*Anal. Chem.* **2000,** *72,* 1224−1227

# Macroporous Photopolymer Frits for Capillary Electrochromatography

Jing-Ran Chen, Maria T. Dulay, and Richard N. Zare*

*Department of Chemistry, Stanford University, Stanford, California 94305-5080*

Frantisek Svec and Eric Peters†

*Department of Chemistry, University of California at Berkeley, Berkeley, California 94720-1460*

**Macroporous polymer frits have been fabricated in fused-silica capillaries by the UV photopolymerization of a solution of glycidyl methacrylate and trimethylolpropane trimethacrylate. This in situ preparation is a simple, rapid, and reproducible process. The frit can be placed at any desired position along the column. Photopolymer frits can withstand the short exposure to a high pressure (over 6000 psi). Bubble formation is not observed to occur with these frits under our experimental conditions. By choice of porogens, it is possible to control the porous properties. The use of such frits in capillaries to retain particles of chromatographic packing has been demonstrated to be stable and robust with continuous operation over 3 days.**

Despite the many advantages of capillary electrochromatography[1,2] (CEC) that have been demonstrated, several technical problems have slowed the development and general acceptance of CEC. One such problem is frit preparation.[3−5] The conventional method of frit fabrication for a particle-packed column involves thermal sintering of a section of the packing material, such as octyldecyl silica (ODS). This approach has several disadvantages, including (1) difficulty in generating the frit reliably and reproducibly, (2) alteration of the characteristics of the stationary phase within the frit itself, (3) difficulty in controlling the porosity of the frit, (4) weakness of the capillary at the location of the frit, (5) band broadening caused by the frit, (6) bubble formation and adsorption of polar analytes on the frit. These problems can directly affect the column performance and column-to-column reproducibility.

Four approaches toward solving this problem have been reported to date: (1) use of surface-functionalized open-tubular columns,[6−9] (2) preparation of "fritless" monolithic columns by a polymerization process that proceeds directly within the confines of a capillary,[10−13] (3) sintering of silica particles within a column,[5] and (4) entrapment of silica particles using various sol−gel techniques.[14−16]

In this paper, we describe a simple and reproducible procedure for fabricating frits by photoinitiated free-radical polymerization of glycidyl methacrylate and trimethylolpropane trimethacrylate in the presence of a porogenic solvent that affords a monolithic plug within the column that serves as a frit.

## EXPERIMENTAL SECTION

**Materials.** The monomers trimethylolpropane trimethacrylate [TRIM] and 2,3-epoxypropyl methacrylate [glycidyl methacrylate, GMA] were of the highest purity available from Aldrich (Milwaukee, WI). Toluene and 2,2,4-trimethylpentane (isooctane) from Sigma (St. Louis, MO) were used as porogenic solvents. The fused capillaries used in this study were purchased from Polymicro Technologies (Phoenix, AZ). The 1.5-$\mu$m spherical octadecyl silica (ODS) particles were provided by Micra Scientific, Inc. (Lafayette, IN). α-Methoxy-α-phenylacetophenone (benzoin methyl ether, 99%), thiourea, benzaldehyde, benzyl alcohol, 2-methylnaphthalene, sodium phosphate, and acetonitrile (HPLC grade) were purchased from Aldrich (Milwaukee, WI). Water was purified with an Ultrapure water system from Millipore (Milford, MA).

**Polymerization Procedure.** The photopolymerization procedure was carried out as previously described.[17] In situ free-radical polymerization was initiated by irradiating the monomer solution in 4-mm i.d. quartz tubes and 75-$\mu$m i.d. capillaries for 60 min at room temperature in a Spectrolinker XL 1500A

* Corresponding author: (phone) 650-723-3062; (fax) 650-723-9262; (e-mail) zare@stanford.edu.

† Novartis Institute for Functional Genomics, San Diego, CA 92121.

