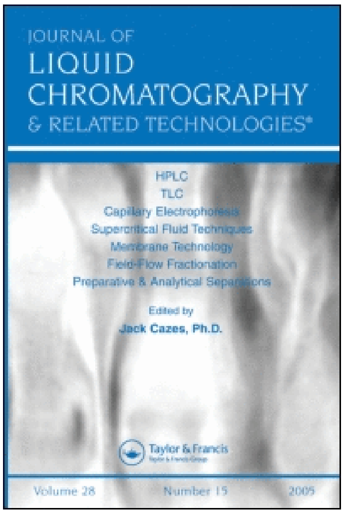

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SUBMICRON SIZED ORGANO-SILICA SPHERES FOR CAPILLARY ELECTROCHROMATOGRAPHY

Kimberly J. Reynolds[a]; Luis A. Colón[a]
[a] Department of Chemistry, State University of New York at Buffalo, Buffalo, NY, U.S.A.

To cite this Article Reynolds, Kimberly J. and Colón, Luis A.(2000) 'SUBMICRON SIZED ORGANO-SILICA SPHERES FOR CAPILLARY ELECTROCHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 1, 161 — 173

To link to this Article: DOI: 10.1081/JLC-100101443

URL: http://dx.doi.org/10.1081/JLC-100101443

J. LIQ. CHROM. & REL. TECHNOL., 23(1), 161–173 (2000)

SUBMICRON SIZED ORGANO-SILICA SPHERES FOR CAPILLARY ELECTROCHROMATOGRAPHY

Kimberly J. Reynolds, Luis A. Colón*

Department of Chemistry
State University of New York at Buffalo
Natural Science Complex
Buffalo, NY 14260-3000, USA

ABSTRACT

Spherical, non-porous organo-silica materials in the submicron size range were synthesized by sol-gel processing and tested for capillary electrochromatography (CEC). The organo-silica spheres were prepared by copolymerization of alkyltriethoxy-silanes and tetraethoxysilane under ambient conditions. The synthetic approach provides silica spheres that are uniform in size (<10% R.S.D.) and contain organic moieties (e.g., C_8) ready to be used as stationary phase for CEC, eliminating the step of stationary phase attachment after particle fabrication. The final particle size is controlled by the water concentration in the initial reaction mixture. The particles were characterized using transmission electron microscopy, gas adsorption, and FT-IR. Fast CEC separations with high efficiency can be achieved using the fabricated submicron particles.

www.dekker.com

INTRODUCTION

Developments concerning uniformly sized silica materials are of interest to a vast audience, including those involved in ceramics research, colloid behavior, catalysis, and chromatography. Ceramic materials can be tailored to have specific properties depending on the characteristics of the starting silica used. Study of colloidal dispersions can yield information about the transport properties of materials in various solvents, aiding in the understanding of both the catalytic properties and the dynamics of these types of systems. Both the base silica material and the affixed stationary phase determine retention characteristics of stationary phase materials for chromatographic applications.

Of key importance for these types of applications is the uniformity of the material of interest. Several different protocols for the fabrication of silica spheres have been made available throughout the literature over the past 30 years.[1-12] Routes to fabrication of pure silica materials include sol-gel processing techniques, in which alkoxysilane precursors are hydrolyzed and condensed to form three-dimensional silica matrices. Uniform sized silica spheres can be produced by the hydrolysis and condensation of alkoxysilane precursors in an ammonia/water catalyzed alcohol system, using the Stöber method.[1] This system has been studied in detail[14-23] due to the control afforded over final particle size, as well as the ease of implementation. Other methods producing uniform silica particles include two phase emulsion-gel techniques.[2,3,5-10,13] These involve emulsification of immiscible mixtures of a silane monomer phase and an aqueous phase, frequently with the addition of a surfactant, to form spherical silica products.

