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Porous Polymer Monoliths Functionalized through Copolymerization of C60 Fullerene-Containing Methacrylate Monomer for Highly Efficient Separations of Small Molecules

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Abstract

Monolithic poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) and poly(butyl methacrylate-*co*-ethylene dimethacrylate) capillary columns, which incorporate the new monomer [6,6]-phenyl-C₆₁-butyric acid 2-hydroxyethyl methacrylate ester, have been prepared and their chromatographic performance tested for the separation of small molecules in the reversed phase. While addition of the C60-fullerene monomer to the glycidyl methacrylate-based monolith enhanced column efficiency 18-fold, to 85,000 plates/m at a linear velocity of 0.46 mm/s and a retention factor of 2.6, when compared to the parent monolith, the use of butyl methacrylate together with the carbon nanostructured monomer afforded monolithic columns with an efficiency for benzene exceeding 110,000 plates/m at a linear velocity of 0.32 mm/s and a retention factor of 4.2. This high efficiency is unprecedented for separations using porous polymer monoliths operating in an isocratic mode. Optimization of the chromatographic parameters affords near baseline separation of 6 alkylbenzenes in 3 minutes with an efficiency of 64,000 plates/m. The presence of 1 wt% or more of water in the polymerization mixture has a large effect on both the formation and reproducibility of the monoliths. Other important factors such as nitrogen exposure, polymerization conditions, capillary filling method, and sonication parameters were all found to be important factors in producing highly efficient and reproducible monoliths.

Keywords

porous polymer monolith; methacrylate; carbon nanostructure; C60; fullerene; HPLC; small molecules; reversed phase

INTRODUCTION

HPLC separations performed with particle packed columns rely on diffusion of the analytes into and out of pores, as well as on partitioning of the analytes between the mobile and stationary phases.^{1,2} Since diffusion-controlled mass transport is relatively slow, particularly for larger molecules, approaches have been developed to diminish diffusion effects including use of sub 2 μ m particles^{3,4} and superficially porous particles.^{5,6} In the early 1990's, we introduced a new type of chromatographic column containing a rigid porous polymer monolith, prepared by *in-situ* polymerization from liquid precursors.⁷ The first generation of these columns possess a unique through pore structure, which enables the

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mass transport to be dominated by fast convection, rather than slow diffusion.^{8,9} This mechanistic change results in very fast separations of large molecules.¹⁰⁻¹³ However, achieving good column efficiency for small molecules has been a challenge due to irregular morphology, a lack of mesopores, and a small surface area typical of these monoliths.¹⁴ This deficiency has led to studies focused on improving the separations of small molecules using polymer monoliths through manipulation of polymerization mixture composition,¹⁵⁻²⁰ termination of the polymerization process before reaching complete conversion,²¹⁻²³ use of reversible addition-fragmentation chain transfer polymerization,²⁴ or polymerization of a single crosslinker.²⁵⁻²⁷ One of the most successful approaches to improve the separations of small molecules is to increase the surface area of organic polymer materials. As shown by our lab, hypercrosslinking poly(styrene-*co*-chloromethylstyrene-*co*-divinylbenzene) monoliths can afford columns with an efficiency exceeding 70,000 plates/m for retained small molecules.^{28,29}