(1) Rathore, A. S.; Horváth, Cs. *J. Chromatogr., A* **1997**, *781*, 185−195.
(2) Colón, L. A.; Reynolds, K. J.; Alicea-Maldonado, R.; Fermier, A. M. *Electrophoresis* **1997**, *18*, 2162−2174.
(3) Behnke, B.; Grom, E.; Bayer, E. *J. Chromatogr., A* **1995**, *716*, 207−213.
(4) Carney, R. A.; Robson, M. M.; Bartle, K. D.; Mayers, P. *J. High Resolut. Chromatogr.* **1999**, *22* (1), 29−32.
(5) Asiaie, R.; Huang, X.; Farnan, D.; Horváth, C. *J. Chromatogr., A* **1998**, *806*, 251−263.
(6) Tsuda, T.; Nomura, K.; Nakagawa, G. *J. Chromatogr., A* **1982**, *248*, 241−247.
(7) Pfeffer, W. D.; Yeung, E. S. *J. Chromatogr., A* **1991**, *557*, 125−136.
(8) Pesek, J. J.; Matyska, M. T. *J. Chromatogr., A* **1996**, *736*, 255−264.
(9) Guo, Y.; Colón, L. A. *Anal. Chem.* **1995**, *67*, 2511−2516.
(10) Peters, E. C.; Petro, M.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1997**, *69*, 3646−3649.
(11) Palm, A.; Novotny, M. *Anal. Chem.* **1997**, *69*, 4499−4507.
(12) Liao, J.; Chen, N.; Ericson, C.; Hjertén, S. *Anal. Chem.* **1996**, *68*, 3468−3472.
(13) Fujimoto, C. *Anal. Chem.* **1995**, *67*, 2050−2053.
(14) Dulay, M. T.; Kulkarni, R. P.; Zare, R. N. *Anal. Chem.* **1998**, *70*, 5103−5107.
(15) Chirica, G.; Remcho, V. T. *Electrophoresis* **1999**, *20* (1), 50−56.
(16) Tang, Q. L.; Xin, B. M.; Lee, M. L. *J. Chromatogr., A* **1999**, *837* (1−2), 35−50.
(17) Viklund, C.; Pontén, E.; Glad, B.; Irgum, K.; Horstedt, P.; Svec, F. *Chem. Mater.* **1997**, *9*, 463−471.



10.1021/ac9911793 CCC: $19.00 
Published on Web 02/11/2000

(Spectronics Corp., Westbury, NY) with six 15 W fluorescent blacklight tubes, producing UV light of predominantly 365-nm wavelength. After 1 h, the porogen, unreacted monomers, and other soluble compounds were removed from the pores by washing with ethanol using an HPLC pump.

**Pore-Size Measurement.** The pore-size distribution of the porous polymer was determined for the samples prepared in the 4-mm tubes from the same polymerization mixtures as the capillaries, by mercury intrusion porosimetry using an automated custom-made combined BET sorptometer-porosimeter (Porous Materials, Inc., Ithaca, NY).

**Frit Fabrication and Column Packing.** The polyimide coating was removed at two small sections (≤2-mm long each) that were 20 cm apart along a fused-silica capillary. An outlet frit was prepared by introducing the monomer mixture into the capillary. The two ends of the capillary were sealed with Parafilm. The capillary was then covered by aluminum foil, leaving 1 mm of the outlet section without polyimide coating exposed to the UV light. The rest of the outlet section that was masked during photopolymerization became the detection window for CE experiments. After an hour of polymerization at room temperature, the unreacted monomer solution was flushed from the column by a syringe pump. After slurry packing[18] the column, 1.5-$\mu$m ODS particles were filled up to the inlet end of the capillary. The same procedure to create the outlet frit was employed to form the inlet frit. The resulting columns were preconditioned with the mobile phase by pressurizing the column inlet to approximately 500 psi with a manual syringe pump (Unimicro Technologies, Inc., Pleasanton, CA) for a few hours prior to their use. It was noticed that the polymerization also took place without removing the polyimide coating, but the process took about 4−6 h.

**Capillary Electrochromatography.** The CEC experiments were performed with a Beckman model 2000 P/ACE capillary electrophoresis system (Beckman Coulter, Fullerton, CA) equipped with an UV absorbance detector. The 75-$\mu$m i.d. packed capillary column was installed in the cartridge holder that was then inserted into the instrument.[19] There is no pressure applied to the packed column in this system. Once the packed column was installed, it was further conditioned by driving the mobile phase through the capillary at an applied voltage of 5 kV for 1 h prior to use. Samples were introduced electrokinetically at the anodic end of the capillary column. The mobile phase employed in these separations was a 5 mM phosphate and 2 mM sodium dodecyl sulfate SDS buffer (pH 7.0) containing 80% (v/v) acetonitrile. Separations were performed at an applied voltage of 10 kV and at a temperature of 20 °C. The analytes were detected by monitoring their absorbance at 254 nm.

A mixture of neutral compounds was separated on two columns, 1 and 2. Column 1 is a 75-$\mu$m i.d. × 27-cm (20-cm packed with 1.5-$\mu$m ODS particles) fused-silica capillary. The frit pore size of this column was 2.5 $\mu$m. Column 2 was the same as column 1 except the frit pore size was 4.0 $\mu$m.

