C18 Modified Monolith Silica Particles of 3-5 μm

Kyoung Moon Han and Won Jo Cheong*

Department of Chemistry, Nano Fine Center, and Institute of Basic Research, Inha University, Incheon 402-751, Korea
**E-mail: wjcheong@inha.ac.kr*
Received June 6, 2008

Key Words : Silica monolith particles, Particles of 3-5 μm, C18 modification, Microcolumn

New stationary phases called monoliths[1-5] have been extensively studied recently. The monolith is an one-body porous solid and it has nanometer-sized inner pores and micrometer-sized through channels. Monolith columns fabricated in silica capillaries have been well used in capillary electrochromatography (CEC).[6-9]

Use of a monolith column enables a high flow to save analysis time since its pressure drop is much lower than that of a conventional packed column. However, formation of commercially available monolith is confined in heat-shrinkable polymer tubing.[5]

We have been studying on home-made microcolumns in our laboratory.[10-13] Especially, we have reported the preparation of C18 modified and endcapped ground monolith particles of 5-10 μm and showed that the C18 ground monolith particles had merits of improved separation efficiency over the conventional C18 particles. However, we were able to produce only the particles larger than 5 μm in that study.

In this study, we have improved the process to obtain ground monolith particles of 3-5 μm resulting in even better separation efficiency.

Experimental

Materials. HPLC grade water and methanol were purchased from Fisher (Pittsburg, PA, USA) and used without purification. Tetramethoxysilane (TMOS), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), acetic acid, PEG 10000 (polyethyleneglycol, MW 10000), chlorooctadecyldimethylsilane, trimethylchlorosilane, toluene, and pyridine were purchased from Aldrich (Milwaukee, IL, USA). The Alltima C18 (3 μm, 80 Å) stationary phase was obtained from Alltech (Deerfield, IL, USA).

Preparation of ground silica monolith particles. The reaction mixture of TMOS, polyethylene glycol, and acetic acid was prepared as in the previous report[13] and the preparation process was modified to obtain 3-5 μm particles. Thus, the reaction mixture composed of PEG 10000 220 mg, urea 225 mg, 0.01 N acetic acid 2.5 mL, and TMOS 1 mL, was heated in a vial at 40 °C for 48 h, then the temperature was raised to 120 °C and remained for 24 h (instead of 6 h in the previous study). Shrinkage during monolith formation enabled easy removal of the completed monolith piece. The monolith was thoroughly powdered with a mortar and pestle, and washed with 50/50 (v/v) methanol/ water (instead of water in the previous study) under reflux for 24 h with vigorous stirring and filtered. The particles were dried, and heated at 330 °C (instead of 300 °C in the previous study) for 24 h to strengthen the monolith structure.[14] The specific surface area and average pore diameters were determined by N_2 physisorption at liquid nitrogen temperature with Micromeritics (Norcross, GA, USA) ASAP 2000. The particles were sieved in a L3P Sonic Sifter Separator of Laval Lab (Laval, Quebec, Canada) and three portions were collected. A portion of particles larger than 10 μm was about 30%, a portion of 5-10 μm, ca 30%, and a portion of 3-5 μm, *ca* 40%. The portion of particles less than 3 μm was negligible.

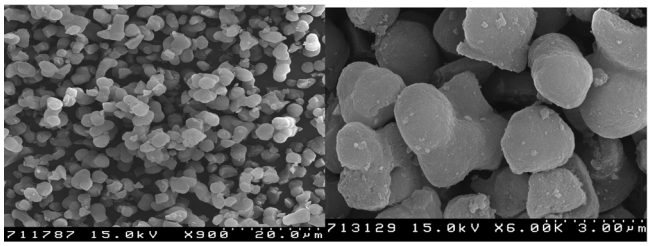
C18 modification and end-capping of the ground silica monolith particles. 200 mg of silica monolith particles of 3-5 μm, pyridine 0.12 mL, xylene 5.0 mL, and chlorodimethyloctadecylsilane 0.12 g were put into a small round bottom flask, and stirred at 110 °C for 24 h. The product was filtered and washed with toluene, THF, and methanol, and dried at 60 °C for 24 h. The dried product, 0.12 mL pyridine, and 5.0 mL xylene, 106 μL HMDS, and 35 μL TMCS were placed in a small flask, and stirred at 110 °C for 24 h. The final product was filtered and washed with toluene, THF, and methanol, and dried at 60 °C for 24 h.