During the last decade, carbon-based nanomaterials have found applications in various analytical applications such as solid-phase extraction,³⁰⁻³⁵ gas chromatography,^{36-39,40,41-46} and electrophoresis.⁴⁷⁻⁵⁰ In contrast, much less work has been performed with carbon-based nanostructures in the field of high performance liquid chromatography (HPLC). One area of study that has been performed with HPLC is the deposition of carbon nanotubes (CNT) onto silica beads to enhance the stationary phase for the separation of aromatic compounds.⁵¹⁻⁵³ In one study, Zhong *et al.* prepared poly(styrene-*co*-divinylbenzene) particles with a small amount of embedded or grafted CNT which provided improved selectivity and efficiency.⁵⁴ Alternatively, Horvath's group entrapped carbon nanotubes into poly(chloromethylstyrene-*co*-ethylene dimethacrylate) monoliths to afford capillary columns with enhanced separations for HPLC and capillary electrochromatography.⁵⁵ We have recently demonstrated enhancements in separations of small molecules, such as alkylbenzenes, using monoliths derived from poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) functionalized with carbon nanotubes.⁵⁶ The CNT could be directly admixed into the polymerization mixture to provide separation enhancement. However, only a limited number could be added before the monolith was negatively affected. Alternatively, the CNT were oxidatively cut to 100-200 long fragments and adsorbed at the pore surface via electrostatic interactions. Despite the impressive effect of the CNT, the presence of the carboxylic acid functionalities on the cut CNT and the limited amount that could be adsorbed onto pore surface limits the extent of enhancement. Therefore, an alternative approach to modification of porous polymer monoliths with carbon nanostructures is desirable.

C60-fullerene has a completely sp²-hybridized three-dimensional structure, analogous to that of carbon nanotubes. In contrast to CNT, C60-fullerene can be made free of metal impurities and can be characterized with relative ease by organic characterization techniques (i.e. NMR and MALDI-TOF). Additionally, and importantly, fullerene is a well-defined, discrete, mono-disperse molecule. Jinno *et al.* was the first to use C60-fullerene packed in a short liquid chromatography column for the separation of polyaromatic hydrocarbons.^{57,58} Although the columns packed with the C60-fullerene showed increased retention and selectivity of polyaromatic compounds, the columns possessed low efficiency. Although seen typically as a positive trait, the inertness of C60-fullerene makes it difficult to permanently anchor C60-fullerene or incorporate it into materials. As a result, only a few instances of fullerene functionalized particulate column packings, based on both silica⁵⁹⁻⁶³ and organic polymers^{64,65}, have been reported. Although the modified columns show separation enhancement over the bare particle substrate or solely C60-fullerene based columns, the modified columns still show relatively low efficiency. Based on our observation of enhanced performance using monoliths containing carbon nanotubes, coupling C60-fullerene with porous polymer monoliths hold the potential to provide highly efficient columns for the separations for small molecules. This report describes the

preparation of monoliths suitable for the fast and highly efficient separation of small molecules through the copolymerization of standard monomers with a new C60 moiety-bearing monomer [6,6]-phenyl-C₆₁-butyric acid 2-hydroxyethyl methacrylate ester.

Experimental Section

Complete description of Chemicals and Materials, Instrumentation, and Synthesis of [6,6]-phenyl-C₆₁-butyric acid 2-hydroxyethyl methacrylate ester (PC₆₁B-HEM) (for structure see Figure 1) can be found in the Supplementary Files.

Purification of the components of the polymerization mixture

As a general procedure, 20 mL of the monomers were passed through basic oven-dried alumina to remove the monomethyl ether hydroquinone inhibitor from the bulk monomers. Approximately 2 g of molecular sieves dried at 180 °C for more than 48 h were added to 20 mL of each purified monomer or porogen. The individual components, with sieves, were then gently agitated, and left in a sealed vial at room temperature for at least 24 h out of direct light. Before use, each component was filtered twice through a 0.20 µm PFTE syringe filter and placed in vial containing approximately 1 g of freshly dried molecular sieves, where they were left for 2-3 h with periodic gentle agitation.

Preparation of the monolithic capillary columns

Monoliths were prepared using a modified procedure and conditions developed previously.⁵⁶ Polymerization mixtures consisted of 24 wt% monovinyl monomers, PCB-HEM and glycidyl methacrylate, a crosslinker, ethylene dimethacrylate (16 wt%), as well as porogenic solvents cyclohexanol (54 wt%), and 1-dodecanol (6 wt%). Alternatively, polymerization mixtures consisted of PCB-HEM (1 wt%), butyl methacrylate (23 wt%), ethylene dimethacrylate (16 wt%), 1-propanol (36 wt%), and 1,4-butanediol (24 wt%).

