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Ground Organic Monolith Particles Having a Large Volume of Macropores as Chromatographic Separation Media

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A reaction mixture was developed for formation of soft organic monolith that was easily smashed, rinsed, refluxed, filtered, and dried to give monolith particles having high pore volume of macropores. This phase was almost without mesopores. The reaction mixture was composed of methacrylic acid, ethylene glycol dimethacrylate, polyethylene glycol (porogen), and an initiator in a mixed solvent of toluene and isooctane. The selection of porogen and its amount was carefully carried out to obtain the optimized separation efficiency of the resultant phase. The median macropore size was 1.6 μm, and the total pore volume was 3.0-3.4 mL/g. The median particle size (volume based) was 15 μm, and the range of particle size distribution was very broad. Nevertheless the column (1 × 300 mm) packed with this phase showed good separation efficiency (N~10,000-16,000) comparable to that of a commercial column packed with 5 μm C18 silica particles.

Key Words : Organic monolith particles, Packed column, Polyethylene glycol, Macropores, High pore volume

Introduction

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 well-made monolith in a metal or silica capillary column can be commonly used as separation media in liquid chromatography, capillary electrochromatography (CEC), and solid phase extraction (SPE). There have been many review articles on organic[1-7] and inorganic[8-11] monoliths as separation media. On the other hand, studies on ground monolith particles have been rare. In our laboratory, C18 modified[12,13] and polystyrene modified[14-19] ground silica monolith particles have been employed as new stationary phases of improved separation efficiency. The very first report on study of organic monolith particles as inexpensive chromatographic separation media was also recently published by our group.[20] However, the separation performance (N~4,000/column) of the column packed with the organic monolith particles was far inferior to that of commercial monolith columns or C18 packed columns (N~10,000-20,000/column). In this study, special organic monolith particles have been prepared to show good separation efficiency (N~10,000-16,000/column) when packed in a 30 cm column.

Experimental

Chemicals and Materials. Glass lined stainless steel tubing (30 cm, 1 mm I.D., 1/8 inch O.D.) was purchased from Grace (Deerfield, IL, USA). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), polyethylene glycol (PEG) 20000 (average molecular weight, MW), PEG 100000, PEG 400000, 2,2,4-trimethylpentane (isooctane), toluene (anhydrous), glacial acetic acid, and trifluoroacetic acid (TFA) 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 (1/8 inch radius, 0.08 mm thickness), 1/8 inch unions, 1/8-1/16 reducing unions, and various PEEK and Teflon tubings were purchased from Valco (Houston, TX, USA).

Preparation of Organic Monolith Particles. A reaction mixture composed of 1.350 mL MAA, 1.14 mL EDMA, 2.00 mL isooctane, and 10.00 mL toluene, was prepared in a vial, purged with nitrogen for 20 min, and heated at 70 °C after tightening the Teflon-lined cap. A determined amount (50, 100, or 200 mg) of PEG (MW 20000, 100000, or 400000) and 50 mg AIBN were rapidly dissolved in the mixture, and the vial was tightly closed with the Teflon-lined cap, and placed in an oven set at 70 °C for 24 h. 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 100000 was found as the optimized porogen, and 100 mg, as the optimized amount of porogen in view of separation efficiency of the resultant phase. After completion of polymerization, the MIP was simply smashed with a spatula, transferred to a round bottom flask, and washed with acetone three times to remove most of the unreacted materials, and refluxed with 5/5 (v/v) water/2-propanol and 9/1 (v/v) 2-propanol/acetic acid for 24 h each to remove trapped porogen molecules. The particles were filtered, rinsed with acetone, dried overnight at room temperature, and stored in a desiccator.

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. A

JEOL (USA) JEM 2100F was used to obtain transmission electron microscopy (TEM) images. The suspension of particles was made in ethanol by sonication, and 10 drops of it were applied on 200 mesh copper grids, dried in a vacuum oven at 60 °C for 24 h and inserted in the TEM instrument.

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. A Micrometrics (Norcross, GA, USA) Autopore IV mercury intrusion porosimetry instrument was used to measure macropore size distribution and total pore volume.