More recently, Unger and co-workers reported a modification of the Stöber method to fabricate porous materials from 0.1 to 2 μm diameter.[12] One of the approaches followed by Unger and co-workers to synthesize porous silica particles was to copolymerize tetraethoxysilane (TEOS) and *n*-alkyltrialkoxysilane; the latter serves as a porogen. The fabricated particles were then subjected to calcination to remove the porogenic organic moiety of the alkysilane, which provided the materials with an increased surface area. After the material was obtained, a post-synthesis modification was necessary to attach an organic functionality (i.e., the stationary phase) at the surface so the particles could be used for chromatographic applications.[24]

It must be pointed out that the possibility of using the uncalcinated material for CEC purposes was not explored. Since the fabricated particles, before calcination, should include an organic moiety, they can be used as the stationary phase without further particle treatment.

In this paper, we demonstrate that submicron organo-silica particles can be prepared for CEC without the calcination step used by Unger and co-workers.[12] Since the synthesized particles already contain the stationary phase, modification of the surface after particle fabrication is not necessary. This makes the process a truly one-step approach to fabricate submicron organo-silica particles for CEC. Herein, we present the characterization of the organo-silica particles and their potential for CEC.

EXPERIMENTAL

Reagents and Equipment

Ethanol (Fisher Scientific, Fair Lawn, NJ), ammonium hydroxide (J. T. Baker, Phillipsburg, NJ), acetonitrile (Sigma-Aldrich, St. Louis, MO), tetraethoxysilane (TEOS) (Aldrich, Milwaukee, WI), and *n*-octyltriethoxysilane (C_8TEOS) (Aldrich, Milwaukee, WI) were used as received. Test compounds for electrochromatography were 1-hydroxypyrene, pyrene, 1-ethylpyrene, and 9-(1-pyrene)nonanol (Molecular Probes, Eugene, OR). Tris(hydroxymethyl) aminomethane (TRIS) and Tris(hydroxymethyl)aminomethane hydrochloride (Fisher Scientific, Fair Lawn, NJ) were used as electrolyte in the mobile phase. Water was purified using a Milli-Q UV Plus water purification system, which was fed from a Milli-RO 10 Plus reversed osmosis system (Millipore, Bedford, MA). KBr for FT-IR experiments was purchased from Fisher Scientific (Fair Lawn, NJ) and was dried at 120°C before use.

The fused silica capillaries used to fabricate the CEC columns were purchased from Polymicro Technologies (Phoenix, AZ). The capillaries were packed with the organo-silica material using the gravitational packing method previously described.[25] The columns were flushed electrokinetically with acetonitrile /50 mM TRIS solution at pH=8 (80/20), which also served as the mobile phase. The CEC experiments were performed in a capillary electrophoresis (CE) apparatus built in-house. The test mixture was injected electrokinetically for 3 seconds at 1 kV. On column detection was performed using a laser-induced fluorescence (LIF) detection system, also built in-house, equipped with a He-Cd laser (325 nm).

Synthesis of Particles and Characterization

The particles were synthesized by mixing 1.0 M NH_3, 0.16 M TEOS, 0.007 M C_8TEOS, and variable water concentration in ethanol. First, the ethanol, ammonium hydroxide, and water were placed in a reaction vial and stirring was initiated at a speed of at least 500-RPM. Freshly prepared mixtures of C_8TEOS and TEOS were rapidly added to the reaction vials with stirring. Within 10 minutes of reaction, the solution takes on a bluish appearance that develops into an opaque white suspension. Mixing is continued until visible aggregation is observed.

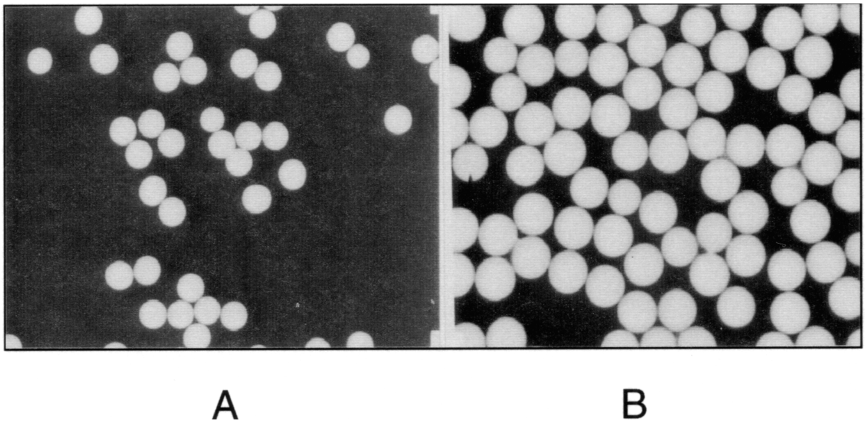

Figure 1. TEM micrographs of two different organo-silica particles fabrication, (A) 440 nm particles magnified 10,000 times, (B) 340 nm particles magnified 20,000 times.

