Chromatographic assessment of two hybrid monoliths prepared via epoxy-amine ring-opening polymerization and methacrylate-based free radical polymerization using methacrylate epoxy cyclosiloxane as functional monomer∗

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Two kinds of hybrid monolithic columns were prepared by using methacrylate epoxy cyclosiloxane (epoxy-MA) as functional monomer, containing three epoxy moieties and one methacrylate group. One column was in situ fabricated by ring-opening polymerization of epoxy-MA and 1,10-diaminodecane (DAD) using a porogenic system consisting of isopropanol (IPA), H2O and ethanol at 65 °C for 12 h. The other was prepared by free radical polymerization of epoxy-MA and ethylene dimethacrylate (EDMA) using 1-propanol and 1,4-butanediol as the porogenic solvents at 60 °C for 12 h. Two hybrid monoliths were investigated on the morphology and chromatographic assessment. Although two kinds of monolithic columns were prepared with epoxy-MA, their morphologies looked rather different. It could be found that the epoxy-MA–DAD monolith possessed higher column efficiencies (25,000–34,000 plates/m) for the separation of alkylbenzenes than the epoxy-MA–EDMA monolith (12,000–13,000 plates/m) in reversed-phase nano-liquid chromatography (nano-LC). Depending on the remaining epoxy or methacrylate groups on the surface of two pristine monoliths, the epoxy-MA–EDMA monolith could be easily modified with 1-octadecylamine (ODA) via ring-opening reaction, while the epoxy-MA–DAD monolith could be modified with stearyl methacrylate (SMA) via free radical reaction. The chromatographic performance for the separation of alkylbenzenes on SMA-modified epoxy-MA–DAD monolith was remarkably improved (42,000–54,000 plates/m) when compared with that on pristine epoxy-MA–DAD monolith, while it was not obviously enhanced on ODA-modified epoxy-MA–EDMA monolith when compared with that on pristine epoxy-MA–EDMA monolith. The enhancement of the column efficiency of epoxy-MA–DAD monolith after modification might be ascribed to the decreased mass-transfer resistance. The two kinds of hybrid monoliths were also applied for separations of six phenols and seven basic compounds in nano-LC.

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1. Introduction

Monoliths, generally in situ formed within a capillary column, are considered as the fourth-generation chromatographic material after the earlier synthesized polysaccharide-based, cross-linked and coated and monodisperse stationary phases [1–3]. Current monoliths can be mainly divided into three types based on the nature of the matrix chemistry, namely, organic polymer monoliths, silica monoliths and organic-silica hybrid monoliths. Organic polymer monoliths and silica monoliths have been widely used in separation field during the past decades [4] and successfully applied to the analysis of small molecules [5,6] and large biomolecules [7,8]. The organic polymer monoliths, mainly composed of polyacrylamides, polymethacrylates and polystyrenes [9,10], possessed excellent pH stability and could be easily fabricated by single-step polymerization reaction. Unfortunately, this type of monoliths...
was apt to swell in some organic solvents such as tetrahydrofuran [11,12], which led to the change of pore structure and the decrease of mechanical stability and further resulted in a short lifetime [13–15]. In contrast, silica-based monoliths showed good solvent resistance and high mechanical stability, while tedious fabrication procedures were time-consuming and difficult to control, which greatly encouraged the researchers and limited the further application [13,16,17].

Organic-silica hybrid monoliths, somewhat combining advantages of the other two types of monoliths, including ease of preparation, less shrinkage, good mechanical stability, good pH stability and high surface area [14], have gained great popularity in solid-phase extractions (SPEs) [18,19], enzyme microreactors [20], high performance liquid chromatography (HPLC) [21] and capillary electrophromatography (CEC) [22] in recent years. The preparation of hybrid monoliths could be exemplified into three approaches [23], that is, (i) general sol–gel process using trialkoxysilanes and tetraalkoxysilanes as coprecursors. (ii) “one-pot” process of alkoxysilanes and organic monomers concomitantly proceeding sol–gel chemistry and free radical polymerization. (iii) Other polymerization approaches of silane-containing organic monomers. In general sol–gel process, the functional group from trialkoxysilanes was directly introduced to the surface of hybrid monolith and could be employed for further post-column modification. Compared to the traditional sol–gel method in the preparation of hybrid monoliths, the “one-pot” reaction manner would introduce the desirable functional groups into the monoliths. In addition, the hybrid monoliths prepared by other polymerization approaches could also be easily synthesized by ring-opening polymerization [24], free radical polymerization [25–27] or some other polymerizations by using a variety of monomers and crosslinkers.

