanik, I. et al. "Use of Full-Column Imaging Capillary Isoelectric Focusing for the Rapid Determination of the Operating Conditions in the Preparative-Scale Continuous Free Flow Isoelectric Focusing Separation of Enantiomers", *J. Chromatogr. A.*, 960, 241-246 (2002).
- Strausbauch, M. A. et al. "Mechanism of Peptide Separation by Solid Phase Extraction Capillary Electrophoresis at Low pH", *Anal. Chem.*, 68, 306-314 (1996).
- Sumpter, S.R. et al. "Static Coating of 5 to 50 μ m I.D. Capillary Columns for open Tubular Column Chromatography", *J. Chromatogr.*, 517, 503-519 (1990).
- Sun, P. et al. "Chitosan Coated Capillary with Reserved Electroosmotic Flow in Capillary Electrophoresis for the Separation of Basic Drugs and Proteins", *J. Microcol. Sep.*, 6, 403-407 (1994).
- Toussaint, B. et al. "Enantiomeric Separation of Clenbuterol by transient Isotachopheresis-capillary zone Electrophoresis-UV Detection New Optimization Technique for Transient Isotachopheresis", *J. Chromatogr. A*, 173-180 (2000).
- Tu, C. et al. "Determination of Nitrate in Seawater by Capillary Zone Electrophoresis with Chloride-Induced Sample Self-Stacking", *J. Chromatogr. A.*, 966, 205-212 (2002).
- Veraart, J. B. et al. "At-Line Solid-Phase Extraction Coupled to Capillary Electrophoresis: Determination of Amphoteric Compounds in Biological Samples", *J. High Resol. Chromatogr.*, 22, 183-187 (1999).
- Wang, D. et al., "Sol-Gel Column Technology for Single-Step Deactivation, Coating, and Stationary-Phase Immobilization in High-Resolution Capillary Gas Chromatography", *Anal. Chem.*, 69, 4566-4576 (1997).
- Wei, W. et al. "One-Step Concentration of Analytes Based on Dynamic Change in pH in Capillary Zone Electrophoresis", *Anal. Chem.*, 74, 934-940 (2002).
- Woolley, C. L. et al. "Deactivation of Fused Silica Capillary Columns with Polymethylhydrosiloxanes", *J. High Resol. Chromatogr./Chromatogr. Comm.*, 7, 329-332 (1984).
- Ogden, M. W. et al. "Characterization of Fused-Silica Capillary Tubing By Contact Angle Measurements", *J. Chromatogr.*, 354, 7-18 (1986).
- Yang, H. et al. "Sample Stacking in Laboratory-on-a-chip Devices", *J. Chromatogr. A.*, 924, 155-163 (2001).
- Clifford, A. A. "Introduction to Supercritical Fluid Extraction in Analytical Science", in *Supercritical Fluid Extraction and its Use in Chromatographic Sample Preparation*, Westwood, S. A. (ed.), 1-38 (1993).
- Furton, K. G. et al. "The Use of Solid-Phase Microextraction-Gas Chromatography in Forensic Analysis", *Journal of Chromatographic Science*, 38, 297-306, (2000).

- Hayes, J. D. et al. "Sol-Gel Process Mediated Advanced Column Technology for Microcolumn Separations", *18th International Symposium on Capillary Chromatogr.*, 1, 496-504 (1996).
- Markides, K. E. et al. "Deactivation of Fused Silica Capillary Columns with Phenylhydrosiloxanes", *J. High Res. Chromatography & Chromatography Comm.*, 8, 378-384 (1985).
- Reighard, T. S. et al. "Bridging the Gap Between Supercritical Fluid Extraction and Liquid Extraction Techniques: Alternative Approaches to the Extraction of Solid and Liquid Environmental Matrices", *Critical Reviews in Analytical Chemistry*, 26(2&3), 61-99, (1996).
- Rotzsche, H. "Chemically Bonded Stationary Phases" in Stationary Phases in Gas Chromatography, *Journal of Chromatography Library*, vol. 48, 142-159, (1991), Elsevier.
- Schomburg, G. et al. "Alkylpolysiloxane Glass Capillary Columns Combining High Temperature Stability of the Stationary Liquid and Deactivation of the Surface; Thermal Treatment of Dealkalinized Glass Surfaces by the Stationary Liquid Itself", *Chromatographia*, 12(10), 651-660, (1979).
- Schutjes, C. P. M. et al. "Deactivation and Coating of Non-Polar 50µm I.D. Capillary Columns", *J. Chromatogr.*, 279, 49-57 (1983).
- Soderquist, A. Office Action mailed Jul. 2, 2003 in U.S. Appl. No. 09/763,419.
- Soderquist, A. Office Action mailed Jan. 5, 2004 in U.S. Appl. No. 09/763,419.
- Soderquist, A. Office Action mailed May 26, 2004 in U.S. Appl. No. 09/763,419.
- Soderquist, A. Office Action mailed Feb. 11, 2005 in U.S. Appl. No. 09/763,419.
- Soderquist, A. Office Action mailed Sep. 20, 2005 in U.S. Appl. No. 09/763,419.
- Stark, F. O. et al. "The Interactions between Trialkylsilanes and E-Glass or Aerosil Surfaces. Reactions of Trimethylsilanol, Trimethylchlorosilane, and Hexamethyldisilazane", *The Journal of Physical Chemistry*, 72(8), 2750-2754, (1968).
- Van Der Vlis, E. et al. "Combined liquid-liquid electroextraction and isotachopheresis as a fast on-line focusing step in capillary electrophoresis", *Journal of Chromatography A*, 687, 333-341, (1994).
- Vorotilov, K. A. et al. "ORMOSIL Films: Properties and Microelectronic Applications" *Journal of Sol-Gel Science and Technology*, 8, 581-584, (1997).
- Wang, D. et al. "Preparation and GC Performance of Sol-Gel Technology-Based Open Tubular Columns" *Eighteenth International Symposium on Capillary Chromatography*, vol. 1, May 20-24, 1996, pp. 505-513.
- Wang, Z. et al. "High-performance polyethylene glycol-coated solid-phase microextraction fibers using sol-gel technology" *Journal of Chromatography A*, 893, 157-168, (2000).
- Welsch, T. et al. "The Thermal Immobilization of Hydroxy-Terminated Silicone Phases in High-Temperature-Silylated Glass Capillaries. A Study of Reaction Mechanisms", *Journal of High Resolution Chromatography*, 14, 153-159, (1991).
- Wercinski, S.A.S. et al. "Solid Phase Microextraction Theory" in *Solid Phase Microextraction, a Practical Guide*, 1999, 1-26, Marcel Dekker, Inc., New York.
- Wilkes, G. L. et al. "'Ceramers': Hybrid Materials Incorporating Polymeric/Oligomeric Species into Inorganic Glasses Utilizing a Sol-Gel Approach", *Polymer Preprints* 26(2), 300-302 (1985).
- Wu, J. et al. "Polypyrrole-Coated Capillary Coupled to HPLC for In-Tube Solid-Phase Microextraction and Analysis of Aromatic Compounds in Aqueous Samples", *Anal. Chem.*, 73, 55-63, (2001).
- Wu, J. et al. "Polypyrrole-Coated Capillary In-Tube Solid Phase Microextraction Coupled with Liquid Chromatography-Electrospray Ionization Mass Spectrometry for the Determination of β -Blockers in Urine and Serum Samples", *J. Microcolumn Separations*, 12(4), 255-266, (2000).
- Wu, J. et al. "Speciation of Organoarsenic Compounds by Polypyrrole-Coated Capillary In-Tube Solid Phase Microextraction Coupled With Liquid Chromatograph/Electrospray Ionization Mass Spectrometry", *Analytica Chimica Acta*, 424, 211-222 (2000).
- Yakabe, Y. et al. "Immobilization Method for Polyethylene Glycol Using a Cross-linking Co-agent", *J. Chromatogr.*, 558, 323-327 (1991).
- Zapf, A. et al. "GC Analysis of Organic Acids and Phenols Using On-Line Methylation with Trimethylsulfonium Hydroxide and PTV Solvent Split Large Volume Injection", *J. High Resol. Chromatogr.*, 22, 83-88 (1999).
- Zeng, Z. et al. "Solid-Phase Microextraction Using Fused-Silica Fibers Coated with Sol-Gel-Derived Hydroxy-Crown Ether", *Anal. Chem.*, 73, 2429-2436 (2001).
- Zhang, J. et al. "Development of the Personal Aldehydes and Ketones Sampler Based upon DNSH Derivatization on Solid Sorbent", *Environ. Sci. Technol.*, 34, 2601-2607 (2000).
- Zhang, Z. et al. "Solid-Phase Microextraction", *Anal. Chem.* 66, 844A-853A (1994).

