

Preparation of Poly(styrene-divinylbenzene) Monolith Frits Encased in Common Polymer Tubing and Their Application in μ LC

Sung Soon Kim 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

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Porous disc-type frits are placed in both ends of a chromatography column to keep the stationary phase in and to let the mobile phase penetrate in common columns. A very special column in view of frit technique is the monolith column. Its whole stationary phase is one body with various multiple porous channels, thus no additional frits are necessary since the whole stationary phase functions as a frit.^{1,2} Details of monolith columns and their applications can be found in some excellent review articles and the references cited thereof.³⁻⁶ Studies on home-made microcolumns and disposable microcolumns have been being carried in our laboratory.⁷⁻¹⁰ Especially we introduced the preparation method of dependable tubing-frits and a column of a new simple design by installing the tubing-frits at the column inlet and outlet unions⁸ instead of sintered silica capillary frits.⁷

In this study, we introduce an easy and cheap preparation method of tiny poly (styrene-divinylbenzene) monolith frits encased in polymer tubing. Common polymer tubing (1/16 inch O.D.) was filled with the monolith reaction mixture, heated, washed, dried and cut into thin slices to yield the frits. It should be noted that organic monolith frits were fabricated in silica capillaries for CEC application¹² and that polystyrene-divinylbenzene monolith frits were also fabricated in silica capillaries in μ LC application.¹³ In those studies, however, frits were permanently fabricated in the capillary columns. In this study, separate monolith frits encased in polymer tubing have been prepared.

Experimental

Materials. Fused silica capillaries (50 μ m I.D., 365 μ m O.D.), glass lined stainless steel tubing (30 cm, 0.5 mm I.D., 1.6 mm O.D.), Alltima C18 stationary phase (5 μ m, 80 Å), Teflon tubing (180 μ m I.D., 1.6 mm O.D.), and Tefzel tubing (180 μ m I.D., 1.6 mm O.D.) were purchased from Alltech (Deerfield, IL, USA). Styrene, divinylbenzene, *n*-octanol, ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Azobisisobutyronitrile (AIBN) was obtained from Junsei Chemical (Tokyo, Japan). HPLC grade acetonitrile, tetrahydrofuran and analytical grade 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) were purchased from

Valco (Houston, TX, USA).

Fabrication of monolith frits in polymer tubing. The monolith mixture reported in the literature¹¹ in preparation of a capillary monolith column was used in this study. A mixture composed of styrene 17 μ L, divinylbenzene 17 μ L, THF 8 μ L, 1-octanol 60 μ L, and AIBN 1 mg was prepared, sonicated for 10 min, filled in a piece of 30 cm polymer tubing (180 μ m I.D., 1.6 mm O.D.), and heated at 60 °C for 48 hr. The tubing was then washed thoroughly with toluene and 2-propanol overnight each at a flow rate of 5 μ L at 80 °C, and dried under nitrogen flow at 60 °C. The tubing was cut into thin (0.1-0.25 mm) slices to result in complete frits. A Hitachi (Tokyo, Japan) X-650 SEM was used to obtain SEM photos of the cross-section of the frit.

μ LC. 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, an ISCO (Lincoln, NE, USA) CV4 capillary window detector, and the home-made 0.5 mm I.D. glass-lined microcolumn were combined to compose the μ LC system. 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.

An Alltech (Deerfield, IL, USA) slurry packer was used to pack the microcolumns. In order to pack a column, a frit (home-made monolith frit or 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 stationary phase (Alltima C18) was dried at 90 °C for 4 hours. The slurry was made by mixing 80 mg particles with 10 mL methanol, and was sonicated for 20 min before packing. The slurry was transferred to the slurry reservoir, and the pressure of the slurry packer was raised to 14,000 psi instantly. The pressure was maintained for 2 min, and decreased to 10,000 psi, and the pressure was maintained for 10 min. The reservoir and the column were continuously vibrated while packing. Finally, the column was conditioned at 8,000 psi for 30 min. The tubing was then connected to the injector through a union with a piece of 5 cm stainless steel tubing (100 μ m I.D., 1.6 mm O.D.) between the column and the injector. Of course, another frit (home-made or commercial) was placed in the union. The 1/32 inch outlet of the other column end was connected to the detector by

installing a 1/32 inch graphite ferrule and a connecting capillary (50 μm I.D., 365 μm O.D.).