In this study, methacrylate epoxy cyclosiloxane (epoxy-MA) (Fig. S1, Supporting information) was first adopted to prepare two kinds of hybrid monoliths via ring-opening polymerization and free radical polymerization. Ring-opening polymerization was carried out by the reaction between epoxy-MA and 1,10-diaminodecane (DAD) to prepare hybrid epoxy-MA–DAD monolith, while free radical polymerization was performed by the reaction between epoxy-MA and ethylene dimethacrylate (EDMA) to prepare hybrid epoxy-MA–EDMA monolith. Both hybrid monoliths were further modified and evaluated in nano-liquid chromatography (nano-LC).

2. Experimental
2.1. Reagents and materials

Epoxy-MA, EDMA (98%), DAD (>98%), stearyl methacrylate (SMA) (contains 90–150 ppm monomethyl ether hydroquinone as inhibitor, technical grade), methacryloxypropyl trimethoxysilane (γ-APTS) (>98%) and (3-aminopropyl)trimethoxysilane (APTM) (>98%) were purchased from Sigma (St Louis, MO, USA), and used directly without further purification. Azobisobutyronitrile (AIBN) was gotten from Shanghai Chemical Plant (Shanghai, China), and recrystallized in ethanol prior to use. Octadecylenyl (ODA) was gotten from Alfa Aesar (Ward Hill, MA, USA), 1,4-Butanediol, 1-propanol, 2-propanol (IPA), ethanol, thiourea, benzeno, toluene, ethylbenzene, propylbenzene, butylbenzene, hydroquinone, pyrocatechol, phenol, p-cresol, 2,6-xenol, 4-tert-butyl-phenol, 1,4-phenylenediamine, 2,4-diaminotoluene, carbamazepine, barbionate, benzidine, 2,4-dinitroaniline, 4-aminobiphenyl and other standard analytes were of analytical grade, and obtained from Tianjin Kernel Chemical Plant (Tianjin, China). Methanol and acetonitrile (ACN) were HPLC-grade and acquired from Merck (Darmstadt, Germany). Deionized water was prepared with a Milli-Q system (Milli-pore, MA, USA).

The fused-silica capillaries with 50 and 75 μm i.d. were the products of Reafine Chromatography Ltd. (Hebei, China).

2.2. Preparation and modification of two kinds of hybrid monoliths

2.2.1. Preparation of hybrid epoxy-MA–DAD monolith (Route I in Fig. 1)

Prior to preparation of hybrid epoxy-MA–DAD monolith, the capillary was pretreated with APTMS to immobilize a layer of amino groups into its inner wall, using the procedure developed previously [28]. In brief, the capillary was rinsed sequentially with 0.1 mol L⁻¹ NaOH (3 h), H₂O₂ (1 h), 0.1 mol L⁻¹ HCl (5 h), H₂O (1 h) and methanol (2 h). The washed capillary was brimmed with APTMS/methanol (50%, v/v) for 0.5 h, and then sealed with rubber septa at both ends and placed in the water bath kept at 60 ± 0.2 °C for 24 h. Using an HPLC pump, the obtained capillary was purged with methanol at a flow rate of 20 μL/min for 1 h. Then, it was dried using a stream of nitrogen at 2 MPa pressure for 3 h, and sealed until used.