Caution: Several methacrylates and solvents are known sensitizing agents. Proper precautions should be taken during the physical handling of these materials.

For concentration studies, PCB-HEM (0.5-2% w/w) and azobisisobutyronitrile (AIBN, 1% w/w with respect to total monomers) were weighted in a 3 mL brown vial. The liquid monomers were transferred into the vial using a plastic syringe fitted with a 0.2 µm PFTE syringe filter. After a quick stirring of the mixture by hand, the solution was sonicated for 5 min before the porogens were added individually using, again, a plastic syringe fitted with a 0.2 µm PFTE syringe filter. The vial was then capped with a rubber septum.

The mixture was then sonicated for another 12 min to remove oxygen and to thoroughly mix all components. To avoid evaporation of the volatile components, only the headspace of the solution was purged for a few seconds with dry 99.99% nitrogen every 4 min instead of the typical purge through the liquid contents. To achieve the best column performance, the sonicated mixture has to be left resting for 5 min and then introduced into the vinylized capillaries. Detailed explanation of processes occurring in the aging mixture are available in the Supplemental Files.

Filling the vinylized capillaries, pierced through the septum, was achieved using slight overpressure of nitrogen to drive the polymerization mixture into the capillary.⁵⁶ Both ends of the filled capillaries were sealed with a piece of rubber, and the capillary was placed into a thermostated water bath. After completion of the polymerization at 70 °C for 24 h, a few centimeters were cut from both ends of the capillary, the monoliths were flushed with acetonitrile and, then, used for separations.

RESULTS AND DISCUSSION

Poly(glycidyl methacrylate-co-ethylene dimethacrylate-co-PCB-HEM) monoliths

To discern the effect of the C60-fullerene methacrylate units on chromatographic performance of the monoliths in reversed phase separations, monolithic capillary columns were first prepared from the relatively polar monomers glycidyl methacrylate and ethylene dimethacrylate. For simplicity, the PCB-HEM was directly admixed into a polymerization mixture with a composition previously used for the preparation of monoliths with added CNT.⁵⁶ While native C60-fullerene does not dissolve in the mixture of the monomers and porogens, we found that up to 1 wt% PCB-HEM dissolves in glycidyl methacrylate after light sonication to form a dark purple solution. The PCBHEM monomer remains dissolved even after addition of the porogens, cyclohexanol and 1-dodecanol. It is worth noting, mixtures containing even the smallest percentage of PCB-HEM did not polymerize at 55 °C and useful monoliths could be obtained from mixtures containing less than 1 wt% of PCB-HEM at a polymerization temperature of at least 70 °C due to the strong inhibiting effect of fullerene.^{66,67}

The efficiency of the glycidyl methacrylate based monolithic columns increases with the addition of PCB-HEM until a maximum is reached at 1 wt% of this monomer added to the polymerization mixture. Further increases are not possible due to limited solubility and complete inhibition of polymerization at higher concentrations. Figure 2A shows a very poor separation of alkylbenzenes characterized with mere 4,400 plates/m for benzene, which is the first retained compound exhibiting the highest efficiency, when using the parent poly(glycidyl methacrylate-co-ethylene dimethacrylate) monolith. In contrast, a good separation of six alkylbenzenes with a rather high efficiency of 72,000 plates/m for the retained compound benzene at a flow rate of 0.15 $\mu\text{L}/\text{min}$ (0.46 mm/s) is achieved using the column prepared from a mixture containing 1 wt% PCB-HEM (Figure 2B). It is worth noting that all column efficiencies reported in this paper are calculated from the widths of peaks obtained by injecting benzene alone under conditions identical to those used in the separation of mixtures of all analytes.