## RESULTS AND DISCUSSION

**General Polymerization Conditions.** Despite several reports on monolithic porous polymer capillary columns, to our knowledge, photoinitiated polymerization has never been used for the preparation of polymeric frits in CEC columns. Various polymers used in monolithic columns are not suitable for fabrication of the frit for two reasons: (1) most of the polymeric network cannot withstand high pressure (>1000 psi); (2) most of the polymerization procedures described in these papers are thermally initiated, so it is difficult to localize the position of the polymer in the capillary columns.

Some of the most important variables affecting the photopolymerization and the properties of the monolithic material have already been studied for larger HPLC systems,[17,20] such as the intensity and wavelength of the light source, the percentage and composition of porogen in the polymerization mixture, the components of the porogenic solvent, and the cross-linker (TRIM) to reactive monomer (GMA) ratio. Our research concentrated on the preparation of the suitable frits and their effects on the CEC separations. We chose a mixture of isooctane and toluene that has proven to be the best-suited porogenic solvent for the preparation of porous GMA/TRIM monoliths.[17] By varying the proportions of these solvents, the frit pore sizes of 2.5 and 4.0 $\mu$m were chosen for our study. Photopolymerization can be achieved even in silica capillaries without removal of the polyimide coating. This procedure is particularly advantageous for the preparation of inlet frits. It was found that, by the hydrolysis of glycidyl methacrylate, the hydrophilicity of the frit could be increased.[21] This minimized the retaining effect of frits on the columns with reversed-phase chromatographic materials.

**Scanning Electron Microscopy.** Images of the photopolymer frit with and without ODS particles are illustrated by the electron micrographs shown in Figure 1 parts a−c. Figure 1 part a shows the photopolymer in a 75-$\mu$m i.d. capillary, and Figure 1 part b provides a magnified view of the polymer. Figure 1 part c shows the polymer structure in the presence of 1.5-$\mu$m ODS particles. This micrograph also demonstrates that the photopolymer loses its spherical shape in the presence of the ODS particles and the pores entrap the silica beads and hold them within their domains.

**Frit Stability.** After the polymerization had been completed, buffer solution was flushed (using a hand-held manual syringe pump) through a capillary column with one frit and no ODS particles present. There was almost no increase in the back-pressure observed as compared with the empty capillary without a photopolymer frit. This observation indicates that the frit does not significantly increase the flow resistance of the system. Although no specific functionalization of the inner capillary surface preceding the polymerization was carried out, the outlet frit easily withstands a short exposure of a high pressure (>6000 psi) used during the column packing. This behavior demonstrates that the monolithic porous polymer frit is strongly bound to the inner surface of the bare capillary wall. Similar observations have been made for the monolithic polymer columns for CEC.[10] No particles of the packing were found to pass through the frit pores during either the packing procedure or the CEC applications, despite their size, which is smaller than that of the mean pore size. This results from the cooperative action of several effects such as the tortuous paths of the pores and the presence of bottleneck-shaped pores within the monolithic structure. Five columns were prepared with

(18) Dittmann, M. M.; Rozing, G. P. *LC−GC* **1995**, *13*, 800−814.
(19) Dulay, M. T.; Yan, C.; Rakestraw, D. J.; Zare, R. N. *J. Chromatogr., A* **1996**, *725*, 361−366.
(20) Yu, C.; Svec, F.; Fréchet, J. M. J. *Electrophoresis* **2000**, *21*, 120−127.
(21) Peters, E. C.; Lewandowski, K.; Petro, M.; Svec, F.; Fréchet, J. M. J. *Anal. Comm.* **1998**, *35*, 83−86.