* cited by examiner

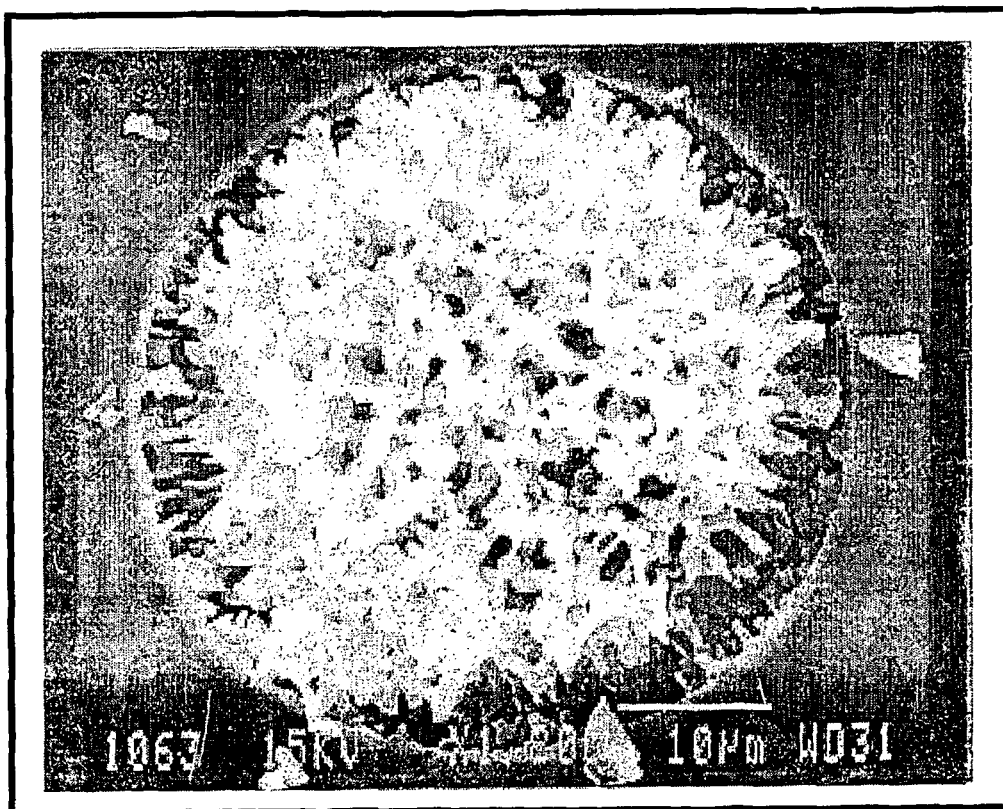


Figure 1

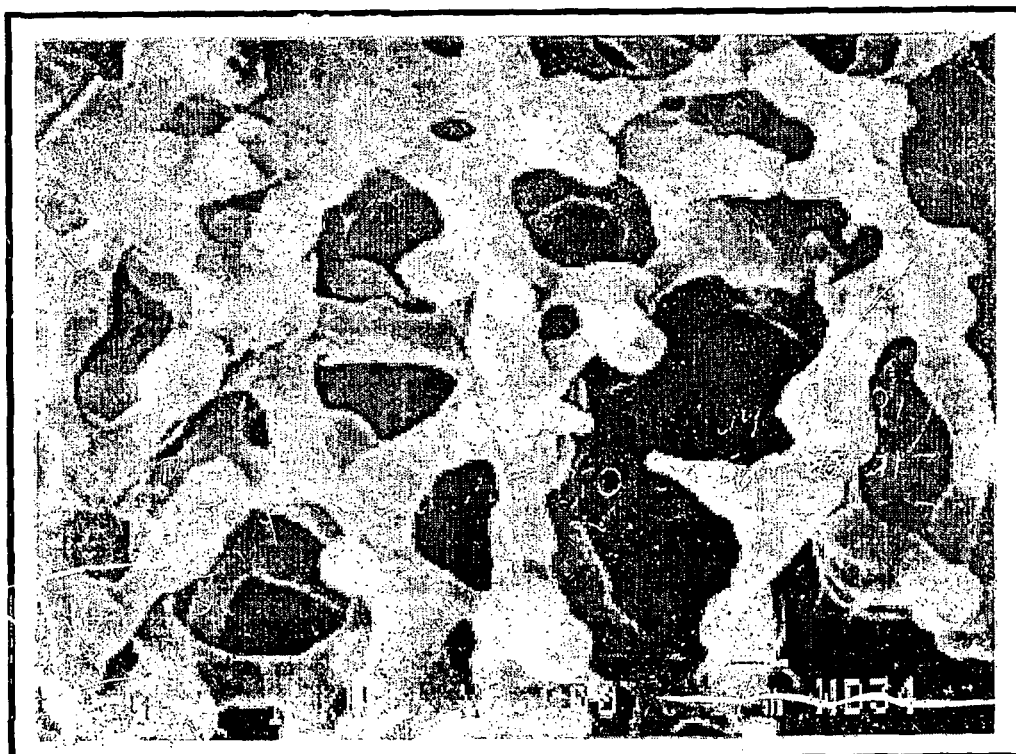


Figure 2

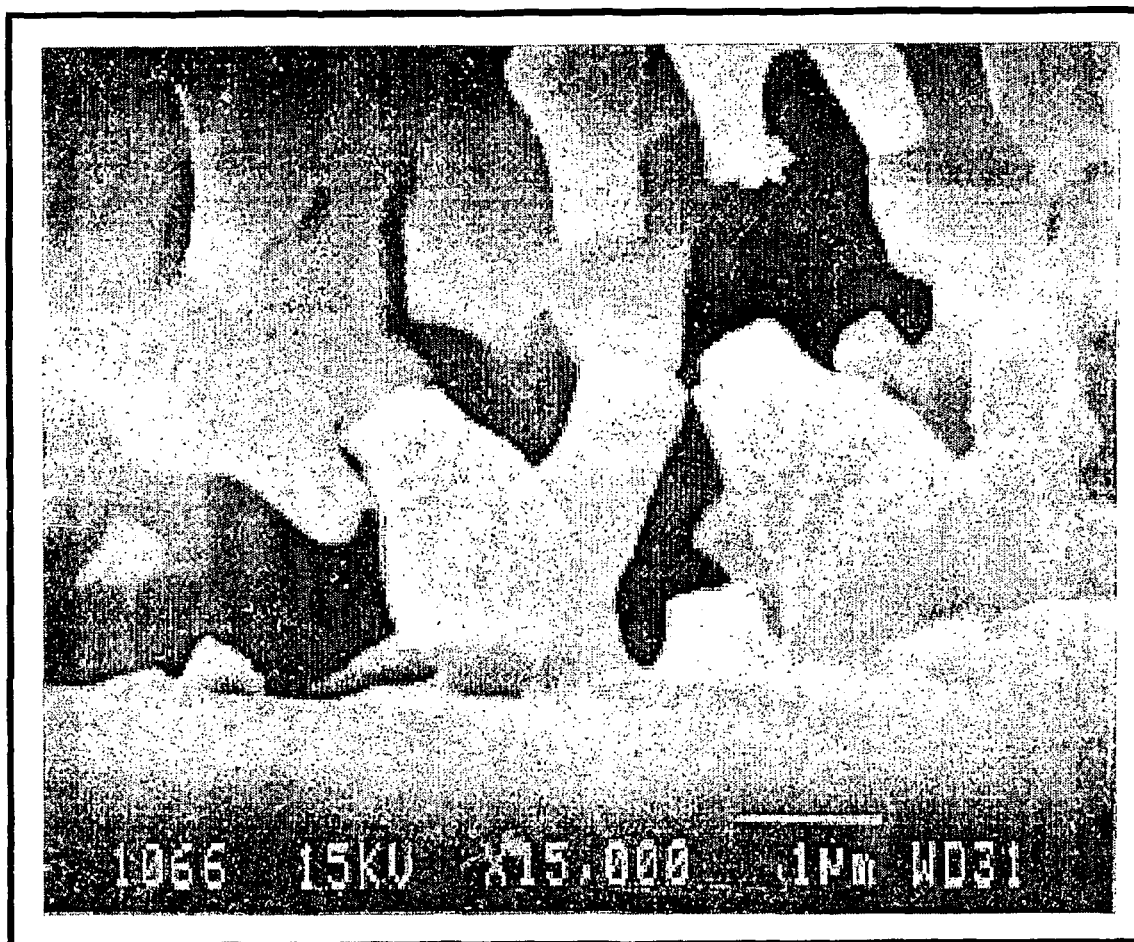


Figure 3

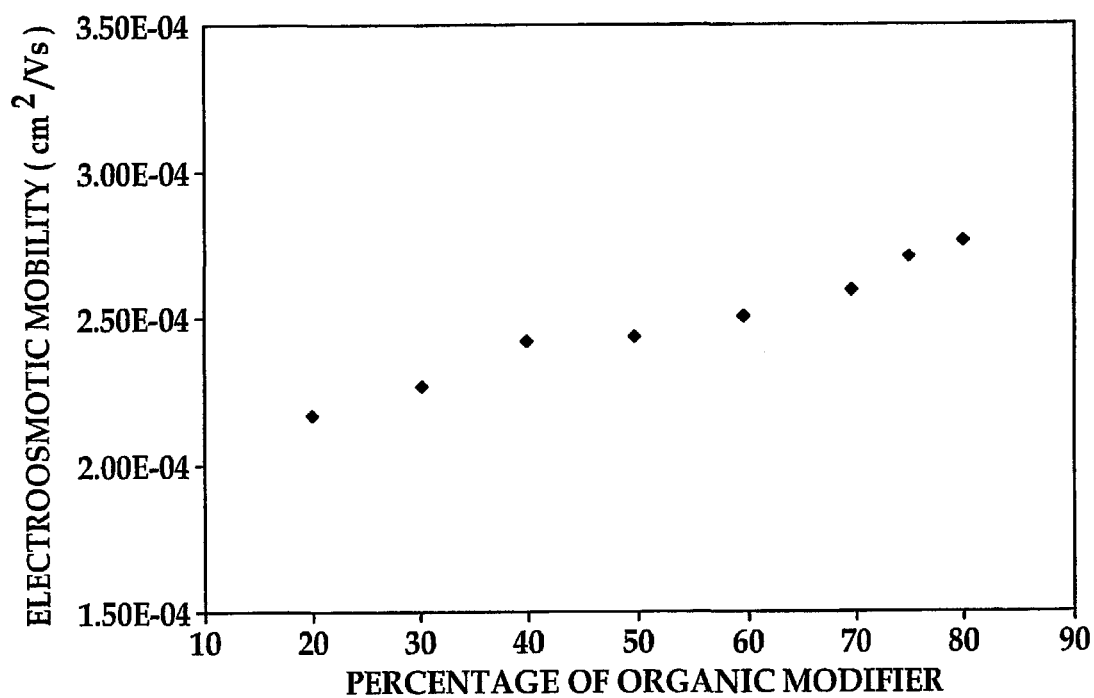


Figure 4

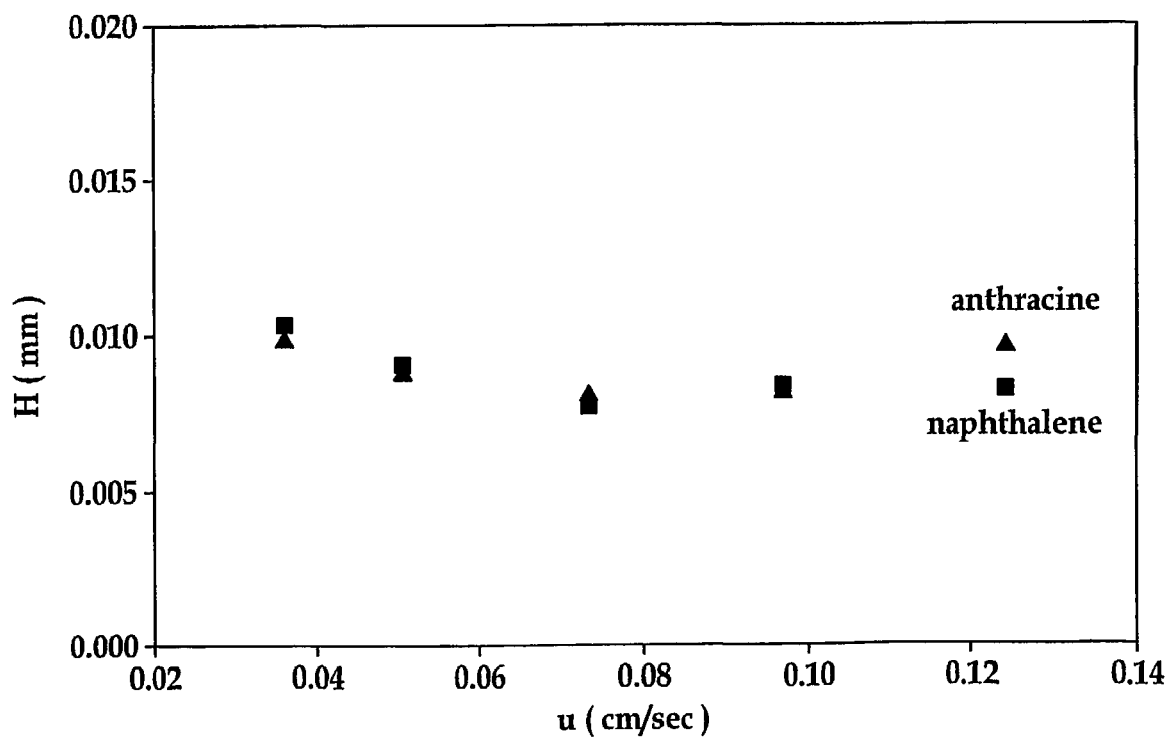


Figure 6

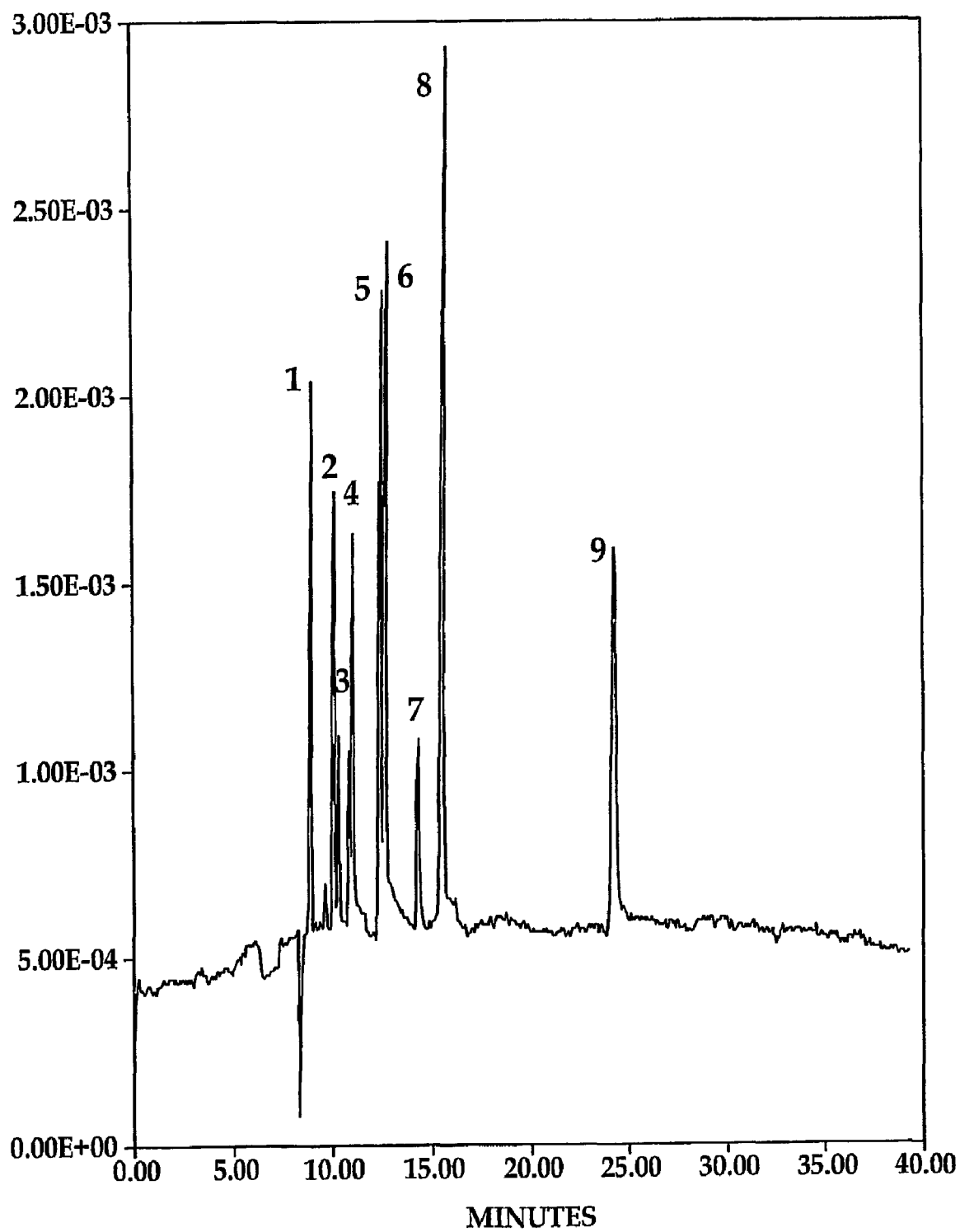


Figure 5

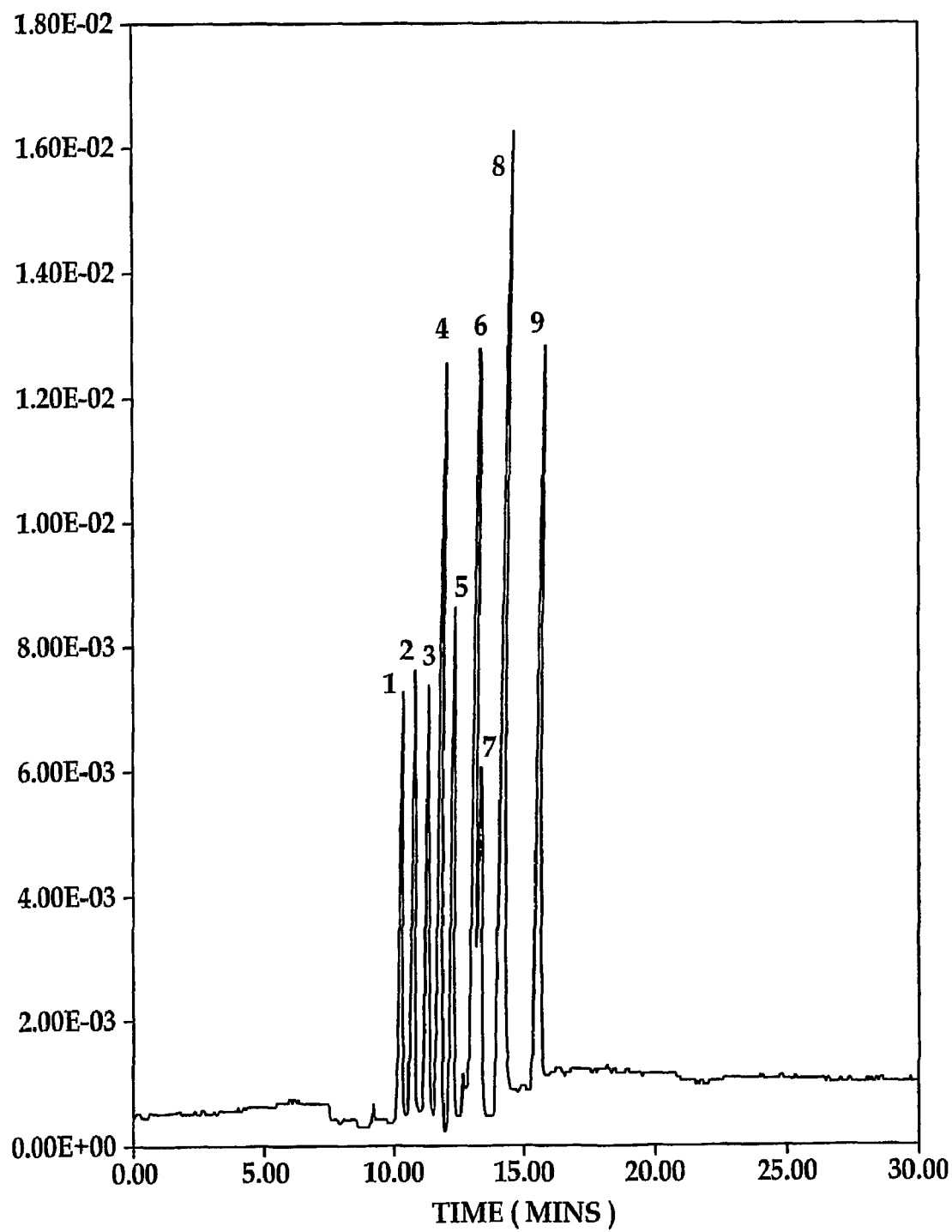


Figure 7

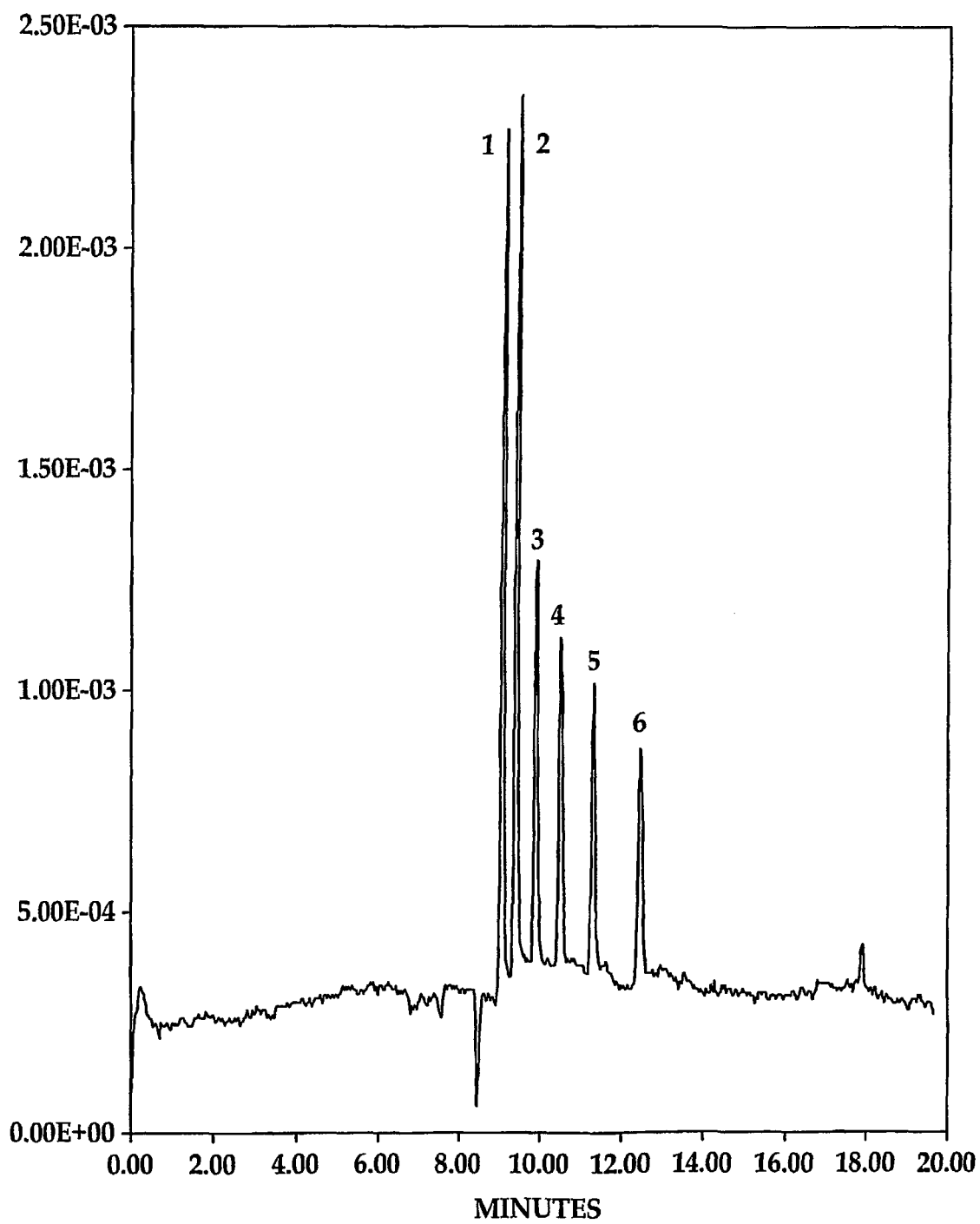


Figure 8

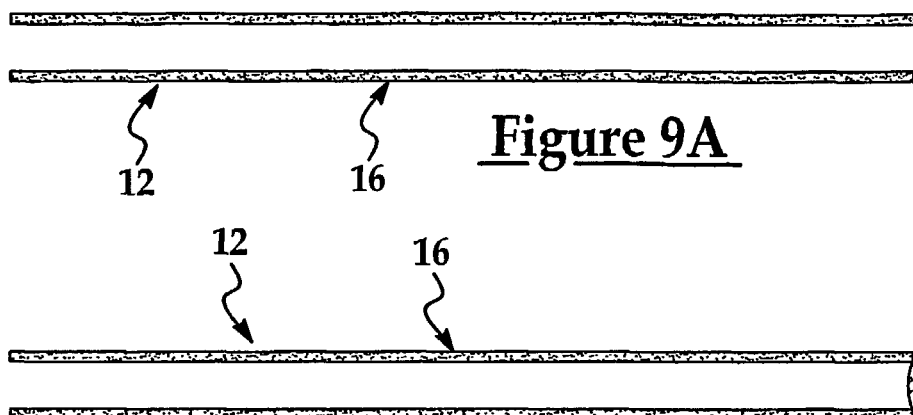


Figure 9A

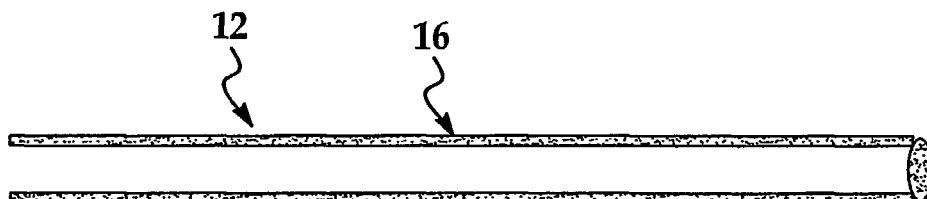


Figure 9B

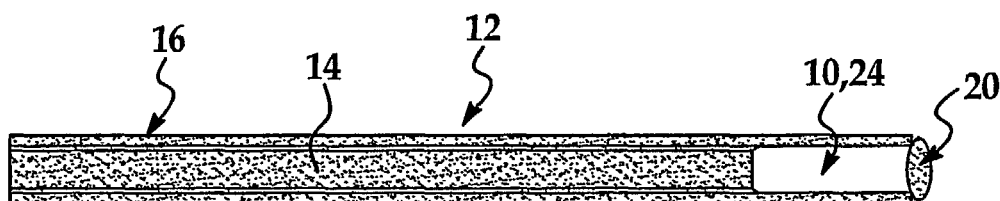


Figure 9C

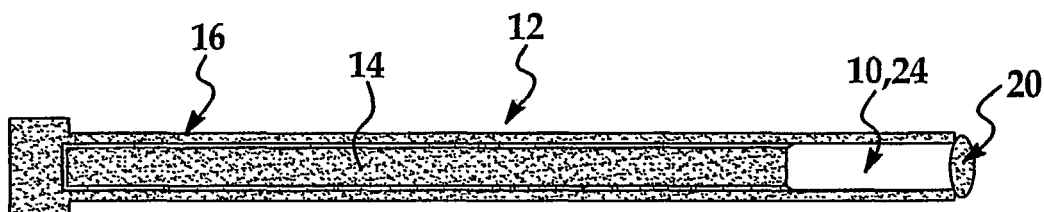


Figure 9D

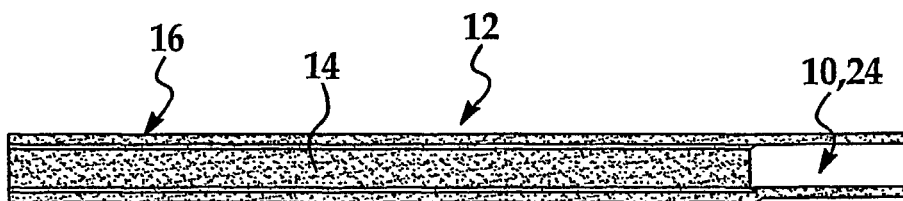


Figure 9E

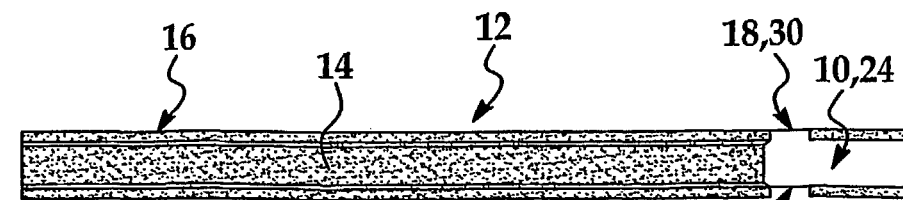


Figure 9F

1

SOL-GEL MONOLITHIC COLUMN WITH OPTICAL WINDOW AND METHOD OF MAKING

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is a National Phase Concerning a Filing Under 35 U.S.C. 371, claiming the benefit of priority of PCT/US01/04271, filed 9 Feb. 2001, which claims the benefit of priority of U.S. Provisional Application Ser. No. 60/181,371, filed 9 Feb. 2000 and U.S. Provisional Application Ser. No. 60/181,642, filed 10 Feb. 2000, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to columns and methods of making columns for separation techniques and apparatus. More specifically, the present invention provides a separation bed and method of making the same for use in various electromigration and non-electromigration separation columns, such as high-performance liquid chromatography, gas chromatography, capillary electrophoresis, capillary electrochromatography, and supercritical fluid chromatography.

2. Description of Related Art

Capillary electrochromatography or CEC is a fairly novel electrokinetic separation technique representing a hybrid of high-performance liquid chromatography or HPLC and capillary electrophoresis, known as CE. In CEC, the electroosmotic flow, or EOF is used to drive the mobile phase through the capillary, using typical HPLC mobile and stationary phases that provide the essential chromatographic interactions. Because of the flat plug-like profile of the electroosmotic flow, CEC offers greatly enhanced separation efficiencies relative to HPLC. Unlike CE, CEC is not restricted to charged solutes. Thus, the potential for CEC, as a separation technique, is much wider.

Capillary electrochromatography is a rapidly growing area in analytical separations. A great deal of research effort is currently being devoted to materialize the great analytical potential that this new hybrid technique has to offer. In order for CEC to achieve success as an independent chromatographic separation technique significant advancements are needed in the area of column technology. This is explained by the fact that in CEC, the column not only serves as the separation chamber, but also as the pumping device to drive the mobile phase through the system. This makes the column the "heart" of the CEC system both in the functional and literal sense of the word.

