

Ground Organic Monolith Particles as Chromatographic Separation Media

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A monolith is one body 3-dimensional organic or inorganic polymer network where macroporous pores (through flow channels, over 1 μm) and mesoporous pores (less than 1 μm) are included. A properly made monolith in a metal column or a silica capillary can be commonly used as separation media in liquid chromatography or capillary electrochromatography (CEC). The monolith column can also be used in solid phase extraction (SPE). There have been many review articles on inorganic and organic monoliths as separation media, and the number of such review articles has proven to increase recently¹⁻¹⁸ as interest in monoliths has kept growing. On the other hand, studies on ground monolith particles have been rare. C18 modified^{19,20} and polystyrene modified²¹⁻²⁴ ground silica monolith particles have been employed as new stationary phases of improved separation efficiency. Nevertheless, there has been no report on organic ground monolith particles as chromatographic or SPE media so far. In this study, special organic monolith particles have been prepared by a simple procedure and their possibility as separation media has been explored. This study is the very first report of using organic monolith particles as separation media. The separation performance ($N \sim 4,000$) of organic monolith particles of this study is inferior to that of commercial monolith columns or C18 packed columns ($N \sim 10,000$ - $20,000$) at present. The simple and inexpensive preparation procedure of this study may be the primary merit of organic ground monolith particles. In addition, their separation performance is subject to improvements in the future.

Experimental

Materials. Glass lined stainless steel tubing (30 cm, 0.5 mm I.D., 1.6 mm O.D.) was purchased from Grace (Deerfield, IL, USA). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), polyethylene glycol (PEG) 10000, 2,2,4-trimethylpentane (isooctane), toluene (anhydrous), glacial acetic acid, and trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Azobisisobutyronitrile (AIBN) was obtained from Junsei Chemical (Tokyo, Japan). HPLC grade acetonitrile and water were obtained from SK Chemicals (Ulsan, Korea). All the reagents were used as received. Screen frits (1.6 mm radius, 0.08 mm thickness), 1/16" unions, and 1/16-1/32 reducing unions were purchased from Valco (Houston, TX, USA).

Preparation of Organic Monolith Particles. A reaction

mixture composed of 270 μL MAA, 228 μL EDMA, 400 μL isooctane, 1.5 mL toluene, 10 mg AIBN, and a determined amount (24, 36, or 48 mg) of PEG 10000 was prepared in a vial. Such formulation was determined after a series of preliminary experiments to result in MIP of compromised softness and hardness for easy smashing and proper particle size distribution. PEG 10000 was used as porogen for mesopores. The mixture was purged with nitrogen for 10 min. The vial was tightly closed with a Teflon-lined cap, and placed in an oven set at 70 $^{\circ}\text{C}$ for 24 h. After completion of polymerization, the MIP was simply smashed with a spatula, transferred to a round bottom flask with a reflux condenser, and stirred under reflux for 24 h after addition of 30 mL 9/1 (v/v) 2-propanol/acetic acid. Such reflux washing step was repeated twice, and the particles were filtered, rinsed with acetone, and dried at room temperature.

Characterization of Monolith Particles. A HITACHI (Tokyo, Japan) S-4200 field emission scanning electron microscopy (FE-SEM) was used to obtain SEM images of the phase. The BET/BJH nitrogen adsorption/desorption isotherms were measured at 77 K using a BEL-Japan (Osaka, Japan) BELSORP-Max for the ground organic monolith particles. The amount of N_2 adsorbed at a relative pressure of $P/P_0 = 0.98$ was used to determine the total pore volume. A Malvern (Worcestershire, UK) Mastersizer 2000 particle size analyzer was used to measure the size distribution of the monolith particles.