HPLC. A chromatography system composed of a Shimadzu (Tokyo, Japan) 10AD pump, a Valco (Houston, TX, USA) CI4W.05 injector with a 50 nL injection loop, a Jasco (Tokyo, Japan) UV-2075 UV/Vis capillary window detector, and a Shimadzu DGU-14A degasser, was used. The C18 modified silica monolith microcolumn (0.5 mm × 300 mm) was packed as in the previous studies.[10-13] The chromatographic data were obtained by a PC system, and a software, Multichro 2000 from Youlin-Gisul (Sungnam, Korea), was used to acquire and process the data.

Three different batches of monolith C18 columns (3-5 μm) and three batches of Alltima C18 columns (3 μm) were prepared to examine the separation performance of the C18 ground monolith phase in comparison with the commercial C18 phase.

Results and Discussion

Figure 1 shows the SEM images of C18 modified and endcapped monolith particles of 3-5 μm. The broken pieces of typical three-dimensional monolith network are observed in Figure 1b. Packing a column with such particles would



(A) (B)

Figure 1. The SEM images of (A) sieved silica monolith particles (3-5 μm) and (B) the expanded view of A.

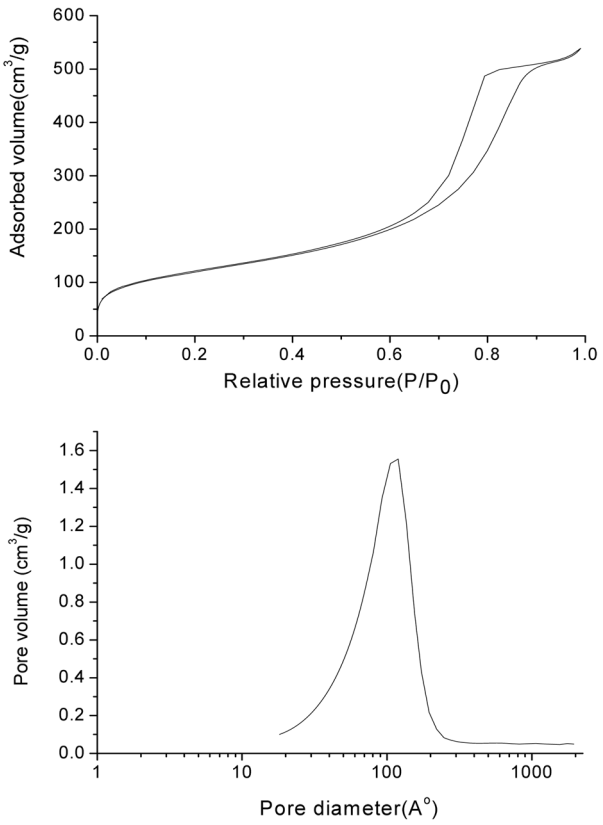


Figure 2. The N_2 adsorption-desorption isotherm and the BJH adsorption pore size distribution of the powdered silica monolith particles (3-5 μm).

result in a packed column with some through flow channels to give better separation efficiency than a column packed with a spherical C18 phase, and it was actually observed as will be shown later. Figure 2 shows the BET analysis. An average pore diameter of 105 Å with wide pore size distribution was observed, and the specific surface was 330 m^2/g. According to the results of elemental analysis shown in Table 1, the carbon load of our phase (17.8%) was found a little higher than that of a commercial phase (Alltima C18, 15.4%), and it may be owing to a little larger pore size of our phase (105 Å) compared to that of the commercial phase (80 Å). Figure 3 compares chromatographic separation between the C18 ground monolith phase and the Alltima C18 phase. Figure 4 shows the Van Deemter plots (based on 3 batches of data) of the selected solutes obtained in 80/20

Table 1. Comparison of elemental analyses between the C18 ground monolith and Alltima C18 phases

Type of particles	Element	%
C18 modified and end-capped ground monolith (3-5 μm)	C	17.8
	H	3.5
Alltima C18 (3 μm)	C	15.4
	H	3.1