Two major types of columns are used in current CEC practice. These are packed and open tubular types. Packed columns comprise the predominant class of CEC columns. Most often the packed capillaries contain 1.5-5 μm , non-polar, octadecylated or ODS particles. The ODS particles possess both the chemically bonded octadecyl stationary phase, providing the essential chromatographic interactions, and the silanol moieties, responsible for the generation of electroosmotic flow to drive the mobile phase and the solutes through the packed capillary. The commercial availability of the ODS-bonded particles and the previously established liquid chromatography or LC separation protocols are two advantages attracting many researchers to use these packed capillaries in CEC. However, the most significant advantage of packed columns in CEC is the possibility of using small micrometer and nanometer size particles. High separation

2

efficiency during fast analysis is achieved in packed CEC columns without requiring ultra-high pressures, as in HPLC to drive the mobile phase through the columns packed with the small particles.

The greatest challenge is the preparation of a uniform packing bed using the small particles. Researchers currently use a variety of packing procedures ranging from slurry packing, electrokinetic, centripetal, and supercritical fluid packing methods. These all involve a plurality of steps to effect the packing process and even with close monitoring do not give as uniform a bed as desired for many applications.

Furthermore, a great degree of difficulty still remains associated with the ability to pack long, narrow bore capillaries. In addition, most packed capillaries require end frits of a different material to retain the packing particles within the packed capillary bed. Creation of those frits remains to be a problem in column preparation as these frits must be rigid enough to retain the packing particles under a wide range of column packing, rinsing and operating conditions. Yet these frits must also possess a highly porous structure to permit a uniform mobile phase flow through the entire cross-section of the column. A further problem arises in that the presence of the frit material makes the packing in the column non-homogeneous due to the presence of a different material and this can cause problems with the separation characteristics of the final column.

Monolithic column technology can effectively overcome both of the difficulties associated with conventional packed capillary column technology. In the monolithic approach, a continuous separation bed is created inside the capillary tube using a solution, which undergoes both chemical and physical changes in the capillary environment to produce the separation bed. In addition, the choice of appropriate chemistry allows the porous bed to chemically bond to the inner walls of the capillary by a condensation reaction and the resulting packed tube is also homogeneous in nature.

The use of monolithic columns has been reported in gas and liquid chromatography and is also currently being used in CEC to alleviate the extensive labor involved with packed column fabrication. Moreover, the greatest inherent advantage of the monolithic capillary columns is the elimination of the need for the end frits. The elimination of these end frits allows the entire column to remain homogeneous, rather than exhibiting different properties by the packing particles and the end frits. It has also been demonstrated that the end frits reduce the column's separation efficiency and are responsible for bubble formation during the analysis.