Column Packing and HPLC. The micro columns (0.5 mm \times 300 mm) were packed according to the procedure of previous reports.¹⁹⁻²⁴ Thus, a commercial screen frit was placed in the 1/16 inch outlet of a 1/16-1/32 reducing union, a piece of 30 cm glass lined stainless steel tubing was fitted to the outlet, and the tubing was connected to the packer. The 100 mg particles were suspended in 5 mL methanol, sonicated for 1 min, stood calm for 30 s, and the supernatant was decanted out to remove very fine particles. This process was repeated three times. The sedimented particles were suspended in 1.2 mL methanol and fed into the reservoir of a slurry packer. The pressure of the slurry packer was instantly raised to 14,000 psi for 5 min, adjusted to 10,000 psi for 10 min, to 8,000 psi for 40 min, and finally to 14,000 psi for 5 min. Then, the compressor was turned off to release the pressure on standing for a while. The column was then connected to the injector, and the 1/32 inch outlet of the column was connected to the capillary window detector by installing a graphite ferrule and a connecting capillary (50 μm I.D.,

365 μm O.D.). A piece of short Teflon tubing was used to connect the two capillary ends.

A Shimadzu (Tokyo, Japan) 10AD pump, a Shimadzu DGU-14A membrane degasser, 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 the home-made 0.5 mm I.D. glasslined micro column were assembled to construct the μ HPLC system. A PC system with the software Multichro 2000 from Youlin-Gisul (Seoul, Korea) was used to acquire and process the chromatographic data.

A test mix composed of *N*-methylaniline, phenol, acetophenone, benzene, and toluene was used.

Results and Discussion

Properties of Organic Monolith Particles. Obtaining a proper size distribution useful for HPLC application (*ca* 5 μm on average) was the primary strategy of this study, and preliminary experiments were carried out to achieve such a goal. The SEM photo and particle size distribution plot are given in Figure 1 and 2.

The major portion of particles is within the range of 1-10 μm . The volume weighted mean particle diameter was 4.8 μm . This is rather promising for the initial result of pioneering study although the preferred range for good HPLC performance is 2-5 μm (monodispersity is ideal). The exist-

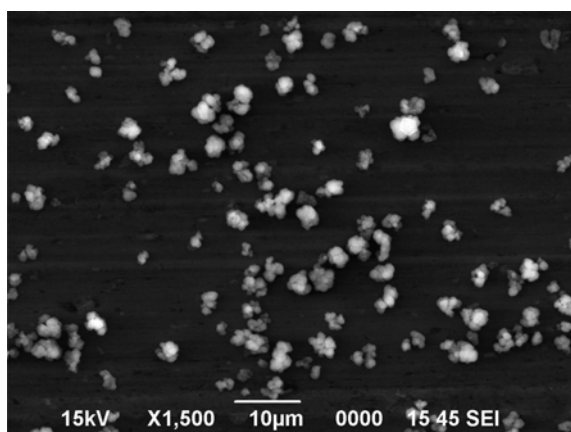


Figure 1. The SEM photograph of the organic monolith particles.

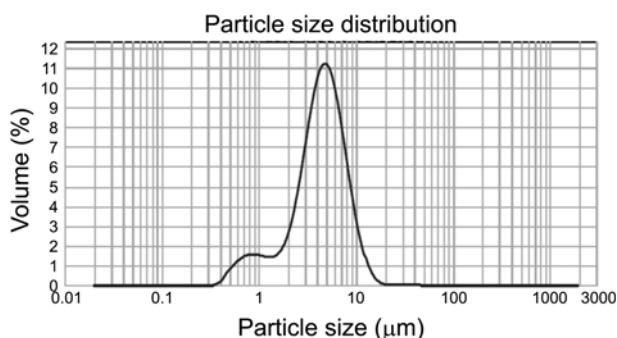


Figure 2. The particle size distribution of the organic monolith particles.

ence of some very fine particles (less than 1 μm) is of no problem since the portion can be easily removed when preparing slurry for column packing.

On the other hand, the result of BET/BJH was rather disappointing. The total pore volume was found only 0.022 cm^3/g , and the BET specific surface area, 3.41 m^2/g . Despite the too small total pore volume, a proper pore size distribution was obtained as shown in Figure 3. The mean pore size is *ca* 400-500 Å although there is an abnormal difference in distribution pattern between BJH adsorption and desorption results. The reason for selective appearance of a sharp peak at 100 Å only in the BJH desorption pore distribution is not clear. Whether the existence is real or not, pores