The silica product was washed three times with ethanol and then three times with hexane as follows. The suspension containing the silica material was centrifuged and the supernatant removed. The material was re-suspended in the wash liquid (ethanol or hexane) and centrifuged again. The particles were dried in a vacuum oven at 80°C for at least 24 hours.

Particle sizing was performed using a Jeol JEM 100CX transmission electron microscope (TEM). Particles were mounted by dipping copper/formvar grids into a suspension of the particles in ethanol. Micrographs were taken at magnifications of 10,000 to 40,000x. Sizing of at least 100 particles was done directly from the negative. Surface area of the material was determined by gas adsorption using a Micromeretics ASAP 2010 (Micromeritics, Norcross, GA). The particle samples were prepared by drying for 24 hours at 80°C under vacuum. Nitrogen was used as the analysis gas and surface area determined by BET analysis. FTIR spectra were collected using a Perkin-Elmer 1760X FT-IR. Samples were prepared in KBr.

RESULTS AND DISCUSSION

Sol-gel processing has been traditionally an effective way to fabricate purely inorganic materials; however, this process also provides a means to produce inorganic-organic hybrid composites.[26] Using such an approach, our laboratory has reported on the preparation of organic-inorganic films inside capillary columns suitable for chromatographic applications.[27-30] Others have followed the use of sol-gel technology to prepare chromatographic columns,

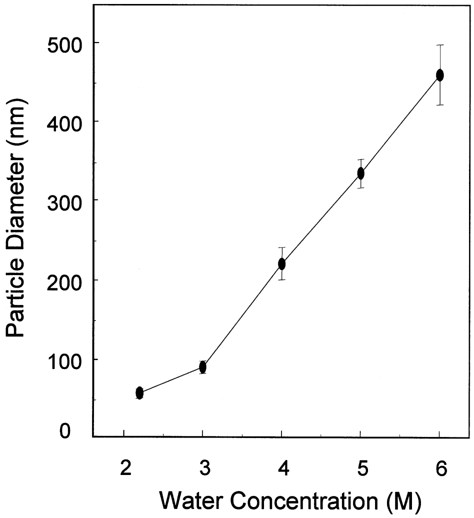

Figure 2. Effect of water concentration on final particle size. Other conditions are 1.0 M ammonia, 0.16 M TEOS, 0.007 M C_8TEOS in ethanol.

particularly for CEC.[31-34] We have modified our previous sol-gel processing method to produce spherical organo-silica particles, produced by hydrolysis and condensation of a mixture containing *n*-octyltriethoxysilane (C_8TEOS) and tetraethoxysilane (TEOS) under basic conditions. The spherical organo-silica material was prepared by mixing ethanol, ammonium hydroxide, water, and freshly prepared mixtures of C_8TEOS and TEOS, according to the procedure described in the experimental section.

Figure 1 shows TEM micrographs of 440 and 340 nm particles as representative examples. The images indicate that the particles are spherical and uniform in shape. The particles have a relatively low size distribution (<10% R.S.D.) and are regular in morphology.