Although much simpler than particle packed capillaries, monolithic columns derived by organic polymerization also possess certain limitations. One critical drawback associated with this type of monolithic capillary is the tendency of the polymer network to swell during exposure to certain organic solvents, which are contained in the running mobile phase. This swelling may result in reductions in the permeability of the monolith as a result of alterations in the porosity of the monolith. Such structural change ultimately leads to changes in the column performance during the course of its use.

Unlike the monolithic separation beds from organic polymers, columns containing a porous silica-based monolithic matrix prepared through sol-gel chemistry do not suffer from the swelling phenomena thus offering a versatile and promising alternative to organic packed capillaries. In addition, monolithic columns, since they are prepared without end frits can produce a homogeneous separation column, which is highly desirable for a wide variety of separation techniques.

Pretorius was one of the first influential pioneers of CEC who, in 1974, demonstrated the advantages of electroosmosis

as a pumping mechanism for chromatographic separations. Jorgenson and Lukacs published CEC analyses of 9-methylanthracene and perylene on an ODS-packed capillary column. Meanwhile, a 1987 report by Tsuda demonstrated the possibility of achieving CEC separations by the simultaneous use of both electroosmotic and pressure-driven flows in the separation column. Yet Knox and Grant made another significant contribution to the development of this technique. Following this publication, the term "electrochromatography" became generally accepted and numerous researchers refocused their attention to CEC.

As described earlier, two types of monolithic columns have been developed: (1) organic polymer-based and (2) bonded silica-based. In the first approach, fabrication of a monolithic capillary column is accomplished by polymerization reaction of organic monomeric precursor(s). Hileman et al used Carbowax coated open pore polyurethane monolithic capillaries for the separations of several classes of analytes including aromatic hydrocarbons, aliphatic alcohols and metal chelates through gas chromatography. Hjerten et al prepared monolithic capillaries with compressed polyacrylamide gels for separation of proteins using HPLC and of low molecular mass compounds and basic proteins using CEC. Frechet and coworkers reported a series of publications on the use of methacrylate monomers for the preparation of HPLC and CEC monolithic capillaries through copolymerization. Palm and Novotny prepared CEC monoliths using mixtures of polyacrylamide/polyethylene glycol, derived with either C₄ or C₁₂ ligands, which were used to separate alkyl phenones and peptides. Additionally, Fujimoto et al reported the usage of cross-linked polyacrylamides for the separation of small dansylated amino acids and neutral steroids on monolithic CEC capillaries.