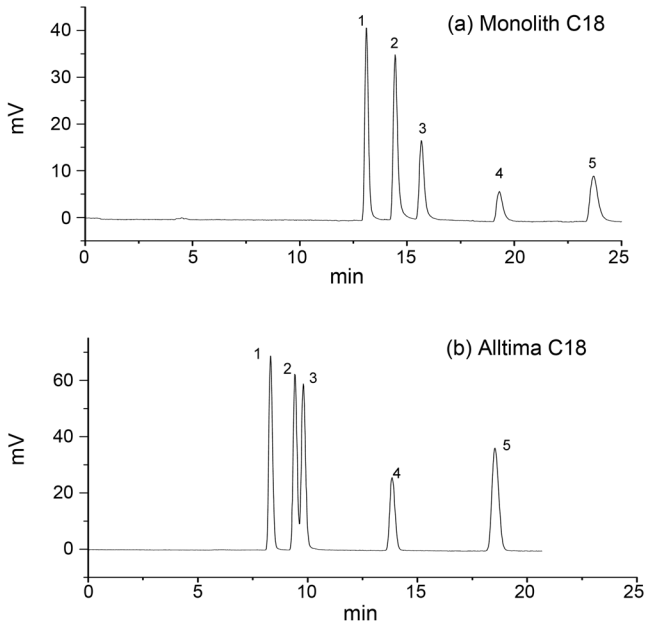


Figure 3. Comparison of separation efficiencies between (a) the column (300 × 0.5 mm) packed with the powdered C18 silica monolith particles (3-5 μm) and (b) the column packed with the Alltima C18 stationary phase (3 μm). The chromatograms of selected solutes were obtained at a flow rate of 0.01 mL/min in 80/20 (v/v) methanol/water with 0.1% TFA. 1; phenol, 2; 2-nitro-aniline, 3; acetophenone, 4; benzene, 5; toluene.

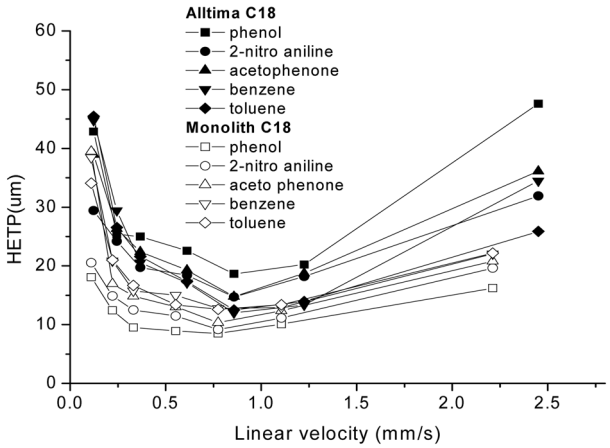


Figure 4. Comparison of van Deemter plots (height equivalent to theoretical plates vs mobile phase linear velocity) measured in 80/20(v/v) methanol/water with 0.1% TFA between the Alltima C18 (3 μm) and the Monolith C18 (3-5 μm) columns (0.5 × 300 mm).

(v/v) methanol/water with 0.1% TFA for the microcolumn packed with the C18 monolithic particles (3-5 μm, open

Table 2. Comparison of the values (averages and standard deviations, n = 3) of number of theoretical plates measured at the flow rate of 0.007 mL/min between the Alltima C18 and the monolith C18 columns

	Phenol	2-nitroaniline	Acetophenone	Benzene	Toluene
Alltima C18 (0.007 mL/min)	16100 ± 340 (2.1%)	20400 ± 400 (2.0%)	20300 ± 330 (1.6%)	25000 ± 450 (1.8%)	24400 ± 410 (1.7%)
Monolith C18 (0.007 mL/min)	35300 ± 850 (2.4%)	32200 ± 680 (2.1%)	26400 ± 540 (2.0%)	23500 ± 420 (1.8%)	23800 ± 450 (1.9%)

symbols) in comparison with those for the microcolumn packed with the commercial (Alltima C18, 3 μm) C18 particles (closed symbols). The numerical HETP values of our phase and the commercial phase are compared in Table 2 for a flow rate of 0.007 mL/min. Chromatographic separation efficiency of our phase was either comparative or better than that of the commercial phase based on the data of Figures 3 and 4, and Table 2 despite its larger average particle size (3-5 μm *vs*. 3 μm). It means that the ground monolith C18 phase shows quite faster mass transfer rates than the commercial C18 phase. Separation performance of monolith particles of 3-5 μm (HETP as low as 8-10 μm) was, of course, better than that of 5-10 μm particles (HETP as low as 15 μm).[13]

It should be noted that the column efficiencies obtained with home-packed microcolumns (0.5 mm I.D.) in this study were inferior to those obtained with commercially available conventional columns (4.6 mm I.D.) reported in the literature[15,16] probably owing to the fact that home-made microcolumns of a narrow I.D. were used in this study. Extra-column band-broadening contribution to HETP will be more significant when a column of limited volume is used. However, the same conditions were applied to preparation of three batches of both columns packed with the ground monolith C18 and Commercial spherical C18 phases, respectively, thus the observed superiority of the ground monolith C18 phase over the commercial C18 phase is statistically valid.