Controlling the conditions under which they are formed can influence the properties of the final sol-gel processed materials (i.e., size, shape, and surface area). In general, the rates of hydrolysis and condensation of the precursor in a sol-gel process are dependent on the ratio of water to alkoxy groups on the silane species. In our experiments, we kept the concentration of the silane species constant and varied the amount of water added to the reaction mixture. The amount of water that is added to the reaction mixture has a profound impact on the particle size of the material produced (Figure 2). The final particle size is controlled by the initial water concentration in the reaction mixture. We also found that particle size is independent of mixing speed above stirbar speeds of 500 RPM (minimum speed used to mix the reaction mixtures). The batch to

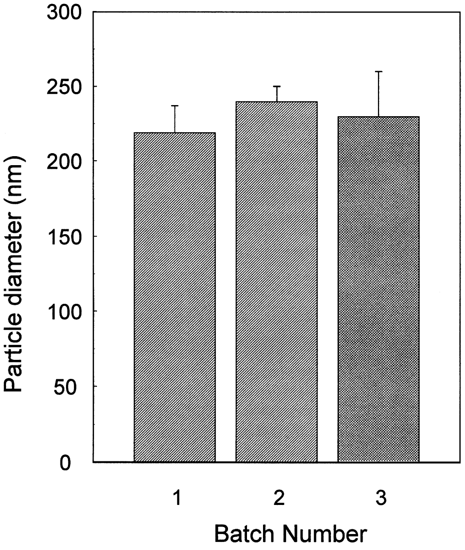

Figure 3. Particle size for three different batches of organo-silica particles. The fabrication conditions were 1.0 M ammonia, 4 M water, 0.16 M TEOS, 0.007 M C_8TEOS, ethanol as solvent.

Table 1

Surface Area of Selected Organo-Silica Particles

Particle Diameter (nm)	Surface Area by B.E.T. (m^2/g)
220	10.8
330	11.1
460	10.0

batch reproducibility can be seen in Figure 3, where three different batches of silica particles were prepared (~225 nm). Table 1 indicates the surface area of three different samples of organo-silica spheres fabricated in our laboratory. Particles with such surface area are considered to be non-porous in nature.

The use of C_8TEOS in the reaction mixture affords inclusion of octyl moieties within the particle matrix. To confirm the incorporation of octyl species into the material, infrared spectra were collected. In order to identify bands that resulted from the octyl species, control samples were fabricated under identical reaction conditions with the exception that C_8TEOS was not added to

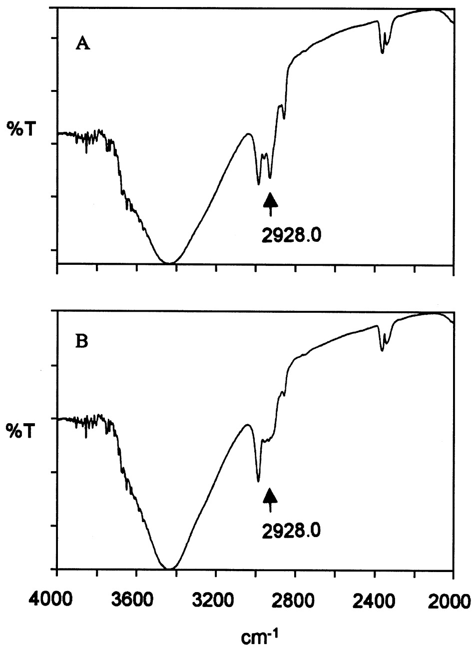

Figure 4. FTIR spectra of (A) organo-silica material and (B) bare silica fabricated by the sol-gel processing.

the reaction vessel. The spectra obtained from the organo-silica were compared with spectra obtained from these control samples (Figure 4). The absorption band evident in Figure 4A (C_8TEOS/TEOS composite) at 2928.0 cm^{-1} is indicative of an aliphatic stretch, and is notably absent in Figure 4B (TEOS composite). This indicates the presence of the C_8 aliphatic species in the silica particles fabricated by our dual-precursor system.

Organo-silica is of considerable interest in the field of liquid chromatography, as it is the most commonly used packing support material for the stationary phase. Reports providing modification of pure silica particles with organic moieties are numerous in the literature.[35-38] Frequently, these modifications are several step processes which can be quite time consuming as well as labor intensive.

It is relevant to indicate that the material produced by the above-mentioned procedure already contains the stationary phase in place. Contrary to the approach of Unger and co-workers,[12] we do not perform calcination. Although the result is a non-porous material, there is no need for particle modification and it is suitable for use in CEC.