An alternative to a column with an organic polymer-based stationary phase column is one with a bonded silica stationary phase prepared by sol-gel chemistry. Cortes and coworkers prepared porous beds by polymerizing potassium silicate solutions in situ. The columns containing the porous beds were then packed with 5 μ m Spherisorb ODS particles for use in LC. Fields used solutions of potassium silicate and formamide to create a porous bed that was further reacted with dimethyloctadecylchlorosilane, and achieved plate heights of 65 μ m in LC.

Tanaka and coworkers used the sol-gel technique for the development of an octadecylsilylated, porous monolithic column for use in LC. In this study, poly(ethylene oxide), PEO, was incorporated into a mixture of tetramethoxysilane (TMOS) and acetic acid to develop porous silica rods, followed by an in-column octadecylsilylation reaction. Following washings, and drying at 50° C. for three days, the silica rods were then treated for two hours at 600° C.

Dulay et al used sol-gel technology for the preparation of monolithic columns loaded with 3 μ m ODS particles. The sol-gel solution served as a retaining matrix immobilizing and shielding the ODS stationary phase particles. Sol-gel capillary columns containing the ODS embedded particles yielded CEC separation efficiencies on the order of 80,000 plates/m (16,000 plates/column) for a test mixture of six uncharged polyaromatic hydrocarbons or PAHs.

Lee and coworkers also used sol-gel chemistry to glue 7 μ m ODS particles thereby creating a continuous large-pore CEC column. The sol-gel technology in this approach was used to create a bridge between adjacent particles, as well as the capillary wall and particles in its vicinity, thereby eliminating the need for retaining end-frits, thus result being efficient separations of small organic and aromatic amine compounds on such "sol-gel-glued" monolithic columns.

SUMMARY OF THE INVENTION

It is, therefore, an object of the invention to create a monolithic column from a sol-gel process.

It is also an object of the invention to prepare a sol-gel column having a porous, separation bed without the use of particles being incorporated into the bed.

It is a further object of the invention to create a sol-gel column in a single-step process that obviates the need for a plurality of processing steps.

It is another object of the invention to produce a monolithic sol-gel column, which is chemically bonded to the capillary wall.

It is a further object of the invention to produce a monolithic sol-gel column that does not require high temperature processing steps.

It is another object of the invention to produce a monolithic sol-gel column having an optical window useful for on-column detection studies of the analytes separated by the separation column, using various spectral techniques.

BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention are readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIG. 1 is a scanning electron micrograph of a sol-gel monolithic column, cross-sectional view, magnified 1,800 times;

FIG. 2 is a scanning electron micrograph of a sol-gel monolithic column, longitudinal view, magnified 7,000 times;

FIG. 3 is a scanning electron micrograph of a sol-gel monolithic column, longitudinal view, magnified 15,000 times;

FIG. 4 is a graph of the effect of the change of electroosmotic mobility with an increase in percentage of acetonitrile and Tris-HCl in a mobile phase;

FIG. 5 represents the CEC analysis of a mixture of PAHs on a 50 cm \times 50 μ l ODS sol-gel monolithic column;

FIG. 6 shows plate height versus flow rate within a sol-gel mediated ODS monolithic capillary;

FIG. 7 is a separation analysis of a mixture of benzene derivatives on a sol-gel mediated ODS monolithic column;

FIG. 8 is a CEC separation of a mixture of aldehydes and ketones on a sol-gel mediated ODS monolithic column; and

FIG. 9 is a schematic showing the various steps in preparing a monolithic separation column having an additional optical window in the structure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, monolithic sol-gel columns are prepared by an in situ creation of chromatographic stationary phases with surface-bonded ligands. Unlike conventional techniques, various column preparation processes, such as deactivation, coating/packing, stationary-phase immobilization and end frit making, are carried out in one single step, thus reducing the time and labor associated with column fabrication. In addition, the process produces a column that is homogenous since there are no particles included in the sol-gel and the sol-gel monolithic bed actually forms bonds with the fused silica capillary surface, making a unitary structure across the diameter of the tube.

In order to achieve the desired sol-gels of the instant invention, certain reagents in a reagent system were preferred for the fabrication of the gels for the monolithic columns of the present invention. The reagent system included two sol-gel

5

precursors, a deactivation reagent, one or more solvents and a catalyst. For the purposes of this invention, one of the sol-gel precursors contains a chromatographically active moiety selected from the group consisting of octadecyl, octyl, cyano-
propyl, diol, biphenyl, phenyl, cyclodextrins, crown ethers
and other moieties. Representative precursors include, but are
not limited to: Tetramethoxysilane, 3-(N-styrylmethyl-2-aminoethylamino)-propyltrimethoxysilane hydrochloride,
N-tetradecyldimethyl(3-trimethoxysilylpropyl)ammonium
chloride, N(3-trimethoxysilylpropyl)-N-methyl-N,N-diallyl-
lammonium chloride, N-trimethoxysilylpropyltri-N-butyl-
lammonium bromide, N-trimethoxysilylpropyl-N,N,N-trimethylammonium
chloride, Trimethoxysilylpropylthiuronium chloride, 3-[2-N-benzylaminoethylaminopropyl]trimethoxysilane hydrochloride,
1,4-Bis(hydroxydimethylsilyl)benzene, Bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane, 1,4-bis(trimethoxysilyl)ethyl)benzene, 2-Cyanoethyltrimethoxysilane, 2-Cyanoethyltriethoxysilane,
(Cyanomethylphenethyl) trimethoxysilane, (Cyanomethylphenethyl)triethoxysilane,
3-Cyanopropyltrimethoxysilane, 3-Cyanopropyltriethoxysilane, 3-Cyanopropyltrimethoxysilane, n-Octadecyltrimethoxysilane,
n-Octadecyldimethylmethoxysilane, Methyl-n-Octadecyldimethoxysilane, Methyl-n-Octadecyltriethoxysilane, n-Dodecyltriethoxysilane, n-Dodecyltrimethoxysilane, n-Octyltriethoxysilane,
n-Octyltrimethoxysilane, n-Octyldiisobutylmethoxysilane, n-Octylmethylmethoxysilane,
n-Hexyltriethoxysilane, n-isobutyltriethoxysilane, n-Propyltrimethoxysilane, Phenethyltrimethoxysilane,
N-Phenylaminopropyltrimethoxysilane, Styrylethyltrimethoxysilane, 3-(2,2,6,6-tetramethylpiperidine-4-oxy)propyltriethoxysilane, N-(3-triethoxysilylpropyl)acetyl-glycinamide, (3,3,3-trifluoropropyl)trimethoxysilane, and (3,3,3-trifluoropropyl)methylmethoxysilane.

A second sol-gel precursor, N-Octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride, was found to be critical since it possessed an octadecyl moiety that allowed for chromatographic interactions of analytes with the monolithic stationary phase. Additionally, this reagent served to yield a positively charged surface thereby providing the relatively high electroosmotic flow necessary in capillary electrophoresis. However, it is considered within the scope to use any other reagent as known to one of ordinary skill in the art that would contain the octadecyl moiety for the purposes already set forth.

The deactivation reagent, Phenyltrimethoxysilane, and the catalyst, Trifluoroacetic acid, were selected for the preparation of the columns of the instant invention, however, any known deactivation reagent and/or catalyst as known to those of ordinary skill in the art may be used.

The sol-gel solutions were prepared by mixing 100 μ L of Tetramethoxysilane (TMOS) with 100 μ L of C₁₈-TMS (N-Octadecyl-dimethyl[3-(trimethoxy-silyl)propyl]ammonium chloride), 10 μ L of PheDMS (Phenyltrimethoxysilane), 100 μ L of 99% Trifluoroacetic acid (TFA) (containing 10% water), and 100 μ L of 90% TFA (containing 10% water) in a micro vial. This mixture was thoroughly vortexed for 5 minutes, and the precipitate was then separated from the sol-gel solution through centrifugation at 13,000 rpm for 5 minutes. The supernatant was decanted into another micro vial and used for the creation of the monolithic separation bed.

A standard fused silica capillary tube, as known to those of skill in the art, was selected to be filled with the sol-gel solution. The tube used here was externally coated with polyimide, however it is within the scope of the invention to use a

6

tube coated with any polymer or other coating such as metal, as known in the art; the external coating serving as a structural integrity device for the tube.

Prior to filling with the sol-gel solution, the inner surface of the capillary was first treated with deionized water. For this, an approximately 5 meter long section of 50 μ m internal diameter fused silica capillary was rinsed with deionized water for approximately 15 minutes under a helium pressure of 200 psi. The capillary was then emptied by expelling the water from within by using the same helium pressure. Both ends of the capillary tube were then fused using an oxyacetylene torch, and the capillary was placed in a GC oven for thermal conditioning by raising the temperature at 0.5° C./min from 40° C. to a final temperature of 250° C. with a hold time of 60 minutes at 250° C. The column was then removed from the GC oven, and the ends were opened, followed by purging of the column with helium under 200 psi pressure for an additional 30 minutes.

Next, a desired length, for example 60 cm, of the hydrothermally pretreated fused-silica capillary was taken and installed into the capillary filling chamber, containing a polyethylene microcentrifuge vial with the desired sol-gel solution. It is, of course, within the scope of the invention to use any desired length as desired by one of skill in the art. Using 100 psi helium pressure, the sol solution was pushed into the column. The column, containing the sol solution, was then allowed to remain installed in the pressurized capillary chamber and left undisturbed for approximately four hours until gelation of the sol solution was visually apparent. Following this, the pressure was slowly released and the column was removed from the capillary filling/purging chamber. It was then affixed perpendicular to the bench top. A 60-s epoxy seal was then applied to the ends of the capillary to ensure adequate sealing prior to its thermal conditioning. Next, a very slow thermal conditioning program was used. An example of this thermal conditioning consists of a programmed temperature heating at 0.2° C./min from 35° C. (1 minute hold time) to a final temperature of 150° C., where the column has held for 120 minutes. Following heating, the ends were cut open and the monolithic capillary was then installed into a Bio-Rad CE system for subsequent rinsing at 100 psi. It is, of course, contemplated that any CE system as known to those of skill in the art may also be used. The monolithic column was initially rinsed with 100% HPLC grade acetonitrile, followed by a 50:50 acetonitrile/deionized water solution for periods of 5 minutes each, and finally the desired running mobile phase for 15 minutes prior to conducting column evaluation and/or analysis.