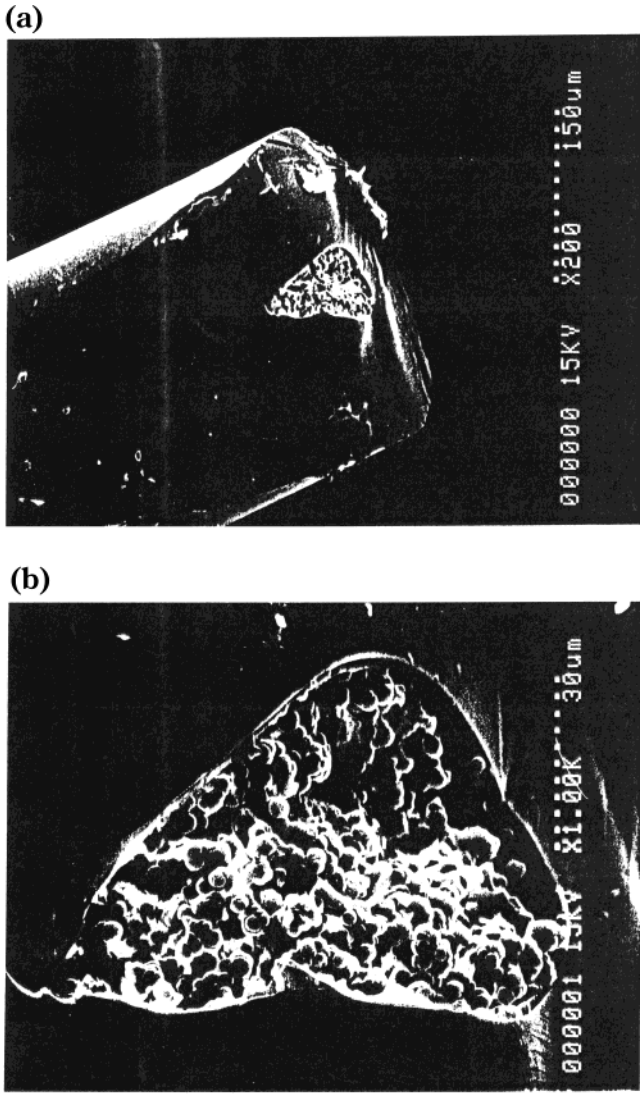


Figure 1. SEM micrographs of (a) an oblique view of a photopolymer (spherical structure) outlet frit in a 75-$\mu$m i.d. × 365-$\mu$m o.d. capillary (no ODS particles present), (b) a magnified (5×) view of (a), and (c) a cross section of a photopolymerized inlet frit (1-mm long) with embedded 1.5-$\mu$m ODS particles.

Table 1. Column Efficiency, Plate Height, and Reduced Plate Height of Column 2[a]

| analyte | $N$/m | $H$ ($\mu$m) | $h$ (dimensionless) |
|---|---|---|---|
| thiourea | 220 000 | 4.5 | 3.0 |
| 2-methylnaphthalene | 205 000 | 4.9 | 3.3 |

[a] Column 2: 75-$\mu$m i.d. × 27 cm (20 cm packed with 1.5-$\mu$m ODS particles). Mobile phase: 20% (v/v) 5 mM phosphate with 2 mM SDS (pH 7.0) and 80% (v/v) acetonitrile.

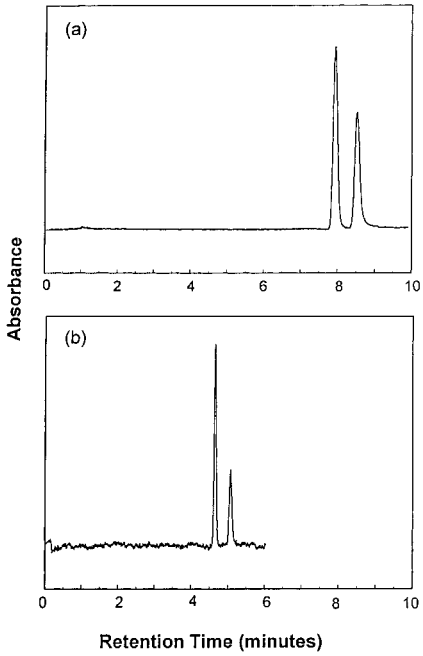


Figure 2. A plot of absorbance versus retention time for (a) column 1, and (b) column 2. The two analytes are thiourea and 2-methylnaphthalene (in order of elution).

this packing procedure and, in all cases, the photopolymer frits exhibited similar mechanical stabilities under our experimental conditions.

In contrast to some other procedures, the UV photoinitiated polymerization does not require elevated temperature for the reaction to be completed. Therefore, the mobile phase used for packing remains in both the outlet frit and the packing during polymerization. Consequently, the conditioning time for the column prior to its use is shortened significantly.

No bubble formation occurs during all the separation runs using 1.5-$\mu$m ODS packed capillary columns with photopolymer frits. The suppression of bubble formation might be due to both the addition of SDS in the running buffer and the replacement of sintered-silica frits with photopolymer frits. It is suggested that under pressures of 10 bar and with the addition of SDS, bubble formation is eliminated.[22] Further studies are underway to determine the effect of the photopolymer frit on bubble suppression.