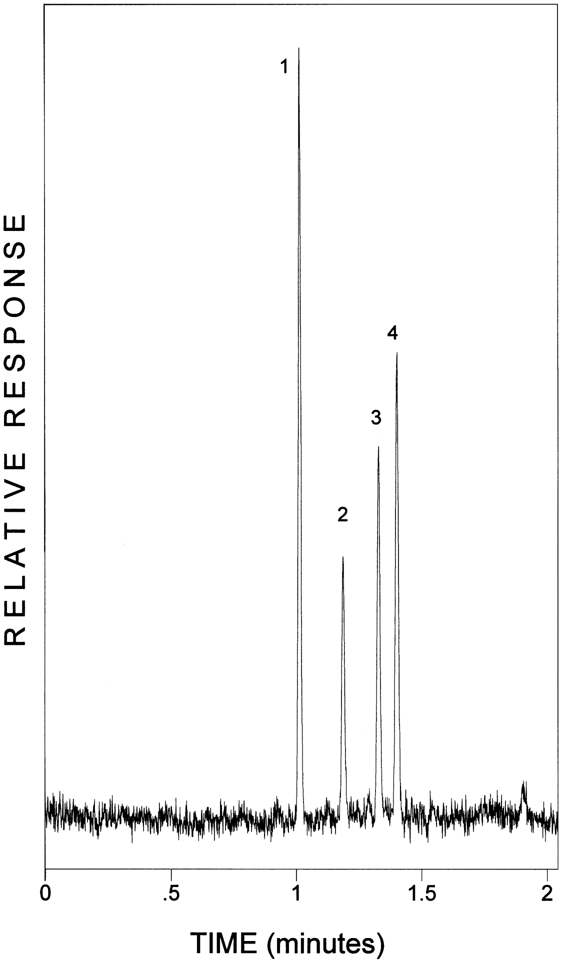

Figure 5. Electrochromatogram of a test mixture separated by CEC on a 50 µm i.d. column packed with 450 nm organo-silica particles. Separation conditions: total column length = 34.5 cm, separation length = 12.7 cm. Mobile phase = 80% acetonitrile 20% 50 mM Tris (pH = 8), injection = electrokinetic, 3 s @ 1 kV, detection using LIF with 325 nm excitation, separation voltage = 27 kV. Peak identification: (1) 1-hydroxypyrene, (2) pyrene, (3) 1-ethylpyrene, (4) 9-(1-pyrene)nonanol.

The potential use of submicron packing material in liquid chromatography has been hindered in the past by the availability of such materials and by the high pressures required to drive solvent through the packed bed. The particles fabricated by our sol-gel process can be used to study the submicron particle regime in liquid chromatography using capillary electrochromatography (CEC),

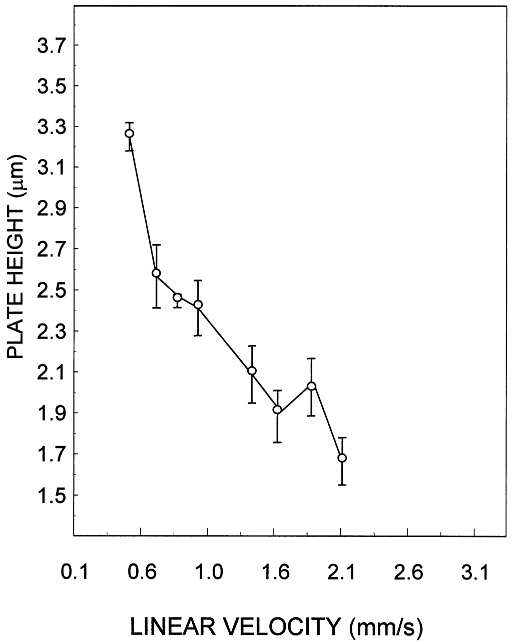

Figure 6. Plot of plate height versus linear velocity for 9-(1-pyrene)nonanol.