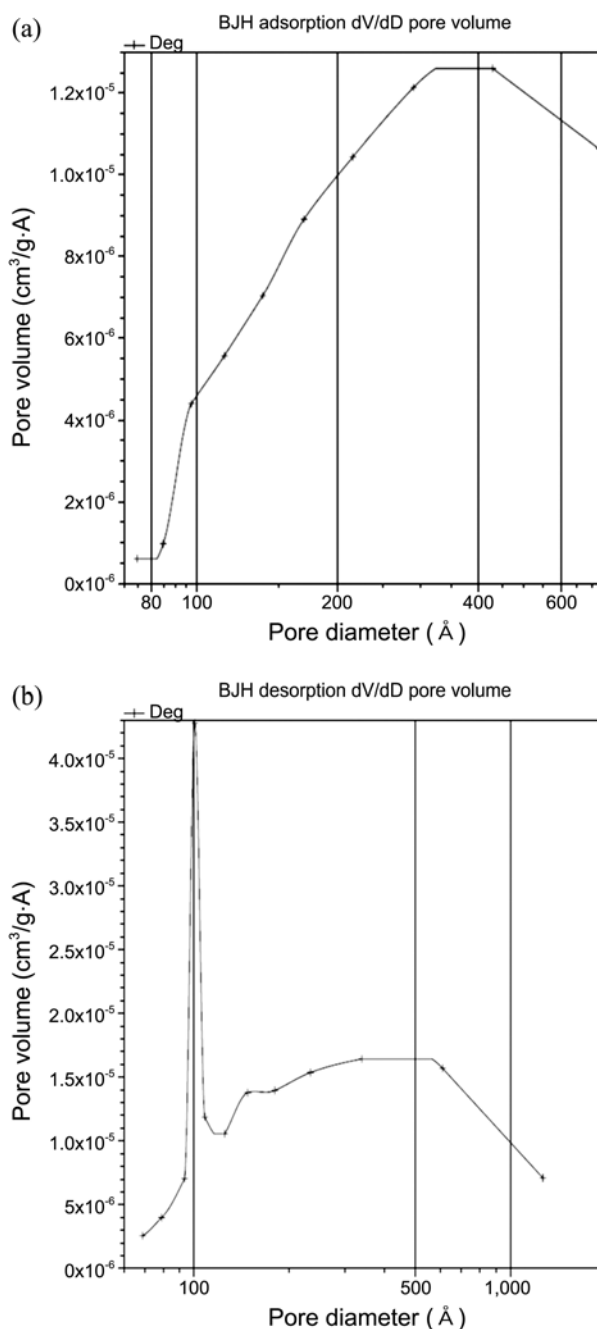


Figure 3. The BJH adsorption (a) and desorption (b) dV/dD pore size distribution.

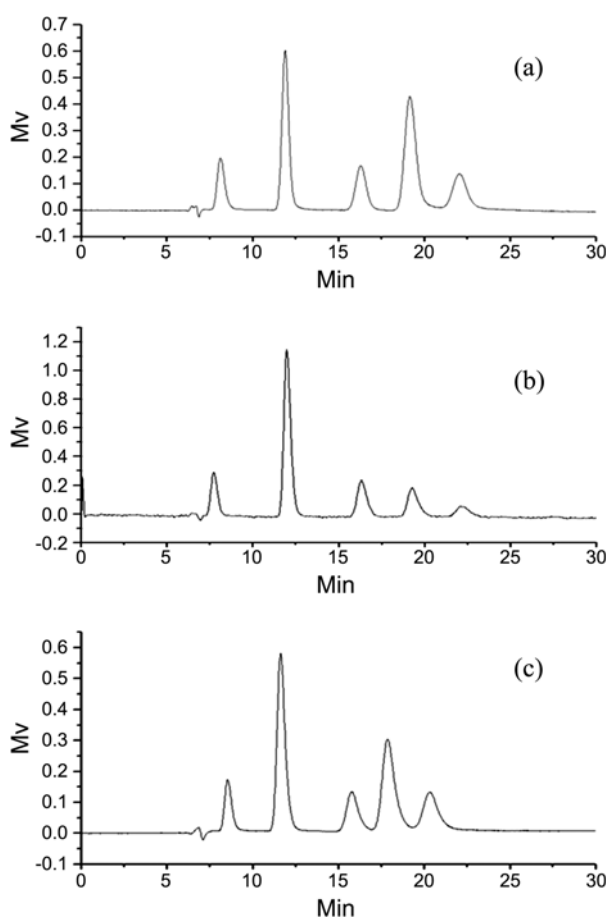


Figure 4. Comparison of chromatograms obtained in the eluent of 40/60 (v/v) acetonitrile/water (0.1% TFA) at a flow rate of 7 $\mu\text{L}/\text{min}$ with the columns packed with the organic monolith particles prepared by the reaction mixture including (a) 24 mg (b) 36 mg, and (c) 48 mg PEG 10000. The solutes are *N*-methylaniline, phenol, acetophenone, benzene, and toluene in the elution order.