The procedures for preparation of hybrid epoxy-MA–DAD monolith were as follows. Briefly, epoxy-MA, DAD, IPA, H₂O and ethanol were mixed together in a centrifuge tube. Then the mixture was ultrasonicated for 15 min at ice bath to be degassed and form a homogeneous solution. After the resultant transparent solution was introduced into the APTMS-pretreated capillary to an appropriate length, both ends were sealed with a rubber septum, followed by immersion into a water bath at 65 ± 0.2 °C for 12 h. The capillary was washed with methanol at a flow rate of 20 μL/min for 3 h to remove the residuals. Finally, the ends of the capillary were dipped in water until further use.

2.2.2. Preparation of hybrid epoxy-MA–EDMA monolith (Route II in Fig. 1)

Prior to fill the capillary with prepolymerization solution, the fused-silica capillary was also pretreated, as similar to the above-mentioned procedures except that APTMS was replaced by γ-MAPS.

The preparation of hybrid epoxy-MA–EDMA monolith was carried out in the light of following procedures. First, epoxy-MA, EDMA, 1-propanol, 1,4-butaneal and AIBN were mixed together in a centrifuge tube. The mixture was ultrasonicated for 30 min in order to be degassed. After the resultant solution was introduced into the γ-MAPS-pretreated capillary to an appropriate length, both ends were sealed with a silicon rubber, followed by immersion into a water bath at 60 ± 0.2 °C for 12 h. The capillary was washed with methanol at a flow rate of 20 μL/min for 3 h to remove the residuals, and the ends of the capillary were dipped in water until further use.

2.2.3. Modification of two kinds of hybrid monoliths

To modify the hybrid epoxy-MA–DAD hybrid monolith, an ethanol solution containing 10.0% (v/v) SMA and 0.5% (w/v) AIBN was pushed through the hybrid monolith and reacted in a water bath at 50 ± 0.2 °C for 12 h, while a solution consisting of 16.2 mg ODA and 300 μL ethanol was pushed through the hybrid epoxy-MA–EDMA monolith for post-column derivatization reaction, which was also carried out in a water bath at 50 ± 0.2 °C for 12 h. Finally, they were washed with methanol at a flow rate of 20 μL/min for 1 h to remove the residuals.

2.3. IR and SEM

The excess of the polymerization mixture remaining in the centrifuge tube was also polymerized under the same conditions as the preparation of the monolithic capillary columns. To remove the
residuals, the synthesized matrix was cut into small pieces with scissors. Then the pieces were dissolved into ethanol, and ultrasonicated for 3 h. The cleaning step was repeated for 4 times. Ultimately, the matrix was dried under vacuum at 60 °C for 24 h. The obtained matrix would be used for FT-IR. FT-IR spectra were measured on Thermo Nicolet 380 spectrometer with KBr pellets (Nicolet, Wisconsin, USA). KBr pellets, containing around 1 mg monolith sample and 100 mg KBr, were prepared by powder compressing machine. SEM images were obtained using a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). Metal plating of capillary monoliths was necessary for SEM.

2.4. Chromatographic characterization

A split-injection HPLC system used in this study consisted of two LC-10AD VP pumps (Shimadzu, Kyoto, Japan), a UV detector (K-2501, Knauer, Berlin, Germany) operated at 214 or 254 nm, a chromatography workstation (Beijing Cairo Scientific Instrument Ltd., Beijing, China) and a 7725I injection valve equipped with a T-union which served as a splitter, with one end connected to the capillary monolithic column and the other end to a blank capillary (95 cm length and 50 μm i.d.). The split ratio was controlled at about 1/400 for the 50–200 μm capillary columns. Chromatographic measurements were made at room temperature. The retention factor (k) was defined as (tR − t0)/t0, where tR and t0 represent the retention times of the analytes and theoria in this work, respectively.

A binary solvent system containing appropriate mixed ratio of ACN and H2O was applied to study chromatographic behaviors of the hybrid monoliths. To inspect the permeability of the monoliths, pressure drop measurements were carried out on an Eksigent one dimensional Plus Nano-HPLC system (Eksigent, Dublin) at room temperature using ACN/H2O (40/60, v/v) as the permeating fluid. The permeability was calculated according to Darcy’s Law [29] by the following, \( B_0 = \frac{F \eta \lambda}{(\pi \rho^2 \Delta P)} \), where \( F \) (m³/s) is the flow rate of mobile phase, \( \eta \) is the viscosity of mobile phase (0.801 cP [30]), \( L \) and \( r \) (m) are effective length and inner radius of the column, \( \Delta P \) (Pa) is the pressure drop of column.