Visualization of the monolithic microstructure within the capillary tube was accomplished through the use of a scanning electron microscope. All scanning electron micrographic images were acquired from sections of the monolithic column initially cut into equal lengths, those being approximately 2.5 mm, and positioned perpendicularly within a retractable aluminum stage using a double-sided tape. These samples were then used to obtain cross-sectional views of the monolithic CEC columns. Longitudinal sections were acquired by dissecting approximately 1.0 cm sections of capillary at approximately 45°, thus yielding a capillary segment revealing a protruding portion of the monolithic matrix without the top portion of the fused silica present. These sections were then mounted parallel on an aluminum stage with the aid of double-sided carbon tape. Both stages, with all mounted capillary segments, were then consecutively placed into a Balzers SCD 050 sputter coating chamber and coated with a gold/palladium alloy at 40 mA for 60 seconds to avert subsequent charging.

reactions proceed, the products of the hydrolysis can then undergo polycondensation reactions in a variety of ways: (a) between hydrolyzed products of the same original precursor, (b) between hydrolyzed products of two different original precursors, and (c) between the hydrolyzed products of either precursor with the silanol groups on the inner capillary surface. A simplified representation of a polycondensation reaction between the hydrolysis products of both precursors is depicted below:

Condensation of Tetrahydroxysilane with N-Octadecyldimethyl[3-(trihydroxysilyl)propyl]ammonium chloride

$$\begin{array}{c}
 \text{CH}_3 \\
 | \\
 20 \times \text{CH}_3-(\text{CH}_2)_{17}-\text{N}^+-\text{C}(\text{CH}_3)_2-\text{Cl}^--\text{Si}(\text{OH})_2-(\text{CH}_2)_3- \\
 | \quad \quad \quad | \\
 \text{CH}_3 \quad \quad \quad \text{OH} \\
 \\
 \text{OH} \\
 | \\
 25 \quad y \text{ HO}-\text{Si}-\text{OH} \xrightarrow{-\text{H}_2\text{O}} \\
 | \\
 \text{OH}
 \end{array}$$

$$\begin{array}{c}
 \text{CH}_3 \\
 | \\
 30 \quad \text{CH}_3-(\text{CH}_2)_{17}-\text{N}^+-\text{C}(\text{CH}_3)_2-\text{Cl}^--\text{Si}(\text{OH})_2-\text{O}-\text{Si}-\text{OH} \\
 | \quad \quad \quad | \quad \quad \quad | \quad \quad \quad | \\
 \text{CH}_3 \quad \quad \quad \text{OH} \quad \quad \quad \text{O} \quad \quad \quad \text{O} \\
 \vdots \quad \quad \quad \vdots \quad \quad \quad \vdots \quad \quad \quad \vdots
 \end{array}$$

25

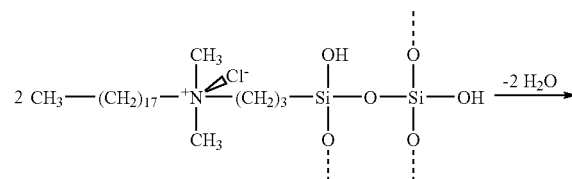
30

35

(a)



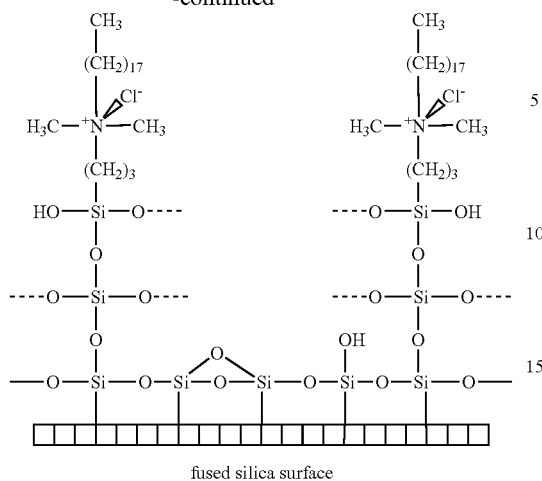
(b)



n-Octadecyldimethyl[3-(trihydroxysilyl)propyl]ammonium chloride

9

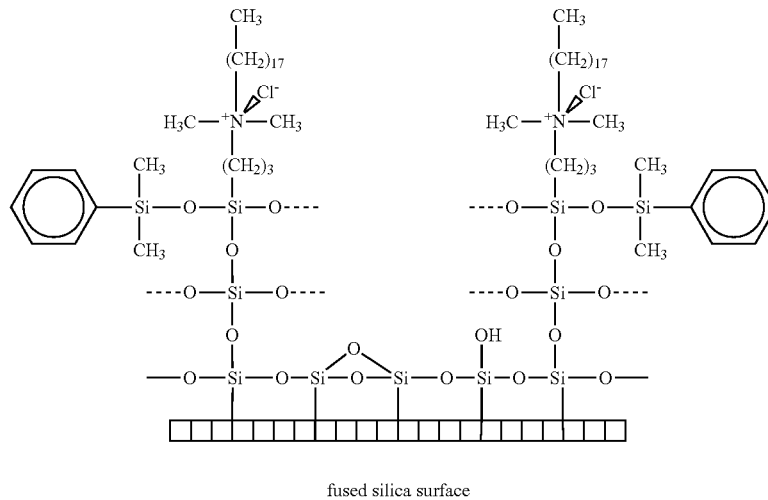
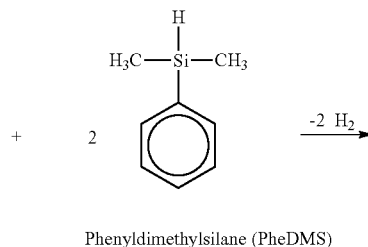
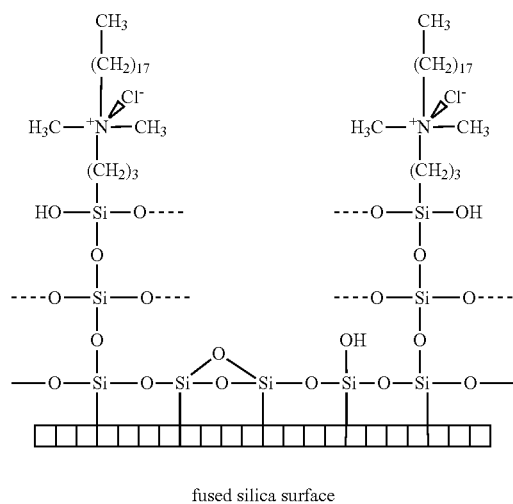
-continued



10

Finally, the incorporation of the PheDMS into the sol solution serves as a deactivating reagent for the monolithic bed. This deactivation reagent is initially added to the sol solution. The mobile hydrogen bonded to silicon atom in the structure of this reagent is reactive toward silanol groups, especially at elevated temperatures. It can be assumed that during the sol-gel process, this reagent becomes physically incorporated in the monolithic structure but subsequently, during thermal treatment of the column, reacts with the residual silanol groups in the monolithic structure providing deactivation, as shown below:

Deactivation of the Sol-Gel ODS Monolith with Phenyltrimethylsilane



11

Thus it has been shown that the sol-gel process provides a chemical anchorage of the monolithic matrix to the inner walls of the capillary (FIG. 3). This thoroughly illustrates a significant attribute of the sol-gel monolithic column. Another aspect is that the sol-gel monolithic bed is completely held in the column chemical bonding with the walls of the capillary, thus obviating the need for any end frits to hold the column material in place within the capillary.

Several analyses were performed using the monolithic columns of the instant invention. Each mobile phase was prepared by mixing the desired volumes of acetonitrile with a Tris-HCl background electrolyte solution. The organic solvent and the background electrolyte solution were thoroughly degassed individually via simultaneous ultrasonication and helium purging for approximately one hour prior to mixing and usage. Thorough degassing of the mobile phase was necessary to prevent subsequent bubble formation/generation during usage. This initial degassing procedure allowed for electrochromatographic experiments to be continuously performed without pressurization of the mobile phase. To achieve the desired concentration of aqueous electrolyte, a 50 mM solution was initially prepared followed by dilution to achieve the 5 mM concentration. The pH of this 5 mM solution was then measured and adjusted to approximately 2.3 by using concentrated HCl. This 5 mM Tris-HCl, having a pH of approximately 2.3 solution, in conjunction with 100% acetonitrile was individually degassed by simultaneous ultrasonication and helium purging, followed by mixing the solution in appropriate volume ratios (e.g., 75% acetonitrile/25% 5 mM Tris-HCl, etc.) to prepare the running mobile phase.

Experiments were conducted for the investigation of the electroosmotic flow (EOF) in sol-gel monolithic columns. The first measurements obtained using the monolithic ODS capillary was an evaluation of the effect of acetonitrile percentage in the running mobile phase on the electroosmotic mobility. For this, a set of mobile phases containing varying percentages of acetonitrile and 5 mM aqueous Tris-HCl was utilized. In addition, DMSO was used as the neutral electroosmotic flow marker. The results obtained from these experiments are depicted in FIG. 4. As illustrated, the electroosmotic mobility within the ODS monolithic capillary consistently increased with the acetonitrile content in the mobile phase. Such an increase in EOF is indicative of an increase in the net positive surface charge within the monolithic columns. One possibility for this to occur is the reduction of effective negative surface charge due to an increase in acetonitrile concentration in the mobile phase, resulting in an equivalent increase of the effective positive surface charge due to the quaternary ammonium groups. This is possible because the negative charge on the monolith/capillary is reduced due to the interaction of acetonitrile with the negative-charge generating surface groups such as the silanols.

In CEC, a consistent EOF is essential to drive the analyte(s) through the separation column. This EOF is generated due to an electrical double layer at the interface of the solid support with the liquid mobile phase. Most commonly, silica is used as the solid support and develops a negative surface charge under CE/CEC running conditions, presumably as a result of the deprotonation of the silanol groups. The negatively charged substrate attracts cations from the electrolyte in the mobile phase thereby forming the electrical double layer.

In this invention, the positively charged quaternary ammonium moiety contained in the N-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride provided a positively charged surface on the monolithic matrix, which, in turn, counteracted on the effects of the residual silanol groups

12

residing both on the monolith and on the inner capillary surface. Under the experimental conditions used, a strong EOF was observed in the reversed direction (from cathode to anode), suggesting that the surface positive charge due to quaternary ammonium functionality in the surface-bonded C₁₈TMS moieties is the EOF-determining factor in the prepared sol-gel monolithic columns.

The CEC analysis of a mixture of PAHs on a sol-gel ODS monolithic column is shown in FIG. 5. A separation column 50 cm×50 μm inner diameter (46.1 cm effective length) is used. The separation conditions were as follows:

15	Injection	-12 kV for 0.03 min
	Run	-15 kV, 2.68 μA
	Mobile phase	80% acrylonitrile/20% 5 mM Tris-HCl, pH 2.34, DMSO used as the EOF marker
	Analyses	(1) benzene 4.4053×10^{-6} M (2) naphthalene 2.7087×10^{-6} M (3) impurity (4) fluorene 1.5433×10^{-6} M (5) phenanthrene 1.5748×10^{-6} M (6) anthracene 9.6850×10^{-7} M (7) fluoranthene 1.1654×10^{-6} M (8) pyrene 1.2283×10^{-6} M (9) benzo[a]pyrene 1.5118×10^{-6} M

The monolithic sol-gel column allowed for the use of a mobile phase containing a higher percentage of acetonitrile (up to 80%) and simultaneously rendered sufficient solute-stationary-phase interactions. The separation efficiencies acquired for naphthalene in the mixture of PAH analytes in this analysis were on the order of 145,800 theoretical plates per meter (73,000 plates/column). Because monolithic columns with overall lengths of up to several meters can be easily prepared by the presented sol-gel technology and that the prepared columns can be operated using commercially available CE instrumentation, new possibilities for generating extremely high efficiencies per column in CEC separations are created.