The permeability of the monolith with addition of the PCB-HEM is only slightly reduced from 2.1×10^{-15} to $1.6 \times 10^{-15} \text{ m}^2$. Similar to our previous observations,^{28,29,56} addition of 2.5% tetrahydrofuran (THF) to the mobile phase reduces both the tailing from asymmetry factor $A_s < 2.0$ in Figure 2B to $A_s < 1.7$ for all the peaks in Figure 2C thus further improving the column efficiency for benzene to 85,000 plates/m. THF also reduces the retention times of all benzene derivatives.

While the column efficiency increased significantly, the back pressure of 29 MPa was rather high, even at slow flow rates, due to the small through pores of the monolith, which did not exceed 500 nm. The restriction imposed by the small pore size prevents the use of higher flow rates needed to reduce the analysis times or the use of longer columns to improve the efficiency per column. Therefore, a different system to generate large pore sizes was chosen.

Poly(butyl methacrylate-co-ethylene dimethacrylate-co-PCB-HEM) monolith

Monolithic columns comprising of poly(butyl methacrylate-co-ethylene dimethacrylate) have been previously optimized by our group.⁶⁸ By changing the porogens and replacing glycidyl methacrylate with butyl methacrylate while holding the amount of ethylene dimethacrylate constant, a monolith with a through pore size of approximately 850 nm is formed using polymerization at 70 °C. This temperature represents an optimum since monoliths do not form when subjected to 55 °C, while the through pore size of the monoliths prepared at 85 °C or greater was too small to enable flow of the mobile phase at pressures tolerable for conventional HPLC systems. The kinetics of the polymerization

reaction is also affected by the initiator. The use of polymerization mixtures containing 0.5 wt% AIBN with respect to monomers affords monoliths with non-uniform morphology (i.e. voids and heterogeneous pore density). In contrast, mixtures containing 1 or 2 wt% AIBN lead to monolithic columns with no difference in performance and, thus 1 wt% was used throughout.

The use of the more hydrophobic butyl methacrylate affords monolithic columns with retention factors for the alkylbenzenes three times higher when compared to the glycidyl methacrylate-based monolith. Compared to separations shown in Figure 2 and for the parent poly(butyl methacrylate-co-ethylene dimethacrylate) monolith in Figure 3A, Figure 3B demonstrates that the resolution of benzene and toluene is significantly improved to $R_s > 1.8$ as calculated from the peak width determined by drawing tangents at 0.607 peak height to provide a width of 2σ followed by extrapolation to the baseline that affords a width of 4σ . The column efficiency for benzene now reaches 106,000 plates/m. The larger through pore size and concomitant decrease in resistance to flow, allows the use of higher flow rates of up to 0.5 $\mu\text{L}/\text{min}$ that are typical of 100 μm i.d. monolithic capillary columns. An efficiency of 120,000 plates/m, unprecedented for organic polymer-based monolithic columns, is achieved for benzene at the minimum of the van Deemter curve represented by a flow rate of 0.10 $\mu\text{L}/\text{min}$ at a flow velocity of 0.33 mm/s (Figure 4).

Effect of water on column-to-column reproducibility

The separation performance of columns originating from the same batch of a freshly prepared polymerization mixture, which used newly purchased components, were reproducible. However, the efficiency could not be duplicated, becoming increasingly worse, if the components of the polymerization mixture were kept in a freezer for a certain period of time. NMR and MALDI-TOF did not indicate any degradation of PCB-HEM even after 6 months of storage and use. The other components were also free of impurities, with the exception of a small amount of water in all of the liquid components. The largest water signals were observed for the hygroscopic 1,4-butanediol. The liquid monomers were stored in a freezer to prevent undesired homopolymerization after removal of the hydroquinone monomethyl ether inhibitor using activated basic alumina. Figure 5 confirms the negative effect of water by comparing the structure of the monoliths using new, dry components with those that have been exposed to air and moisture for 3 weeks. In the presence of water, the morphology of the monolith is altered and the size of the through pores is increased. This change is accompanied by a reduction in back pressure and a drastic decline in performance. By utilizing freshly re-purified monomers and/or new, never opened, bottles of porogens, the column efficiency returned to the original high values exceeding 100,000 plates/m. The significant change in the morphology of the monolith was also observed after the deliberate addition of 1 wt% water to the polymerization mixture. This is not completely unexpected since we observed the dramatic effect of water, which was part of the porogenic solvent mixture, on performance of monolithic columns designed for capillary electrochromatography.⁶⁹ To prevent the undesired morphological changes, freshly purified components dried with molecular sieves were always used for the preparation of our monoliths. Molecular sieves used for drying are fragile and readily produce fine particulate matter suspended in the components of the polymerization mixture, also causing column irreproducibility. Therefore, the individual components were always filtered through a 0.2 μm PTFE syringe filter. Using all the means mentioned above, column-to-column reproducibility of the efficiency is characterized by a relative standard deviation less than 3.3% and 0.5% for run-to-run retention times (Table 1).