3. Results and discussion

3.1. Preparation of two kinds of hybrid monoliths

3.1.1. Preparation of hybrid epoxy-MA–DAD monolith

The hybrid epoxy-MA–DAD monolith was produced by ring-opening polymerization of epoxy-MA and DAD (Fig. 1 and Table 1). The porogenic composition and the polymerization temperature were investigated in detail. In the study, the mixture of IPA/H2O/ethanol was chosen as the porogenic solvents. Columns 1, 2 and 3 (Table 1) were used to interpret the effect of H2O content on the morphology and permeability of the hybrid monolith (the amounts of epoxy-MA and DAD were kept at 50.0 and 10.0 mg, respectively). When the content of H2O decreased from 49.9 to 46.9% (w/w) in the porogenic system, it was found that the morphology of monolith transformed from loose to homogeneous, and the permeability dramatically decreased from a big value (too loose to get the value, column 1) to 2.55 × 10⁻¹⁴ m² (n = 3, RSD = 2.0%, column 2). When the content of H2O was further reduced to 44.9% (w/w), the permeability became so poor that it was impossible to flush through the column (column 3) even at a pressure of 30 MPa, due to the highly dense monolithic bed formed. In addition, columns 2, 4 and 5 were used to investigate the influence of the content of IPA. When the content of IPA was 40.1% (w/w), it was too dense to pump through the obtained monolith, however, when the content of IPA was 35.4% (w/w), it was too loose to get the permeability of the obtained monolith. Only when the content of IPA was 37.9% (w/w), a homogeneous monolith with the permeability of 2.55 × 10⁻¹⁴ m² (column 2) was gotten. Furthermore, the content of ethanol was also investigated using columns

![Figure 1](image-url) Fig. 1. The schematic preparation of two hybrid monoliths via ring-opening polymerization and free radical polymerization using epoxy-MA as functional monomer.

<table>
<thead>
<tr>
<th>Column</th>
<th>IPA (wt%)</th>
<th>H2O (wt%)</th>
<th>Ethanol (wt%)</th>
<th>Temperature (°C)</th>
<th>Permeability (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.8</td>
<td>49.9</td>
<td>14.3</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37.9</td>
<td>46.9</td>
<td>15.2</td>
<td>65</td>
<td>2.55 × 10⁻¹⁴</td>
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<tr>
<td>3</td>
<td>39.4</td>
<td>44.9</td>
<td>15.7</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40.1</td>
<td>45.3</td>
<td>14.6</td>
<td>65</td>
<td></td>
</tr>
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<td>35.4</td>
<td>48.8</td>
<td>15.8</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37.2</td>
<td>46.0</td>
<td>16.8</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>38.7</td>
<td>47.8</td>
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<td>65</td>
<td>5.73 × 10⁻¹⁴</td>
</tr>
<tr>
<td>8</td>
<td>37.9</td>
<td>46.9</td>
<td>15.2</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>37.9</td>
<td>46.9</td>
<td>15.2</td>
<td>70</td>
<td>2.81 × 10⁻¹⁵</td>
</tr>
</tbody>
</table>

a Weight percentage of IPA in the porogenic solvent
b Weight percentage of H2O in the porogenic solvent
c Weight percentage of Ethanol in the porogenic solvent
d Too loose to get the permeability values.
e Too hard to pump through.