Column Packing and HPLC. The micro columns (1.0 mm × 300 mm) were packed as follows. A 1/8 inch commercial screen frit was placed in the 1/8 inch port of a 1/8-1/16 reducing union, a piece of 30 cm glass lined stainless steel tubing (1.0 mm I.D., 1/8 inch O.D.) was fitted to the port, and the tubing was connected to the packer. The 300 mg particles were suspended in 9 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 5.5 mL methanol and fed into the reservoir of a slurry packer. The pressure of the slurry packer was instantly raised to 15,000 psi for 5 min, adjusted to 10,000 psi for 10 min, to 8,000 psi for 30 min, with mechanical vibration, and the supply of the compressor gas was closed to reduce the pressure slowly. The column was detached from the slurry packer, installed with another 1/8-1/16 reducing union (column inlet) with a 1/8 inch column inlet frit, and connected to the injector with a short piece of stainless steel tubing (0.1 mm I.D., 1/16 inch O.D.), and the 1/16 inch port of the column outlet was connected to the capillary window detector by installing a connecting capillary (50 μm I.D., 365 μm O.D.) in a short piece of PEEK tubing (380 μm I.D., 1/16 inch O.D.) with an one-body polymer nut-ferrule tightening around the PEEK tubing. 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 1.0 mm I.D. glasslined micro packed column were assembled to construct the μHPLC system. A PC system with the software Multichro 2000 from Youlin-Gisul (Seoul, Korea) was used to acquire and process the chromatographic data. The stock test mix sample solution was prepared by dissolving *N*-methylaniline (0.5 μL), phenol (0.88 μL), acetophenone (0.14 μL), benzene (2.93 μL), and toluene (1.46 μL) in 1 mL mobile phase and was stored at 4 °C. The sample was further diluted for injection. The mobile phase was 40/60 (v/v) acetonitrile/water containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 15 μL/min. KNO_3 was used to measure t_o.

Results and Discussion

Characterization of Organic Monolith Particles. The organic monolith particles of this study have some specific features. They are rather large particles (Fig. 1 and 2) with wide particle size distribution (Fig. 3), and they have rather large total pore volume composed mostly of macropores (> 1 μm, Fig. 4), and nevertheless they show quite good separation efficiency (N~ 10,000-16,0000) when packed in a 30 cm column (Fig. 5 and 6). There are few mesopores in the organic monolith particles as proved by the very low total mesoporous pore volume (0.007 mL/g) and BET specific surface area (4.2 m^2/g) measured by the BET/BJH N_2 adsorption/desorption isotherms. Macroporous pore volume cannot be measured by the N_2 adsorption/desorption measurement. According to the mercury intrusion porosimetry measurements, the contribution of pores less than 0.5 μm to the total pore volume (mesoporous and macroporous) is negligible (Fig. 4). Mesopores were also hardly observed in the expanded TEM views of organic monolith particles as shown in Figure 2. Although the accuracy of mercury intrusion porosimetry for pore size and total pore volume is known to be limited, the three repeated measurements were rather in good agreement (Fig. 4), yielding the median pore size of 1.6 μm, the total pore volume of 3.0-3.4 mL/g, and the porosity of 76-78%. The particle size is mostly in the range of 5-30 μm (average 15 μm) according to the volume based particle size distribution (Fig. 3). However, the size of basic building units is less than 1 μm, and the particles have the characteristic monolith structure of high porosity and 3-dimensional network, which ensured the compact monolith structure of a diversity of through-flow channels and macropores on packing, enabling high separation efficiency. There is some portion of sub-micron particles, too (Fig. 3), and

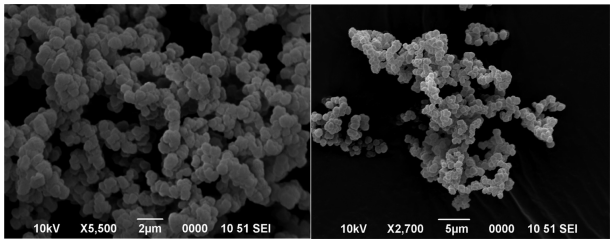

Figure 1. SEM photographs of organic monolith particles prepared by using the optimized formulation. The size of scale bar is 2 μm and 5 μm from the left, respectively.

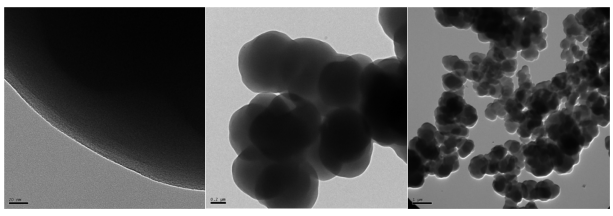

Figure 2. TEM photographs of organic monolith particles prepared by using the optimized formulation. The size of scale bar is 20 nm, 0.2 μm, and 1 μm from the left, respectively.