which uses electroosmosis to drive the solvent through the column.[39,40] This approach has been followed independently by Unger and our laboratory.[24, 41-43] Using a capillary column packed with particles fabricated by our synthesis process a mixture of probe compounds were separated. Figure 5 shows the separation of 4 pyrene derivatives in a column packed with 450 nm organo-silica particles containing the C-8 moiety as the stationary phase. The octyl groups introduced during the particle synthesis serve as the stationary phase and afford separation of the analytes. Notice that the linear velocity on the separation shown in Figure 5 is about 2.1 mm/s. The separation was achieved in a relatively fast analysis time. Even though our material is nonporous, this linear velocity compares very favorably with the 2.8 mm/s reported by Unger and co-workers on porous 0.5μm particles (calculated from the thiourea peak in Figure 7 of reference 24). We can speculate on the relatively similar linear velocities in columns packed with the porous material and our nonporous particles as follows. Free silanol groups at the particles surface are responsible for generation of EOF. The velocity in the porous material can be affected by the lack of free silanol groups contributing to the EOF. This can be due to double layer overlapping inside the pores and/or because of a decrease in free silanol groups inside the pores after the calcination treatment. Our results indicate that higher surface area does not contribute significantly to the generation of EOF.

The theoretical plates obtained in the column packed with 450 nm C_8-organosilica particles varied between 370 000 (hydroxypyrene) and 480 000 (9-(1-pyrene)nonanol) plates per meter. These efficiencies are slightly higher than those observed by Unger and co-workers using similar sized porous materials. Figure 6 shows a plot of plate height vs. linear velocity for 9-(1-pyrene)nonanol (the most retained compound). It is obvious that under our experimental conditions, a minimum on the plot has not been achieved. We are limited by our CEC system, which can only provide voltages up to 30 kV. A higher voltage should provide for an even faster separation and should also approach the minimum in the plate height vs. linear velocity curve.

CONCLUSION

Ammonia catalyzed hydrolysis and condensation of mixtures of C_8TEOS and TEOS produce sub-micron sized organo-silica spheres of uniform size and shape. The synthesis is a truly one-step, relatively facile procedure performed at ambient conditions that produces particles where the stationary phase is an inherent part of the particle. These organo-silica particles are suitable for CEC, without further modifications (e.g., calcination to enhance surface area and then attachment of stationary phase). The CEC results showed that the performance of the non-porous organosilica particles is comparable to the performance reported for porous materials of similar size.[24] Therefore, the less time consuming approach presented here is recommended for the fabrication of submicron sized particles for CEC. A C_8 stationary phase was used here; however, the synthetic approach can be modified to incorporate other selectivities.

ACKNOWLEDGMENTS

The authors thank Mr. Ted Szczesny for the assistance with the TEM work. Financial support by the National Science Foundation under grant number CHE-9614947 is also acknowledged.