Table 1. Effect of synthesis parameters on the formation of hybrid epoxy-MA–DAD monoliths.
2, 6 and 7. When the content of ethanol was 16.8% (w/w), the permeability became so poor that it was too hard to flush through the column (column 6). When the content of ethanol decreased from 15.2 to 13.5% (w/w) in the porogenic system, the morphology of monolith transformed from homogeneous to a little loose and the permeability increased from \(2.55 \times 10^{-14} \text{m}^2\) (column 2) to \(5.73 \times 10^{-14} \text{m}^2\) (column 7). Finally, the ratio of 37.9/46.9/15.2 (IPA/H\(_2\)O/ethanol, w/w/w) was selected as the relatively optimal porogenic system (the condition for column 2) for the preparation of hybrid epoxy-MA–DAD monolith in following experiments.

The polymerization temperature also played an important role in the preparation of the organic–inorganic monolith. Columns 2, 8 and 9 were used to investigate the effect of the temperature on the morphology and permeability of the monoliths. It was observed that the monolith could not be well formed at 60 °C (column 8, Fig. S2A–C, Supporting information) or 70 °C (column 9, Fig. S2D–F, Supporting information). Lower temperature (60 °C) resulted in the loose structure of the monolith, which could be attributed to the incomplete phase separation. Meanwhile, higher temperature (70 °C) resulted in poor permeability \(2.81 \times 10^{-15} \text{m}^2\), \(n = 3\), RSD = 0.01% of the monolith. When the polymerization was performed at 65 °C, a continuous and homogeneous rod (column 2, in Table 1 and Fig. 2A and B) with good permeability could be obtained. Thus, the relatively optimal polymerization temperature was controlled at 65 °C. It is noted that phase separation would be occurred after 30 min at 65 °C, due to the fast ring-opening reaction. So the polymerization mixture of containing epoxy-MA and DAD was mixed together and ultrasonicated at ice bath to form a homogeneous solution, facilitating the repeatability of hybrid epoxy-MA–DAD monolith.

The repeatability of hybrid epoxy-MA–DAD monolith (column 2) was assessed through the relative standard deviation (RSD) with benzene as the test compound. The run-to-run, column-to-column and batch-to-batch repeatabilities were 0.3% \((n = 4)\), 2.5% \((n = 3)\) and 4.1% \((n = 3)\) (correspondingly the average retention factor values of benzene were 0.65, 0.61 and 0.64), respectively. These results suggested the good repeatability of hybrid epoxy-MA–DAD monolith.

3.1.2. Preparation of hybrid epoxy-MA–EDMA monolith

Similar to the preparation of hybrid epoxy-MA–DAD monolith, the porogenic composition and polymerization temperature were also two noteworthy factors. In present work, 1-propanol/1,4-butanol were chosen as porogens for preparing hybrid epoxy-MA–EDMA monolith, referred to the preparation of poly(butyl methacrylate-co-EDMA) monolith [31]. The effect of the content of 1-propanol in the prepolymerization mixture on the morphology and permeability of the monolithic columns (the amounts of epoxy-MA and EDMA were kept at 48.0 and 33.6 mg, respectively) was examined by varying its content from 33.2 to 42.3% (columns 10–12). The lower content of 1-propanol (33.2%, w/w, column 10) in the prepolymer mixture would result in a slack monolith with a permeability of \(8.84 \times 10^{-14} \text{m}^2\), while the higher content of 1-propanol (42.3%, w/w, column 12) would result in a transparent monolith with a poor permeability of \(5.30 \times 10^{-15} \text{m}^2\). The 38.1% (w/w) 1-propanol (column 11) could result in a homogeneous monolith within the confines of the capillary. In addition, columns 11, 13 and 14 were used to investigate the effect of the content of 1,4-butanediol on the permeability of the monolithic columns. As seen in Table 2, an increase of the 1,4-butanediol weight percentage led to an increase of the permeability of the corresponding monoliths. Furthermore, the effect of the temperature on the permeability of monoliths was investigated using columns 11, 15 and 16. Good permeabilities (Table 2) could be obtained when the reaction was carried out at 55 and 60 °C, while a poor permeability of \(5.94 \times 10^{-15} \text{m}^2\) was gotten at 65 °C. Though the permeability value of column 15 (55 °C) was larger (Table 2) than that of column 11 (60 °C), it was found that the morphology of column 15 was a little loose. Ultimately, 60 °C was employed concerning the permeability and the morphology of the monoliths. Therefore, the conditions for column 11 (Table 2) were adopted to prepare the monoliths for further experiments.