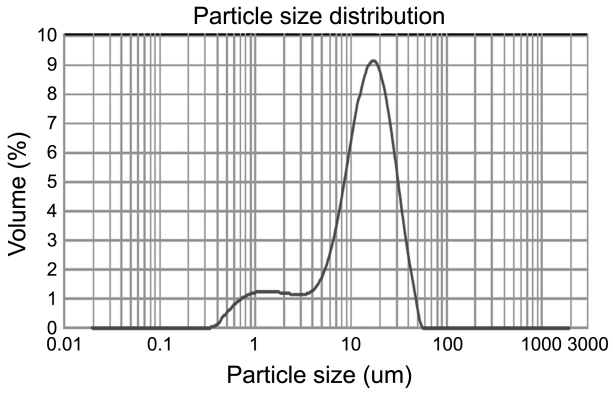

Figure 3. Volume based particle size distribution of organic monolith particles.

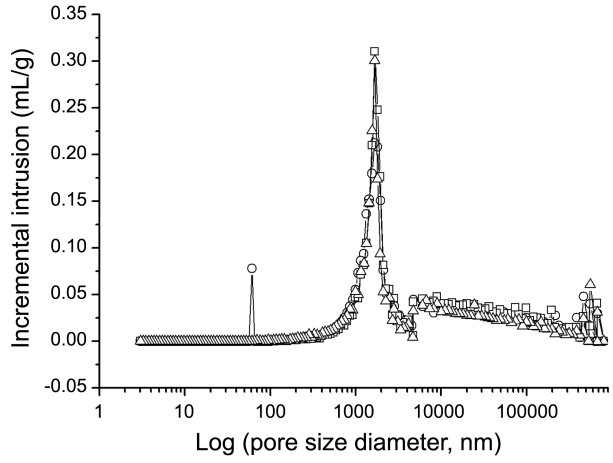

Figure 4. Pore size distribution of organic monolith particles.

these particles should be removed by differential sedimentation before slurry packing.

Chromatographic Separation with the Organic Monolith Particles. As shown in Figures 5 and 6, the chromatographic performance of the organic monolith particles made in this study was much better than expected in view of the large average particle size, wide particle size distribution, and little mesoporous pore volume. The optimized porogen and its amount were found PEG MW 100000 and 100 mg in the reaction mixture (1.350 mL MAA, 1.14 mL EDMA, 2.00 mL isooctane, and 10.00 mL toluene). The separation efficiency (N~10,000-16,000, Tables 1 and 2) of the column packed with the organic monolith particles made by using the optimized formulation was comparable to that of a commercial C18 column packed with 5 μm particles. It is not clear at present how the molecular weight and amount of porogen (PEG) affect the kinetics and thermodynamics of the monolith formation, however, it is clear that there should be an optimum molecular weight and amount of porogen to form a desirable monolith network that can be easily smashed into particles which still maintain the useful monolith structure. The soft bulk monolith made in this study is believed to have some large vacancies that are harmful to separation efficiency when used as a bulk monolith. Such

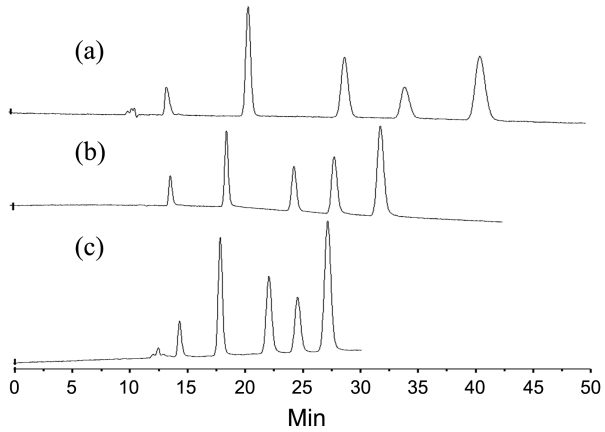

Figure 5. Comparison of chromatograms obtained in the eluent of 40/60 (v/v) acetonitirile/water (0.1% TFA) at a flow rate of 15 μL/min with the columns packed with the organic monolith particles prepared by the reaction mixture including (a) 50 mg (b) 100 mg (c) 200 mg PEG MW 100000. The solutes are *N*-methylaniline, phenol, acetophenone, benzene, and toluene in the elution order. The corresponding N values are in Table 1.