Van Deemer plots, as depicted in FIG. 6, were constructed through variations in the operating voltages, thereby altering the mobile-phase flow rate through the column and measuring the achieved plate heights corresponding to each operating voltage. The conditions were as follows:

50	Injection	-12 kV for 3 sec
	Run	-3 to -19 kV
	Mobile phase	75% acetonitrile/25% 5 mM Tris-HCl, pH 2.34, DMSO used as the EOF marker
	Test solutes	(a) naphthalene (b) anthracene

For the used test solutes, the Van Deemer plots reveal minimal increases in plate heights as the mobile-phase flow rates are enhanced. The relatively flat right-hand portion of the H vs u curves indicate an efficient mass-transfer process between the mobile phase and the monolithic ODS separation bed.

As can be seen in FIG. 6, the optimum linear velocity for the used sol-gel monolithic ODS column was 0.75 mm/s, which corresponds to applied field strength of -240 V/cm (-12 kV) in the sol-gel monolithic columns. This shows a new possibility for use of longer sol-gel columns that produce higher overall column efficiencies without exceeding the upper voltage limits of commercially available CE instruments. Furthermore, the use of sol-gel technology to prepare these monolithic ODS columns for CEC is further accentuated.

13

ated as increased column lengths can be used because the highly porous structure of the monoliths allows for their rinsing and CEC operation using commercially available CE instrumentation without any additional pressurization capability. There was no need for pressurization of both capillary ends during analysis or for increased pressurization for capillary rinsing prior to analysis. No bubble formation was detected during analysis with the monolithic capillaries when using electric field strengths of up to 300 v/cm. In addition, the highly porous structure of the monolithic capillaries allowed for operation without the need for modification to the commercial CE instrument.

A test mixture of benzene derivatives was also used to further evaluate the separation performance of the sol-gel ODS monolithic columns using a mobile phase containing 75% acetonitrile and 25% aqueous 5 mM Tris-HCl at pH of 2.34. Column efficiencies on the order of 163,200 plates/m (81,600 plates/column) were obtained in these analyses as shown in FIG. 7.

FIG. 8 illustrates an analogous separation of a mixture of aldehydes and ketones obtained on a sol-gel monolithic ODS column. As in the case with the benzene derivatives, this probe mixture contained more closely related analytes. Column efficiencies on the order of 174,600 plates/m (87,300 plates/column) were obtained in these analyses. The conditions here were:

Separation column	50 cm × 50 μm (inner diameter) (46.1 cm of effective length)
Injection	-12 kV for 0.03 min -25 kV 0.5 μA run
Mobile phase	70% acrylonitrile/30% Tris-HCl, pH 2.34
Analytes	(a) benzaldehyde, 1.180×10^{-3} M (b) o-tolualdehyde, 1.9655×10^{-3} M (c) butyrophenone, 3.2680×10^{-4} M (d) valerophenone, 1.5263×10^{-4} M (e) hexaphenone, 3.0618×10^{-4} M (f) heptaphenone, 2.986×10^{-4} M.

Repeatability studies were performed using various analyte mixtures. These experiments were essential to evaluate the consistency in solute retention on the sol-gel monolithic ODS columns. The following table presents the CEC characteristics of sol-gel monolithic columns and experimental data on retention time repeatability for a test mixture of seven aromatic aldehydes and ketones.

Analyte	Separation Efficiency, N (plates/ column)		Reten- tion factor, k	Separation Factor, α		R.S.D. (n = 5)
	t_R (min)	s				
Benzaldehyde	89 778	9.144	0.050		0.027	0.295%
Tolualdehyde	91 039	9.526	0.094	0.382	0.029	0.302%
Butyrophenone	83 867	10.048	0.154	0.522	0.025	0.248%
Valerophenone	79 353	10.678	0.227	0.630	0.016	0.154%
Hexaphenone	86 027	11.550	0.327	0.872	0.022	0.194%
Heptaphenone	89 687	12.788	0.469	1.238	0.031	0.244%

As depicted in this table, consistent repeatability values are exemplified by the low RSD (0.15-0.30%) values for solute retention times in a series of five consecutive runs.

An additional embodiment of the invention is shown in FIG. 9. This embodiment provides an optical window that allows for the use of a detection device to monitor the eluted

14

analytes after passing through the separation bed. Such an optical window allows for detection or further analysis of samples.

More specifically, the optical window is formed in an area or segment containing gas 10 of the capillary tube, as generally shown at 12, adjacent the sol-gel bed 14. Normally, to provide structural integrity and strength to the tube, an outer coating 16 is provided around the entire outer surface of the tube 12. This outer coating 16 can be in the form of a polymer, metal or other coating as known in the art. In accordance with this embodiment of the present invention, this outer coating 16 is removed from the tube adjacent the sol-gel bed 14, as shown by 18, defining an optical window 30 in the tube in the area of the gas containing area 10.

Generally, the capillary tube 12 itself is optically transparent. However, the outer protective coating 16 interferes with the optical properties thereof. In order to provide the optical window 30, one end of the tube is sealed, as in 20. Under pressure, the sol-gel solution is introduced, thereby compressing the gas within the capillary tube inner gas containing area 10. This forces the formation of a compressed gas space 24, separating an end portion of the bed 14 from the sealed end portion 20. A portion of the outer coating is removed, by burning or by other means known in the art, in the area of the gas containing area 10, thus forming the optical window 30, in the capillary tube. This window may be of any desired size known to those of ordinary skill in the art.

In this embodiment, the separation bed may also be thermally and/or solvent treated as described earlier, and any further necessary pretreatment steps prior to use of the column is considered here as being within the scope of the invention.

A preferred method for forming this window involves the following steps:

- (a) selection of an externally coated optically transparent capillary tube, such as one containing a fused-silica inner surface, the coating being any as known to those of ordinary skill in the art, such as polymer, metal or other removable coatings;
- (b) sealing of the distal capillary end;
- (c) filling the sealed capillary with sol-gel solution, the pressure of the compressed gas in the sealed portion forming a gas pocket in the distal end thereof;
- (d) allowing the sol-gel solution inside the capillary to transform into a porous monolithic bed;
- (e) sealing the proximal end of the capillary tube to allow thermal conditioning;
- (f) removing the seals at both ends of the filled capillary tube to allow for solvent conditioning; and
- (g) removing a portion of the outer coating of the capillary tube at the distal end in the region of the former gas pocket to provide a window of any desired length for optical spectrometric analyses.

The incorporation of the optical window allows for additional analyses to be performed on the sample as it exits the separation bed. The choice of spectrometer is within the scope of ordinary skill in the analytical chemistry art, and any known instrument is considered within the scope of the invention. It is also understood that any form of pretreatment of the column may be used including thermal and solvent or both, but the choice of pretreatments is solely a matter of choice.

It may be appreciated by one skilled in the art that additional embodiments may be contemplated, including alternate reagents and tubes used for the outer matrix for the sol-gel filling.

In the foregoing description, certain terms have been used for brevity, clarity and understanding, but no necessary limi-

tations are to be implied therefrom beyond the requirements of the prior art, because such words are used for description purposes herein and are intended to be broadly construed. Moreover, the embodiments of the apparatus and reagents used herein are by way of example, and the scope of the invention is not limited to those in either construction or chemistry.

Having now described the invention, the preferred embodiments thereof and the advantageous new and useful results obtained thereby, along with reasonable chemical equivalents thereof as obvious to those of ordinary skill in the art, these are now set forth in the appended claims.

REFERENCES

- Behnke, B.; Bayer, E., *J. Chromatogr. A*, 1994, 680, 93.
 Behnke, B.; Grom, E.; Bayer, E., *J. Chromatogr. A*, 1995, 716, 207.
 Brinker, C. J.; Scherer, G. W., *Sol-gel Science: The Physics and Chemistry of Sol-gel Processing*; Academic Press, San Diego, Calif. 1990.
 Chong, S.; Wang, D.; Hayes, J. D.; Malik, A. *Anal. Chem.* 1997, 69, 3889.
 Colon, L. A.; Fermier, A. M.; Guo, Y.; Reynolds, K. J., *Ninth International Symposium on High Performance Capillary Electrophoresis (HPCE '97)*, Anaheim, Calif. 1997.
 Cortes, H. J.; Pfeiffer, C. D.; Richter, B. E.; Stevens, T. S., *J. High Resolut. Chromatogr. And Chromatogr. Commun.*, 1987, 10, 446.
 Dittmann, M. M.; Rozing, G. P., *J. Chromatogr. A*, 1996, 744, 63.
 Dulay, M. T.; Yan, C.; Rakestraw, D. J.; Zare, R. N., *J. Chromatogr. A*, 1996, 725, 361.
 Dulay, M. T.; Kulkarni, R. P.; Zare, R. N., *Anal. Chem.*, 1998, 70, 5103.
 Ericson, C.; Liao, J.; Nakazato, K.; Hjerten, S., *J. Chromatogr. A* 1997, 767, 33.
 Ericson, C.; Hjerten, S., *Anal. Chem.* 1999, 71, 1621.
 Fields, S. N., *Anal. Chem.*, 1996, 68, 2709.
 Frame, L. A.; Robinson, M. L.; Lough, W. J., *J. Chromatogr. A*, 1998, 798, 243.
 Fujimoto, C.; Fujise, Y.; Matsuzawa, E., *Anal. Chem.*, 1995, 716, 107.
 Fujimoto, C.; Fujise, Y.; Matsuzawa, E., *Anal. Chem.*, 1996, 68, 2753.
 Hayes, J. D.; Malik, A., *J. Chromatogr. B* 1997, 695, 3.
 Hayes, J. D.; Malik, A., manuscript in progress.
 Hileman, F. D.; Sievers, R. E.; Hess, G. G.; Ross, W. D., *Anal. Chem.*, 1973, 45, 1126.
 Hjerten, S.; Liao, J. L.; Zhang, R., *J. Chromatogr.*, 1989, 473, 273.
 Hjerten, S.; Li, Y. M.; Liao, J. L.; Mohammad, J.; Nakazato, K.; Pettersson, G., *Nature*, 1992, 356, 810.
 Ishizuka, N.; Minakuchi, H.; Nakanishi, K.; Soga, N.; Tanaka, N., *J. Chromatogr. A*, 1998, 797, 131.
 Jorgenson, J. W.; Lucas, K. D., *J. Chromatogr. A*, 1981, 218, 209.
 Knox, J. H.; Grant, I. H., *Chromatographia*, 1987, 24, 135.
 Li, Y. M.; Liao, J. L.; Nakazato, K.; Mohammad, J.; Terenius, L.; Hjerten, S., *Anal. Biochem.* 1994, 223, 153.
 Ludtke, S.; Adam T.; Unger, K. K., *J. Chromatogr. A*, 1997, 786, 229.
 Malik, A.; Chong, S. in Pawliszyn, J. (ed.), "Applications of Solid Phase Microextraction," Royal Society of Chemistry, 1999, United Kingdom, Ch. 6, pp. 73-91.
 Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka, N., *Anal. Chem.*, 1996, 68, 3498.
 Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka, N., *J. Chromatogr. A* 1997, 8, 547.
 Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka, N., *J. Chromatogr. A*, 1998, 797, 121.
 Moffatt, F.; Cooper, P. A.; Jessop, K. M., *Anal. Chem.*, 1999, 71, 1119.
 Nakanishi, K. K.; Minakuchi, H.; Soga, N.; Tanaka, N., *J. Sol-gel Sci. and Tech.* 1997, 762, 135.
 Palm, A.; Novotny, M. V., *Anal. Chem.* 1997, 69, 4499.
 Peters, E. C.; Petro, M.; Svec, F.; Frechet, J. M. J., *Anal. Chem.* 1997, 69, 3646.
 Peters, E. C.; Petro, M.; Svec, F.; Frechet, J. M. J., *Anal. Chem.* 1998, 70, 2288.
 Pietrzyk, D. J. in *Packings and Stationary Phases in Chromatographic Techniques*; Unger, K. K., Ed.; Vol. 47; Marcel Dekker: New York 1990; Chapter 10.
 Pretorius, V.; Hopkins, B. J.; Schieke, J. D., *J. Chromatogr. A*, 1974, 99, 23.
 Seifer, R. M.; Kraak, J. C.; Th. Kok, W.; Poppe, H., *J. Chromatogr. A*, 1998, 808, 71.
 Smith, N. W.; Evans, M. C., *Chromatographia* 1994, 38, 649.
 Svec, F.; Frechet, J. M. J., *Anal. Chem.* 1992, 64, 820.
 Svec, F.; Frechet, J. M. J., *J. Chromatogr. A* 1995, 702, 89.
 Tang, Q.; Wu, N.; Lee, M. L., *J. Microcol. Sep.*, 1999, 11, 550.
 Tsuda, T., *Anal. Chem.*, 1987, 59, 521.
 Van den Bosch, S. E.; Heemstra, S.; Kraak, J. C.; Poppe, H., *J. Chromatogr. A*, 1996, 755, 165.
 Wang, Q. C.; Svec, F.; Frechet, J. M. J., *Anal. Chem.* 1993, 65, 2243.
 Wang, D.; Chong, S.; Malik, A. *Anal. Chem.* 1997, 69, 4566.
 Wei, W.; Luo, G. A.; Hua, G. Y.; Yan, C., *J. Chromatogr. A*, 1998, 817, 65.
 Xin, B.; Lee, M. L., *Electrophoresis*, 1999, 20, 67.
 Yan, C.; Dadoo, R.; Zhao, H.; Zare, R. N.; Rakestraw, D. J., *Anal. Chem.*, 1995, 67, 2026.
 Yang, C.; El Rassi, Z., *Electrophoresis* 1998, 19, 2278.
 Yang, C.; El Rassi, Z., *J. Liq. Chromatogr.* 1995, 18, 3373.
 Zhang, M.; El Rassi, Z., *Electrophoresis*, 1998, 19, 2068.
 Zhang, M.; El Rassi, Z., *Electrophoresis*, 1999, 20, 31.
 Zhang, M.; Yang, C.; El Rassi, Z., *Anal. Chem.*, 1999, 71, 3277.
 Zimina, T. M.; Smith, R. M.; Meyers, P., *J. Chromatogr. A*, 1997, 758, 191.