The repeatability of hybrid epoxy-MA–EDMA monolith (column 11) was also measured through the RSD with benzene as the test compound. The values of run-to-run, column-to-column and batch-to-batch repeatabilities were 2.4% \((n = 3)\), 5.5% \((n = 3)\) and 5.6% \((n = 3)\) (correspondingly the average retention factor values of benzene were 0.65, 0.61 and 0.64), respectively. These results suggested the good repeatability of hybrid epoxy-MA–EDMA monolith.

Fig. 2. SEM images of hybrid epoxy-MA–DAD monolith (column 2) and epoxy-MA–EDMA monolith (column 11).
factor values of benzene were 1.55, 1.59 and 1.60, respectively, which meant the good repeatability of hybrid epoxy-MA–EDMA monolith.

3.2. Pristine hybrid monoliths: epoxy-MA–DAD vs epoxy-MA–EDMA

3.2.1. Morphology

Fig. 2 presents the SEM images of the resulting hybrid epoxy-MA–DAD (column 2) and epoxy-MA–EDMA (column 11) monoliths. Although two kinds of monolithic columns were both prepared by epoxy-MA, their morphologies looked rather different. As shown in Fig. 2A and B, bicontinuous structure could be used to depict the morphology of epoxy-MA–DAD monolith, which had a large domain size (~3 μm) [32] with skeletons (~1 μm) and through pores (~2 μm) in the fused-silica capillary (75 μm i.d.). On the other hand, the cauliflower-like globular structure, intertwined by typically micrometer-sized pores [33], could serve as a description for the morphology of epoxy-MA–EDMA monolith (Fig. 2C and D). These different morphologies of two kinds of hybrid monoliths were possibly related to phase separation during the formation of monoliths via both ring-opening polymerization and free radical polymerization. Although epoxy-MA contains silicon atoms, the phase separation of hybrid epoxy–MA–EDMA monolith was exactly same as the common methacrylate-based organic monoliths prepared by free radical polymerization [34–36]. At the beginning of the phase separation for these organic monoliths, the growing number and size of the propagating radicals in poor solvents for the polymer contributed to an early phase separation. Then they grew and became fixed in the cross-linked matrix. At last, the high degree of cross-linking and rapid phase separation led to the formation of cauliflower-like globular structures. However, the hybrid epoxy–MA–DAD monolith was formed via ring-opening polymerization, in which phase separation was similar to spinalodial-like decomposition resulting in bicontinuous-like skeletons typical of silica-based monoliths prepared by sol–gel chemistry [37–39]. The starting system was a homogeneous mixture of IPA/H₂O/ethanol. By the reaction of epoxy-MA and DAD, the molecular weight of the product increased gradually and formed a continuous polymer phase and a solvent phase. The phase separation might follow spinalodial decomposition mechanism, which played an important role in the formation of bicontinuous structure of hybrid epoxy–MA–DAD monolith.

3.2.2. Chromatographic performance

The chromatographic assessment of the resulting pristine hybrid epoxy-MA–DAD and epoxy-MA–EDMA monoliths was performed. Their separation abilities were firstly investigated using alkylbenzenes as probes. Both separations were performed using 50% (v/v) ACN/H₂O as the mobile phase at the flow rate of 110 μL/min (before split) (shown in Fig. S5, Supporting information), and five alkylbenzenes were baseline-separated within 15 minutes with good peak shapes. Corresponding to the hydrophobicities of these analytes, they were all eluted in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene, indicating that hydrophobic interaction plays an important role in the separation of alkylbenzenes on both pristine monoliths. The retentions of alkylbenzenes on two pristine columns were also compared, and the result was shown in Fig. S4 (Supporting information). It was obvious that the retentions of alkylbenzenes on epoxy-MA–EDMA monolith were almost twice as strong as those on epoxy-MA–DAD monolith.