REFERENCES

1. W. Stöber, A. Fink, E. Bohn, J. Colloid Interface Sci., **26**, 62-69 (1968).

2. A. Wojcik, L. Kwietniewski, J. Chrom., **435**, 55-62 (1988).

3. W. Minehan, G. Messing, Colloids and Surfaces, **63**, 181-187 (1992).

4. A. Van Blaaderen, A. Vrij, J. Colloid Interface Sci., **156**, 1-18 (1993).

5. L. Gan, K. Zhang, C. Chew, Colloids and Surfaces A, **110**, 199-206 (1996).

6. R. Lindberg, J. Sjöblom, G. Sundholm, Colloids and Surfaces A, **99**, 79-88 (1995).

7. Q. Huo, J. Feng, F. Schüth, G. Stucky, Chem. Mater., **9**, 14-17 (1997).

8. J. Esquena, R. Pons, N. Azemar, J. Caelles, C. Solans, Colloids and Surfaces A, **123-124**, 575-586 (1997).

9. C. Chang, H. Fogler, Langmuir, **13**, 3295-3307 (1997).

10. J. Esquena, Th. Tadros, K. Kostarelos, C. Solans, Langmuir, **13**, 6400-6406 (1997).

11. H. Izutu, F. Mizukami, P. Nair, Y. Kiyozumi, K. Maeda, J. Mater. Chem., **7(5)**, 767-771 (1997).

12. G. Büchel, M. Grün, K. Unger, Supramolecular Sci., **5**, 253-259 (1998).

13. J. Choi, C. Kim, D. Kim, J. Am. Ceram. Soc., **81(5)**, 1184-1188 (1998).

14. C. Tan, B. Bowen, N. Epstein, J. Colloid Interface Sci., **118**, 290-293 (1987).

15. G. Bogush, M. Tracy, C. Zukoski, J. Non-Crystalline Solids, **104**, 95-106 (1988).

16. G. Bogush, C. Zukoski, J. Colloid Interface Sci., **142**, 1-19 (1991).

17. L. Jelinek, P. Dong, C. Rojas-Pazos, H. Taibi, E. Kováts, Langmuir, **8**, 2152-2164 (1992).

18. A. Van Blaaderen, A. Kentgens, J. Non-Crystalline Solids, **149**, 161-178 (1992).

19. J. Bailey, M. Mecartney, Colloids and Surfaces, **63**, 151-161 (1992).

20. S. Chen, P. Dong, G. Yang, J. Yang, Ind. Eng. Chem. Res., **35**, 4487-4493 (1996).

21. S. Chen, P. Dong, G. Yang, J. Colloid Interface Sci., **189**, 268-272 (1996).

22. R. Lindburg, G. Sundholm, B. Pettersen, J. Sjöblom, S. Friberg, Colloids and Surfaces A, **123-124**, 549-560 (1997).

23. H. Boukari, J. Lin, M. Harris, J. Colloid Interface Sci., **194**, 311-318 (1997).

24. S. Ludtke, T. Adam, K. Unger, J. Chromatogr. A, **786(2)**, 229-235 (1997).

25. K. Reynolds, T. Maloney, A. Fermier, L. Colón, Analyst, **123**, 1493-1495 (1998).

26. C. Brinker, G. Scherer, **Sol-Gel Science.** Academic Press, Boston, 1990.

27. Y. Guo, L. Colón. Anal. Chem., **67**, 2511-2516 (1995).

28. Y. Guo, L. Colón, J. Microcol. Sep., **7**, 485-491 (1995).

29. Y. Guo, G. Imahori, L. Colón, J. Chromatogr. A, **744**, 17-29 (1996).

30. Y. Guo, L. Colón, Chromatographia, **43**, 477-483 (1996).

31. S. Chong, D. Wang, J. Hayes, B. Wilhite, A. Malik, Anal. Chem., **69**, 3889-3898 (1997).

32. D. Wang, S. Chong, A. Malik, Anal. Chem., **69**, 4566-4576 (1997).

33. M. Dulay, R. Kulkarni, R. Zare, Anal. Chem., **70**, 5103-5107 (1998).

34. Q. Tang, B. Xin, M. Lee, J. Chromatogr. A, **837**, 35-50 (1999).

35. A. Van Helden, J. Jansen, A. Vrij, J. Colloid Interface Sci., **81(2)**, 354-368 (1981).

36. R. Badley, W. Ford, F. McEnroe, R. Assink, Langmuir, **6**, 792-801 (1990).

37. L. Sander, S. Wise, CRC Crit. Rev. in Anal. Chem., **18(4)**, 299-413 (1987).

38. E. Bourgeat-Lami, Ph. Espiard, A. Guyot, Polymer, **36(23)**, 4385-4389 (1995).

39. L. Colón, Y. Guo, A. Fermier, Anal. Chem., **69**, 461A-467A (1997).

40. L. Colón, K. Reynolds, R. Alicea-Maldonado, A. Fermier, Electrophoresis, **18**, 2162-2174 (1997).

41. S. Ludtke, A. Thomas, K. Unger, **Ninth International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques**, Anaheim, CA, 1997.

42. L. Colón, A. Fermier, Y. Guo, K. Reynolds, **Ninth International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques**, Anaheim, CA, 1997.

43. L. Colón, A. Fermier, Y. Guo, K. Reynolds, S. Rodríguez, **Nineteenth International Symposium on Capillary Chromatography and Electrophoresis**, Wintergreen, VA, 1997.

Received April 21, 1999
Accepted July 27, 1999
Manuscript 5091