It was observed that the pressure drop of the column was about the same (*ca* 90 bar at the flow rate of 0.007 mL/min) for both phases, therefore superiority of our phase over the commercial phase may not be due to the existence of through flow channels. The column pressure drop data indicates that there is not really sufficient through flow channels in the column packed with the monolith particles of 3-5 μm. The effect of long and branched (or bent) structure of our phase (Figure 1b) would be the real reason of reduced band broadening. The deepest diffusion distance of a monolith particle from its surface is less than 1 μm owing to its unique structure while that of a spherical particle (3 μm) is 1.5 μm. Thus the faster mass transfer of the monolith particles may be a shape-based intrinsic character.

The success of preparation of monolith particles less than 5 μm in this study was done by formation of more ripened monolith with extended reaction time to be easily broken into smaller particles followed by more effective washing of residual reaction mixture with methanol/water to avoid aggregation of particles upon calcination. However the yield of particles of 3-5 μm is rather low (40%, particles larger than 5 μm are the rest) in this study. Further improvement of the process is under way.

Conclusion

We have prepared monolith particles of 3-5 μm by some modifications in the processes of monolith formation and washing. The microcolumn packed with such C18 ground monolith particles showed better separation efficiency than commercial C18 particles of 3 μm. The shorter diffusion distance from the particle surface and existence of some through flow channels of the column packed with the C18 monolith particles seem to be responsible for such superiority.

Acknowledgments. This work was supported by the Korea Research Foundation grant (KRF-2007-521-C00187).

References

1. Hjerten, S.; Liao, J. L.; Zahang, R. *J. Chromatogr.* **1989**, *473*, 273-275.
2. Svec, F.; Frechet, J. M. L. *Anal. Chem*. **1992**, *64*, 820-822.
3. Zou, H.; Huang, X.; Ye, M.; Luo, Q. *J. Chromatogr. A* **2002**, *954*, 5-32.
4. Siouffi, A. M. *J. Chromatogr. A* **2003**, *1000*, 801-818.
5. Cabrera, K. *J. Sep. Sci*. **2004**, *27*, 843-852.
6. Ching, Q. C.; Svec, F.; Frechet, J. M. L. *Anal. Chem*. **1993**, *65*, 2243-2248.
7. Li, Y. M.; Liao, J. L.; Nakazato, K.; Hjerten, S. *Anal. Biochem*. **1994**, *223*, 153-158.
8. Elicson, C.; Liao, J. L.; Nakazato, K.; Hjerten, S. *J. Chromatogr. A* **1997**, *767*, 33-41.
9. Li, W.; Fries, D. P.; Malik, A. *J. Chromatogr. A* **2004**, *1044*, 23-52.
10. Cheong, W. J.; Kang, G. W.; Lee, W. L.; Yoo, J.-S. *J. Liq. Chrom. & Rel. Technol*. **2002**, *25*, 1367-1378.
11. Cheong, W. J.; Seo, Y. J.; Park, S. T.; Kang, G. W. *Bull. Korean Chem. Soc*. **2006**, *27*, 1059-1062.
12. Seo, Y. J.; Kang, G. W.; Park, S. T.; Moon, M.; Park, J. H.; Cheong, W. J. *Bull. Korean Chem. Soc*. **2007**, *28*, 999-1004.
13. Ko, J. H.; Baik, Y. S.; Park, S. T.; Cheong, W. J. *J. Chromatogr. A* **2007**, *1144*, 269-274.
14. Motokawa, M.; Kobayashi, H.; Ishizuka, N.; Minakuchi, H.; Nakanishi, K.; Jinnai, H.; Hosoya, K.; Ikegami, T.; Tanaka, N. *J. Chromatogr. A* **2002**, *961*, 53-63.
15. Moriyama, H.; Anegayama, M.; Komiya, K.; Kato, Y. *J. Chromatogr. A* **1995**, *691*, 81-89.
16. Wu, N.; Dempsey, J.; Yehl, P. M.; Dovletoglou, A.; Ellison, D.; Wyvratt, J. *Anal. Chim. Acta* **2004**, *523*, 149-156.