3.3. Functionalized hybrid monoliths: SMA-modified epoxy-MA–DAD vs ODA-modified epoxy-MA–EDMA

3.3.1. Surface properties

Two kinds of hybrid monoliths were also characterized by FT-IR to establish the reaction sites of epoxy-MA, in which epoxy moieties and α,β-unsaturated ester, as two active functional groups, might take part in reacting with other monomers in the formation of monoliths. Fig. 3A(I) and (II) represents the FT-IR spectra of pristine and SMA-modified epoxy-MA–DAD monoliths, respectively. It could be found from Fig. 3A(I) that the signals at 1721 and 1603 cm⁻¹ implied the presence of α,β-unsaturated carbonyl, which manifested that the epoxy–MA–DAD monolith was formed by the ring-opening reaction between the amino groups of DAD and the epoxy moieties of epoxy-MA, not by 1,4-Michael addition reaction between the amino of DAD and the α,β-unsaturated ester of epoxy-MA. Compared to Fig. 3A(I), the signal of ester carbonyl at 1732 cm⁻¹ was emerged in Fig. 3A(II) instead of the signals of α,β-unsaturated ester (1721 and 1603 cm⁻¹) in Fig. 3A(I), suggesting that the vinyls on the surface of monolith might be consumed after SMA modification via free radical reaction. Fig. 3B(I) and (II) represents the FT-IR spectra of pristine and ODA-modified epoxy-MA–EDMA monoliths, respectively. It could be easily found from Fig. 3B(I) that the signals of α,β-unsaturated ester (~1721 and 1603 cm⁻¹) were disappeared, while the signal of ester carbonyl at 1732 cm⁻¹ was presented. These data indicated that free radical polymerization was carried out between the methacrylates of epoxy-MA and EDMA to form the hybrid epoxy-MA–EDMA monolith. The signal intensity at 3481 cm⁻¹ (assigned to hydroxyl and amino groups) in Fig. 3B(II) was significantly increased by comparison with that in Fig. 3B(I), suggesting that the nucleophilic additional reaction was carried out between the amino group of ODA and the epoxy moieties on the surface of pristine monolith, which produced extra amino and hydroxyl groups.

3.3.2. Chromatographic performance

Fig. S5A and B (Supporting information) show the effect of ACN content in mobile phase on the retention factors of alkylbenzenes on pristine and SMA-modified epoxy-MA–DAD monoliths. As the ACN content increased from 40 to 70%, the retention factors

<table>
<thead>
<tr>
<th>Column</th>
<th>1-Propanol¹ (wt%)</th>
<th>1,4-Butanediol² (wt%)</th>
<th>Temperature (℃)</th>
<th>Permeability (m²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>33.2</td>
<td>66.8</td>
<td>60</td>
<td>8.84 x 10⁻⁴⁴</td>
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<tr>
<td>11</td>
<td>38.1</td>
<td>61.9</td>
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<td>2.82 x 10⁻⁴⁴</td>
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<tr>
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<td>65</td>
<td>5.94 x 10⁻⁴⁵</td>
</tr>
</tbody>
</table>

¹ Weight percentage of 1-propanol in the porogenic solvent.
² Weight percentage of 1,4-butanediol in the porogenic solvent.
³ Monomer/porogenic solvent, 4/5 (w/w).

Table 2

Effect of synthesis parameters on the formation of hybrid epoxy-MA–EDMA monoliths.
of alkylbenzenes decreased, further demonstrating the reversed-phase retention mechanism of alkylbenzenes on two monoliths. Fig. S5C and D (Supporting information) demonstrated that the plots of logarithmic retention factors of alkylbenzenes on these two monoliths versus the number of saturated carbon atoms in the alkyl chain were all linear. The methylene selectivity, $\alpha_{\text{CH}_2}$, which could be conveniently used to characterize the hydrophobicity of a stationary phase, was calculated from the following equation [40]:

$$\log k = n \log \alpha_{\text{CH}_2} + \log \beta$$

where $n$ is the number of saturated carbon atoms in the alkyl chain, and $\beta$ is the retention factor of benzene. As shown in Fig. S5C and

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**Fig. 3.** FT-IR spectra of pristine and modified hybrid monoliths. A (I), pristine epoxy-MA–DAD monolith; A (II), modified epoxy-MA–DAD monolith; B (I), pristine epoxy-MA–EDMA monolith; B (II), modified epoxy-MA–EDMA monolith.