of such size impose no problem in view of mass transfer kinetics since such pore size is the typical one in the commercial C18 stationary phase.

Chromatographic Separation Performance of the Organic Monolith Particles. Despite the too low total pore volume, the ground organic monolith particles showed some separation performance as shown in Figure 4. Incorporation of PEG 10000 in the reaction mixture was very critical. The organic monolith particles prepared without PEG showed very poor separation performance. It was intended to form sufficient mesopores in the monolith by incorporation of PEG 10000. Such intention was only partially fulfilled since the total pore volume was only 0.022 cm^3/g . On the other hand, the total pore volume of ground silica monolith particles was ca 0.4 cm^3/g .¹⁹ There was an optimum amount of PEG in the reaction mixture since use of a too little or too much amount of PEG yielded inferior separation performance of the resultant organic monolith particles as shown in Figure 4. The numbers of theoretical plates (*N*) obtained are assembled in Table 1. The results obtained with the organic monolith particles prepared with 36 mg PEG were better than those of the other cases.

Table 1. The number of theoretical plates obtained with the columns packed with the organic monolith particles prepared with the reaction mixture including 24, 36, 48 mg PEG 10000

PEG (mg)	<i>N</i> -methylaniline	Phenol	Acetophenone	Benzene	Toluene
24	1300	3900	3200	3800	3100
36	1600	4100	3900	4400	2500
48	1600	2900	2600	2900	2600

Perspective of Organic Monolith Particles. The separation performance of the column packed with the organic monolith particles of this study is, of course, inferior to that of commercial C18 packed columns or monolithic columns. The typical *N* values of commercial 25 cm C18 (5 mm) columns are in the range of 10,000-25,000 while *N* values as high as 4,000 or so were obtained with the columns packed with organic monolith particles in this study. Two factors are responsible for this. First, the too small total pore volume and the too low specific surface area caused reduced retention and poor separation. Thus an eluent of quite high water content was needed to obtain proper retention times. Second, the wide particle size distribution surely contributed to poor separation efficiency.

The critical merit of the stationary phase of this study compared to the conventional C18 phase or bulk monolith phase is the potential cost efficiency since all the preparation steps including polymerization, liquid state powdering, and washing can be done in the same reactor. The manufacturing procedure is also compatible with large scale production. The trick that enables such advantages is to prepare soft monolith that can be easily smashed into powders by agitation using delicately formulated reaction mixtures. The final product may be used as separation media without further chemical modification. A hard bulk monolith will be very difficult to handle in a similar fashion. Very delicate care should be taken, however, not to result in too small particles in formulating the reaction mixture.

Organic monolith particles may be very prospective media in separation science after improvements in particle size distribution and total pore volume. Many different monomers, cross-linkers, mesopore porogens, and solvents can be employed for such improvements. In addition, this method can be used for mass production of SPE media without too rigorous improvement since separation media of moderate separation efficiency is not good enough for HPLC or CEC purposes, but may be successfully employed for SPE application. The total pore volume might be increased by creating a situation where porogen molecules form aggregates of proper size (100-500 \AA), uniformly dispersed in the reaction mixture being surrounded by the monomers, and stay stable until a 3-dimensional polymer network is completed. Relevant study is being taken.

Conclusion

The applicability of organic monolith particles as separation

media has been explored in this study. We were able to prepare organic monolith particles of some separation performance in HPLC by a potentially cost-effective process. The soft monolith formed with a reaction mixture of a delicate formulation was easily smashed and washed to give organic monolith particles. Actual realization of organic monolith particles as dependable separation media requires further improvements in narrowing particle size distribution and enhancing total pore volume. Such study is under way.

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