Effect of tetrahydrofuran on separation of alkylbenzenes

It has been observed that the addition of THF in the mobile phase reduces peak tailing and improves the column efficiency in sp^2 containing systems.^{28,56,70} Figure 6 illustrates the effect of increasing the percentage of THF while holding the overall volume of organic components in the mobile phase at 50%. Interestingly, just 2.5% THF has a large impact on the column efficiency with increases from 86,000 to 108,000 plates/m without significantly affecting the retention times, presumably due to the reduction in the asymmetry factor from 1.9 to 1.6. Higher percentages of added THF lead to a continuous decrease in the efficiency while no positive effect on column efficiency is observed with 20% THF. However, the resolution between the critical pair of benzene and toluene is negatively affected and decreases from 3.1 at 2.5% THF to less than 1 with 20% THF.

Rapid separations

The most efficient separation, shown in Figure 3B, was achieved at a flow rate of 0.25 $\mu\text{L}/\text{min}$ and the elution times for benzene and toluene were 13 and 17 min, respectively, using a mobile phase containing 50% of organic solvents. An increase in organic content of the mobile phase decreases retention of the analytes and may lead to faster separations, an effect that follows the linear solvent strength model.⁷¹ As expected, we found that retention decreases with the increase in organic content in the mobile phase. This decrease in retention is accompanied by a concomitant decrease in the column efficiency resulting in reduced resolution of peaks. For example, resolution of benzene and toluene peaks is significantly less than 1 at an organic content of 80% and their peaks largely overlap.

Optimization of the system variables enables a significant acceleration of the separation of all six alkylbenzenes that can be achieved in 3 min using a 70 mm column and a mobile phase with 70% organic content at a flow rate of 0.40 $\mu\text{L}/\text{min}$, which corresponds to a flow velocity of 1.4 mm/s (Figure 7). Impressively, the column efficiency for benzene remains high at 64,000 plates/m.

CONCLUSION

This study demonstrates that chromatographic performance of monolithic poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) and poly(butyl methacrylate-*co*-ethylene dimethacrylate) capillary columns is significantly improved when a small amount (1 wt%) of the C60-fullerene containing PCB-HEM monomer is included in the polymerization mixture. Optimization of both the polymerization and chromatographic conditions of monolithic columns for reversed phase chromatography of small molecules exhibits unprecedented efficiencies in excess of 110,000 plates/m for the retained compound, benzene. Since our calculations of column efficiencies did not take into account the extra column contribution to the peak broadening, it is likely that the “column only” efficiency is even higher. The synergistic effect of the hydrophobic butyl methacrylate and the chemistry of PCB-HEM afford monoliths with properties that enable fast and efficient separation of small molecules when compared to traditional organic polymer monoliths. Thus, our results demonstrate, again, the ability of carbon nanostructures to positively affect the separation performance and elevate the monolithic columns prepared from organic polymers into the arena previously dominated by silica-based monoliths.