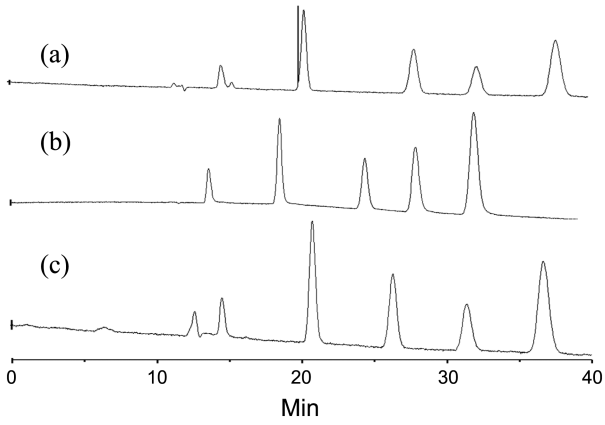

Figure 6. Comparison of chromatograms obtained in the eluent of 40/60 (v/v) acetonitirile/ water (0.1% TFA) at a flow rate of 15 μL/min with the columns packed with the organic monolith particles prepared by the reaction mixture including (a) 100 mg PEG MW 20000 (b) 100 mg PEG MW 100000 (c) 100 mg PEG MW 400000. The solutes are *N*-methylaniline, phenol, acetophenone, benzene, and toluene in the elution order. The corresponding N values are in Table 2.

unnecessary empty spaces can be removed by packing the ground monolith particles. A new strategy of preparing macroporous monolith particles without mesopores may be more adaptable to chromatographic packing material than organic particles composed of mesopores, since the latter may be subject to serious swelling owing to the enhanced surface area while the former, to much less swelling. The organic monolith was constructed with building units of sizes less than 1 μm, thus the macropores (> 1 μm) were not internally formed but rather externally formed (surface macropores) by the curved and twisted 3-dimensional network. The particular 3-dimensional diverse network that bears a large amount of macropores (3.0-3.4 mL/g) was believed to be realized by the porogen optimization.

Table 1. The number of theoretical plates obtained with the columns packed with organic monolith particles prepared with the reaction mixture including 50, 100, 200 mg PEG 100000

PEG content	*N*-Methylaniline	Phenol	Acetophenone	Benzene	Toluene
50 mg	8,900	14,000	12,100	12,900	12,700
100 mg	10,400	16,100	15,300	14,700	13,900
200 mg	8,400	11,000	11,400	11,600	11,000

Table 2. The number of theoretical plates obtained with the columns packed with organic monolith particles prepared with the reaction mixture including 100 mg of PEG 20000, 100000, 400000

PEG MW	*N*-Methylaniline	Phenol	Acetophenone	Benzene	Toluene
20,000	5,300	10,600	10,000	10,200	9,900
100,000	10,400	16,100	15,300	14,700	13,900
400,000	9,200	11,100	9,700	10,900	10,800

Nevertheless, the stationary phase was with few mesopores, and the solute retention times were much reduced in comparison to those of the conventional mesoporous C18 phase. This problem was easily solved by increasing water content in the mobile phase.

Perspective of Organic Monolith Particles. The reproducibility of retention times and plate numbers measured with a fixed column in a day and in different days was better than 2.0% and 3.5%, respectively. The reproducibility of retention times and plate numbers measured with three columns packed with three different batches of organic monolith particles was better than 4.6%.

We propose in this study the strategy of formation of soft organic monolith that can be easily smashed into particles with diverse 3-dimensional monolith network having only macropores and large pore volume. The potential of such monolith particles seems promising since the production can be carried out cost-effectively to yield nevertheless quite useful stationary phase whose performance is comparable to that of conventional C18 phase. The monolith particles can be made in a reactor installed with a replaceable filtering unit at the bottom with a removable cover without transferring to another container. Formation of monolith, smashing into particles, rinsing with acetone, refluxing with washing solvent, additional rinsing, and intermittent filtering steps may be carried out in the same container. The efficiency of washing of residual reaction components from the monolith particles will be much better than that from the bulk hard monolith. The final product can be directly used for chromatographic stationary phase without further modification.

This stationary phase with macropores and without mesopores may be rather favorably adapted to analysis of long retaining analytes of high molecular weights. This phase can also be used as stationary phase media for solid phase extraction (SPE).

There are many possible combinations of functional monomers, crosslinking monomers, porogens, and reaction solvents to make a variety of organic monolith particles as new prospective chromatographic stationary phases.

Conclusion

Useful organic monolith particles of high total pore volume with macropores have been developed as chromatographic separation media for packed columns. Despite the large average particle size and wide particle size distribution, feasible separation efficiency was obtained with a column packed with this phase after appropriate optimization of porogen and its amount in the reaction mixture. Owing to good chromatographic separation performance, potential low-cost production, and unlimited possibilities of selection of raw reaction components, this new research area of organic monolith particles may attract great attention in the fields of HPLC and SPE in the future.

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