In order to estimate the performance of the home-made monolith frits, 5 different columns with these frits were prepared and 15 measurements (3 measurements for each batch) of chromatograms were carried out over 6 months. For comparison purpose, the commercial screen frits were also used to prepare 5 columns to carry out 15 measurements over the same period. A test mix composed of phenol, 2-nitroaniline, acetophenone, benzene, and toluene was prepared in methanol and stored in a refrigerator at 4°C before use.

Results and Discussion

Fabrication of monolith in polymer tubing. The Microscope and SEM photos of the frit are shown in Figure 1. The poly (styrene-divinylbenzene) monolith was well fabricated in the inner space of the polymer tubing as shown in Figure 1A and 1B, and it had the typical monolith structure with macroporous through flow channels as shown in Figure 1C and 1D. Some deformation of the frit cross-section and scattering of cut pieces were observed (Figure 1C) since a common domestic cutting blade was used to cut the fritted tubing into slices. A much better cut could have been achieved if an advanced cutting machine had been used. Nevertheless, the home-made frits sliced by a manual mini-

knife have shown good performances as will be discussed below.

The effect of tubing material on frit preparation. The polymer tubing should be soft enough to be cut into slices very easily and also should be hard enough to avoid accidental escape of the frit element from the polymer frame (tubing) on impact of cutting since there are no chemical bonds but van der Waals forces between the monolith and the tubing. Two polymer tubing was used, that is, Teflon (tetrafluoroethylene polymer) and Tefzel (ethylene tetrafluoroethylene copolymer) tubing. The latter has greater hardness. There was no difference in efficiency of monolith formation between the two tubing materials. However, the yield in cutting successful frit slices with Tefzel tubing (over 90%) was a little better than the yield with Teflon tubing (ca 80%). Nevertheless, more than 50 frits were produced with only 1 cm fritted tubing in both cases since the slice thickness was only 0.1-0.25 mm. Thus very cheap frits are made by the method of this study.

The performance of home made frits in comparison with commercial screen frits. The typical chromatograms of the test mix obtained with the column with monolith frits and the column with screen frits are compared in Figure 2. The scheme of column construction is given in Scheme 1. Nice chromatograms were obtained in both cases although a little better separation efficiency was observed with monolith frits. As shown in Table 1, statistically greater values of

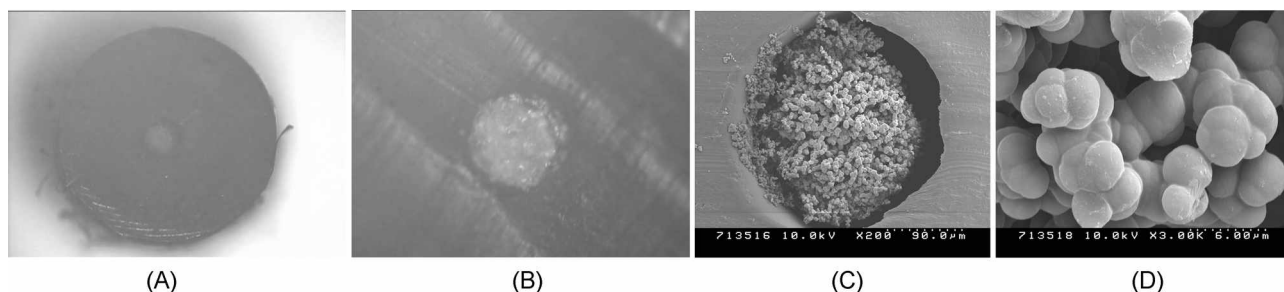


Figure 1. The microscope and SEM photos of the monolith frit. (A) The microscope photo. (B) The expanded view of A. (C) The SEM photo of the cross-section of the monolith frit. (D) The expanded view of C. The A, B images are not sharp owing to light scattering.