Our current experiments aim at determining a mechanism that would explain a substantial effect of very small amount of C60 containing nanostructures on efficiency of monolithic columns. Figure 8 shows significant changes in the morphology of the monoliths without and with the incorporated PCB-HEM. However, we do not know yet whether it is the altered morphology, the C60 chemistry, or both that make the difference. In the ongoing study, we

seek the answer to this question via preparing parent monoliths and grafting their pore surface with PCB-HEM. This approach will eliminate the effect of morphology and discern the function of the C60 chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structure of [6,6]-phenyl-C₆₁-butyric acid 2-hydroxyethyl methacrylate ester.

Figure 2.

Separation of uracil and alkylbenzenes using a parent monolithic poly(glycidyl methacrylate-co-ethylene dimethacrylate) capillary column (A) and using a column containing 1 wt% PCB-HEM (B, C), both prepared at a temperature of 70 °C. Conditions: column 53 mm $\times$ 100 μ m i.d., flow rate 0.15 μ L/min, UV detection at 254 nm; (A) mobile phase 50:50 vol% acetonitrile-water, back pressure 15 MPa (B) mobile phase 50:50 vol% acetonitrile-water, back pressure 25 MPa; (C) mobile phase 47.5:2.5:50 vol% acetonitrile-tetrahydrofuran-water, back pressure 27 MPa; Peaks in order of elution: uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene.

Figure 3.

Separation of uracil and two alkylbenzenes using a parent monolithic poly(butyl methacrylate-co-ethylene dimethacrylate) capillary column (A) and using a column containing 1 wt% PCB-HEM (B), both prepared at a temperature of 70 °C. Conditions: column 108 mm $\times$ 100 μ m i.d., mobile phase 45:5:50 vol% acetonitrile:tetrahydrofuran:water, flow rate 0.25 μ L/min, back pressure 22 MPa, UV detection at 254 nm; Peaks in order of elution: uracil, benzene, toluene.

Figure 4. Van Deemter plot demonstrating effect of flow velocity on efficiency of monolithic column containing 1 wt% PCB-HEM for benzene. Column 108 mm $\times$ 100 μ m i.d.; ternary mobile phase 45:5:50 vol% acetonitrile- tetrahydrofuran-water.

Figure 5. SEM images of poly(butyl methacrylate-co-ethylene dimethacrylate) with 1 wt% PCB-HEMA monoliths using (A) dry components (B) components exposed to air and condensation for 3 months.

Figure 6.

Effect of tetrahydrofuran content in mobile phase on the efficiency of monolithic poly(butyl methacrylate-co-ethylene dimethacrylate) column containing 1% PCB-HEMA. Conditions: column 70 mm $\times$ 100 μ m i.d., mobile phase contains constant 50% organic content, flow rate 0.25 μ L/min.

Figure 7. Fast and efficient separation of alkylbenzenes using a monolithic poly(glycidyl methacrylate-co-ethylene dimethacrylate) capillary column containing 1 wt% PCBHEMA. Conditions: column 70 mm $\times$ 100 μ m i.d., mobile phase 65:5:30 vol% acetonitrile-THF-water, flow rate 0.40 μ L/min, back pressure 20 MPa, UV detection at 254 nm; Peaks in order of elution: uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene.

Figure 8. SEM image of poly(butyl methacrylate-co-ethylene dimethacrylate) monolith (left panels) and its counterpart prepared from polymerization mixture containing 1 wt% PCB-HEMA (right panels).

Table 1Reproducibility of poly(butyl methacrylate-*co*-ethylene dimethacrylate-*co*-PCB-HEM) capillary column.

Flow rate $\mu\text{L}/\text{min}$	RSD, %	
	Column-to-column efficiency (N = 3)	Run-to-run retention (N = 3)
0.40	1.8	0.4
0.25	3.1	0.4
0.15	3.3	0.5

Conditions: column 100 mm $\times$ 100 μm i.d., mobile phase 45:5:50 vol.% acetonitrile-tetrahydrofuran-water.