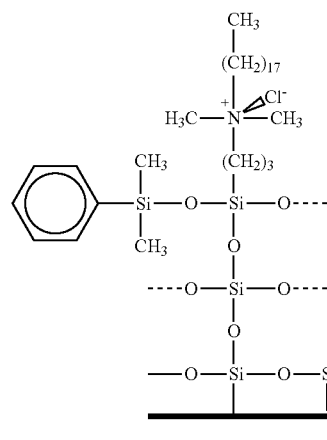
What is claimed is:

1. A monolithic sol-gel column comprising:

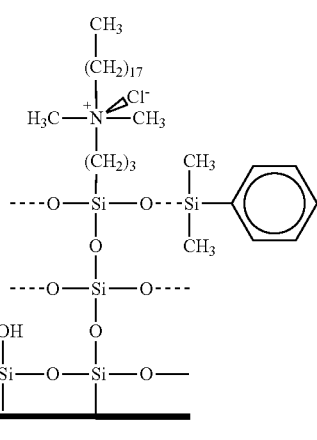
- a) a hydrothermally pretreated fused-silica capillary tube including an inner surface; and
- b) a porous matrix of substantially homogenous composition and being free of chromatographic particles, said porous matrix comprising i) a functional group having a positive or negative charge; and ii) at least one chromatographically active moiety chemically bonded to said porous matrix; wherein said porous matrix is chemically bonded to said inner surface of said capillary tube; wherein said column comprises a gas-filled portion distal to said porous matrix and that has not been contacted by said porous matrix and that has smooth surface characteristics and that is optically transparent, and wherein said column does not comprise an end frit; and wherein said porous matrix is formed in situ by a one-step process inside said capillary tube.

2. The monolithic sol-gel column according to claim 1, wherein said porous matrix comprises:

17



18



3. The monolithic sol-gel column according to claim 1, wherein said matrix is formed using a sol-gel precursor that comprises a strong basic functional group or a strong acidic functional group.

4. The monolithic sol-gel column according to claim 3, wherein said strong basic functional group is a quaternary amine group.

5. The monolithic sol-gel column according to claim 3, wherein said strong acidic functional group is a sulfonic acid group.

6. The monolithic sol-gel column according to claim 1, wherein said chromatographically active moiety is selected from the group consisting of octadecyl, octyl, cyanopropyl, diol, biphenyl, phenyl, cyclodextrins, and crown ethers.

7. The monolithic sol-gel column according to claim 1, wherein said functional group provides a positive charge to said porous matrix.

8. The monolithic sol-gel column according to claim 1, wherein said functional group provides a negative charge to said porous matrix.

9. The monolithic sol-gel column according to claim 1, wherein said matrix is formed using a sol-gel precursor molecule that comprises both said functional group and said chromatographically active moiety.

10. The monolithic sol-gel column according to claim 1, wherein said chemical bond is a covalent bond.

11. A monolithic sol-gel column comprising:

- a) an optically transparent hydrothermally pretreated fused-silica capillary tube;
- b) a porous matrix of substantially homogenous composition and being free of chromatographic particles, said porous matrix comprising i) a functional group having a positive or negative charge; and ii) at least one chromatographically active moiety chemically bonded to said porous matrix; wherein said porous matrix is chemically bonded to said inner surface of said capillary tube; and wherein said porous matrix is formed in situ by a one-step process inside said capillary tube; and
- c) an optically transparent window means within a portion of said tube for providing for optical analysis of analytes after said analytes travel through said porous matrix, wherein said optically transparent window is formed in an area of said capillary tube containing gas, adjacent to said porous matrix; and

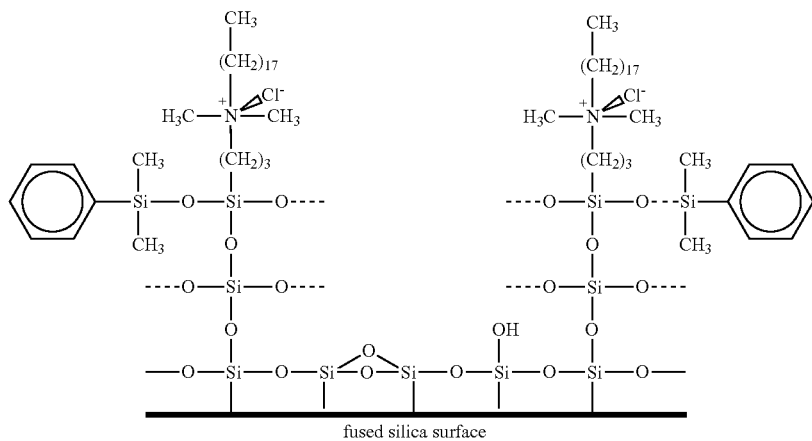
wherein said column does not comprise an end frit.

12. The monolithic sol-gel column according to claim 11, wherein an outer-coating is provided around the outer surface of said capillary tube.

13. The monolithic sol-gel column according to claim 12, wherein said outer-coating is a polymer or a metal.

14. The monolithic sol-gel column according to claim 12, wherein a portion of said outer-coating is removed from said capillary tube adjacent to said porous matrix thereby defining said optical window in the tube.

15. The monolithic sol-gel column according to claim 11, wherein said porous matrix comprises:



19

16. The monolithic sol-gel column according to claim 11, wherein said porous matrix is formed using a sol-gel precursor that comprises a strong basic functional group or a strong acidic functional group.

17. The monolithic sol-gel column according to claim 16, wherein said strong basic functional group is a quaternary amine group.

18. The monolithic sol-gel column according to claim 16, wherein said strong acidic functional group is a sulfonic acid group.

19. The monolithic sol-gel column according to claim 11, wherein said chromatographically active moiety is selected from the group consisting of octadecyl, octyl, cyanopropyl, diol, biphenyl, phenyl, cyclodextrins, and crown ethers.

20

20. The monolithic sol-gel column according to claim 11, wherein said functional group provides a positive charge to said porous matrix.

21. The monolithic sol-gel column according to claim 11, wherein said functional group provides a negative charge to said porous matrix.

22. The monolithic sol-gel column according to claim 11, wherein said matrix is formed using a sol-gel precursor molecule that comprises both said functional group and said chromatographically active moiety.

23. The monolithic sol-gel column according to claim 11, wherein said chemical bond is a covalent bond.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,947,174 B2
APPLICATION NO. : 10/203586
DATED : May 24, 2011
INVENTOR(S) : Abdul Malik and James D. Hayes

Page 1 of 2

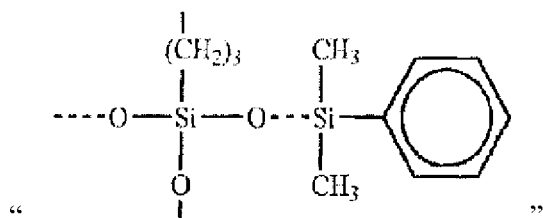
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 4,

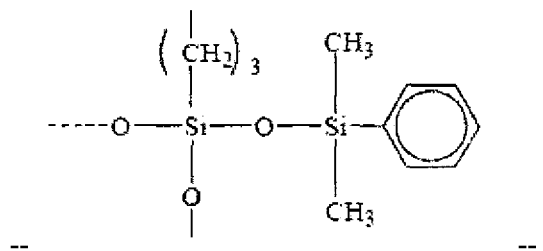
Line 38, "50 cmx50 μ l" should read --50 cm x 50 μ m--.

Column 17,

Line 1, middle section of right side of structure,

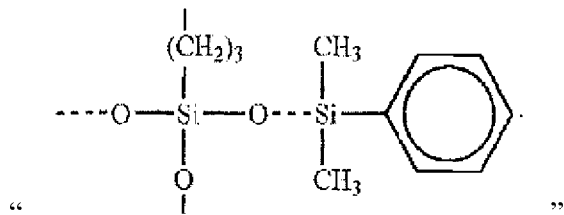


should read



Column 18,

Line 48, middle section of right side of structure,



Signed and Sealed this
Twentieth Day of December, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

should read