**CEC Separations.** The efficiency, $N$, the plate height, $H$, and the reduced plate height, $h$, of a packed column (column 2) with photopolymer frits are listed in Table 1. Figure 2 compares the

(22) Seifar, R. M.; Kok, W. Th.; Kraak, J. C.; Poppe, H. *Chromatographia* **1997**, *46*, (3/4), 131−136.

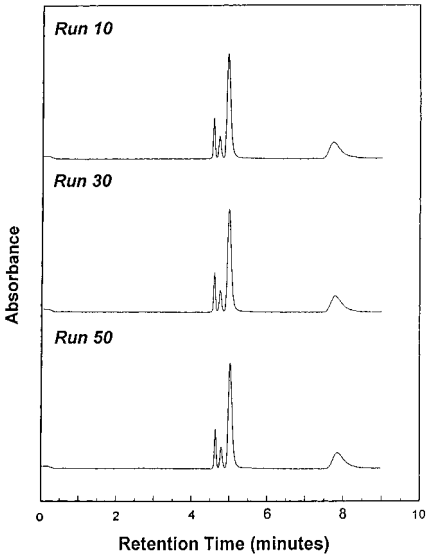


Figure 3. Selected representative CEC electrochromatograms from 60 runs using column 2. All the conditions are the same as in Figure 2 except the mobile phase for these runs is 50% (v/v) 5 mM phosphate with addition of 2 mM SDS (pH 7.0) and 60% (v/v) acetonitrile. The analytes are thiourea, benzyl alcohol, benzaldehyde, and 2-methylnaphthalene (in the order of elution).

separation of two neutral compounds, thiourea and 2-methylnaphthalene, achieved in columns furnished with porous polymer frits characterized by mean pore diameters of 2.5 (Figure 2 part a) and 4.0 $\mu$m (Figure 2 part b), respectively. The column with 4-$\mu$m frits exhibits shorter retention times and a better column efficiency compared with those of the other column. This finding suggests that photopolymer frits may have an effect on the column performance. Detailed studies are underway to determine the mechanism.

**Column Performance.** A systematic study of the run-to-run reproducibility for the analysis of a mixture of neutral molecules consisting of thiourea, benzyl alcohol, benzaldehyde, and 2-methylnaphthalene was carried out over a period of 3 days. Figure 3 shows electrochromatograms of runs 10, 30, and 50. There is almost no variation in retention times of all test compounds. Table 2 shows the relative standard deviations of the capacity factor, $k'$, the efficiency, $N$, and the resolution, $R_s$, for each compound. These RSDs for all three monitored variables, averaged over 60 runs, are 3.5%, 3.3%, and 5.5%, respectively. The integrity of the packed column remained unchanged, and the column is assumed to be useful for a much longer period of time. In Figure 3, the efficiencies of thiourea, benzyl alcohol, benzaldehyde, and 2-methylnaphthalene are 200 000 plates/m, 160 000 plates/m, 60 000 plates/m, and 20 000 plates/m, respectively. The unretained compound, thiourea, gives the highest efficiency. The efficiencies drop for the compounds that are more retained than thiourea. The significant efficiencies drop of the last two peaks compared with those of Figure 2 are mainly due to the use of a lower percentage (50%) of acetonitrile in the running buffer. Furthermore, the peak for 2-methylnaphthalene shows tailing that is mainly due to its adsorption to the surface of the ODS particles.

Table 2. Relative Standard Deviation of Capacity Factor ($k'$), Column Efficiency ($N$), and Resolution ($R_s$) over 60 Separations for Column 2[a]

| relative standard deviation (%) | | | |
|---|---|---|---|
| analyte | $k'$ | $N$ | $R_s$ |
| thiourea | | 2.0 | |
| benzyl alcohol | 3.2 | 5.5 | 3.2 |
| benzaldehyde | 2.1 | 5.3 | 2.7 |
| 2-methylnaphthalene | 2.6 | 3.6 | 2.8 |

[a] Column 2: 75-$\mu$m i.d. × 27 cm (20 cm packed with 1.5-$\mu$m ODS particles). Mobile phase: 50% (v/v) 5 mM phosphate with 2 mM SDS (pH 7.0) and 50% (v/v) acetonitrile.

## ACKNOWLEDGMENT

Support of this research by Beckman Coulter, Inc. (Fullerton, CA) is gratefully acknowledged. We thank Cong Yu for his help on pore-size measurements and Bob Jones and Keisuke Morishima for their assistance with taking the SEM pictures.

Received for review October 12, 1999. Accepted December 29, 1999.

AC9911793