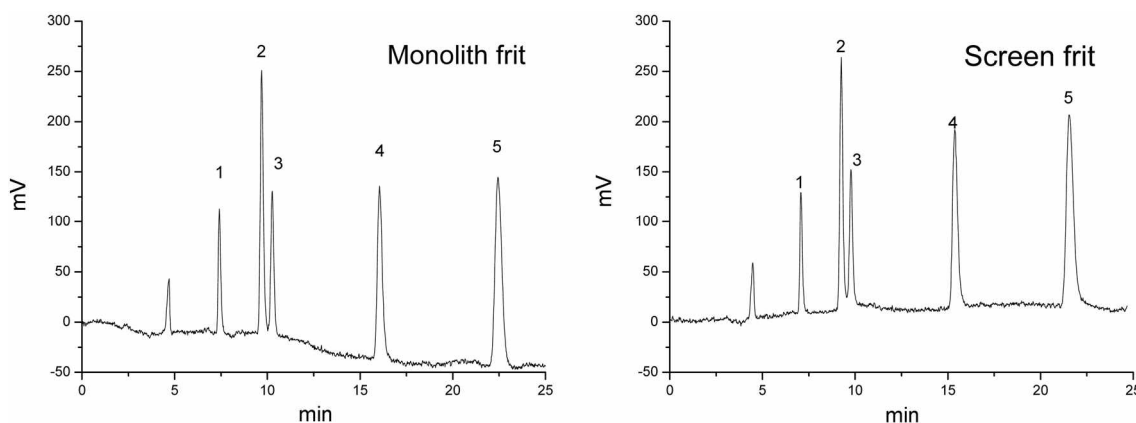
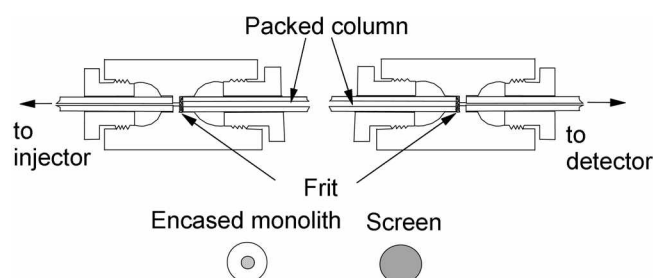


Figure 2. Comparison of chromatograms obtained with the Alltima C18 column (5 μm , 0.5 \times 300 mm) with monolith frits and the column with screen frits in 60/40 acetonitrile/50 mM ammonium acetate at a flow rate of 10 $\mu\text{L}/\text{min}$ at 254 nm. 1; phenol, 2; 2-nitroaniline, 3; acetophenone, 4; benzene, 5; toluene.



Scheme 1. The schematic of the packed microcolumn with either encased monolith frits or screen frits.

Table 1. Comparison of the Values of Number of Theoretical Plates (averages and standard deviations) Based on 15 Measurements over 6 Months with 5 Batches of Microcolumns (0.5×300 mm) Packed with the Alltima C18 Phase between the Monolith Frits and the Screen Frits

Solute	Monolith frit	Screen frit
phenol	14000 ± 1100	12500 ± 900
2-nitroaniline	16600 ± 1300	14600 ± 1000
acetophenone	16800 ± 1500	14800 ± 1200
benzene	17600 ± 1300	15100 ± 1300
toluene	17500 ± 1100	15000 ± 1000

number of theoretical plates were obtained with the monolith frits based on the data obtained with 5 batches of columns over 6 months. The diameter of the home made frit is $180 \mu\text{m}$ while that of the commercial stainless steel screen frit is 1.6 mm ($1600 \mu\text{m}$). Therefore, the area of screen frit is about 80 times larger than the area of home-made monolith frit. On the other hand, the thickness of screen frit (0.08 mm) is comparable to that of home-made frit ($0.1\text{--}0.25 \text{ mm}$). Thus the void volume of screen frit is far greater than that of home made frit. Furthermore, the home made frit seems to be relatively free of solute adsorption since it is made of nonpolar material while the stainless steel screen frit may be subject to some adsorption problem. The above discussion

explains the observation of better separation efficiency with the monolith frits than with the screen frits.

Conclusion

Cheap and simple monolith frits have been prepared by forming poly (styrene-divinylbenzene) monolith in common polymer tubing followed by cutting into slices. The performance of the monolith frits in view of separation efficiency (number of theoretical plates) was found statistically better than that of the stainless steel screen frits that have been generally used in microcolumns.

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