**Fig. 4.** The dependence of plate height of analytes on the linear velocity of mobile phase on (A) pristine epoxy-MA–DAD, (B) SMA-modified epoxy-MA–DAD, (C) pristine epoxy-MA–EDMA and (D) ODA-modified epoxy-MA–EDMA monoliths. The analytes are thiourea, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene with different concentrations. The mobile phase was 50% (v/v) ACN/H$_2$O, and the flow rate ranged from 20 to 110 \(\mu\)L/min (before split). Column dimensions: pristine epoxy-MA–DAD monolith, 36.4 cm x 75 \(\mu\)m i.d.; SMA-modified epoxy-MA–DAD monolith, 35.5 cm x 75 \(\mu\)m i.d.; pristine epoxy-MA–EDMA monolith, 35.4 cm x 75 \(\mu\)m i.d.; ODA-modified epoxy-MA–EDMA monolith, 37.6 cm x 75 \(\mu\)m i.d. On-column UV detection at 214 nm.
D, the linear relationship ($R > 0.998$) of pristine and SMA-modified epoxy-MA–DAD monoliths suggested the equation was valid for further analysis. The slopes of the lines in Fig. 5C represented the logarithmic methylene selectivity of the pristine monolith, giving $\alpha_{\text{CH}_2}$ values of 0.19, 0.15, 0.12 and 0.12 at 40%, 50%, 60% and 70% ACN mobile phase concentration, respectively. Similarly, the SMA-modified monolith was similarly evaluated in Fig. 5D, and $\alpha_{\text{CH}_2}$ was determined to be 0.23, 0.18, 0.15 and 0.14 at 40%, 50%, 60% and 70% ACN in the mobile phase, respectively. These values were higher than those for pristine monolith under the same conditions, demonstrating the higher hydrophobicity of SMA-modified monolith relative to the pristine one. The result also indicated that hybrid epoxy-MA–DAD monolith was successfully functionalized with SMA.

The effect of ACN content in mobile phases on the retention factors of alkylbenzenes on pristine and ODA-modified epoxy-MA–EDMA monoliths were also investigated, and the results were shown in Fig. 5A and B (Supporting information). The retention factors of alkylbenzenes were decreased as the percentages of ACN increased, indicating the reversed-phase mechanism of alkylbenzenes for both pristine and ODA-modified hybrid epoxy-MA–EDMA monoliths. The methylene selectivity, $\alpha_{\text{CH}_2}$, was adopted to evaluate the hydrophobicity of pristine and ODA-modified epoxy-MA–EDMA monoliths. As shown in Fig. 5C and D (Supporting information), the linear relationship ($R > 0.992$) of pristine and modified epoxy-MA–EDMA monoliths indicated the equation was credible for this study. Taking 50% (v/v) ACN/H$_2$O for example, the $\alpha_{\text{CH}_2}$ (0.16) for ODA-modified monoliths was higher than that for pristine monolith (0.12), suggesting the increase of hydrophobicity after modification.

**Fig. 5**. Separations of phenols (A, B) and basic compounds (C, D) on pristine (A, C) and modified (B, D) epoxy-MA–DAD hybrid monoliths. Analytes: (A, B) (1) hydroquinone, (2) pyrocatechol, (3) phenol, (4) p-cresol, (5) 2,6-xylene, (6) 4-tert-butyl-phenol; (C, D) (1) 1,4-phenylenediamine, (2) 2,4-diaminotoluene, (3) carbamazepine, (4) barbitone, (5) benzidine, (6) 2,4-dinitroaniline, (7) 4-aminobiphenyl. Experimental conditions: Mobile phases, (A, B) ACN/H$_2$O (40/60, v/v), (C, D) ACN/H$_2$O (37.5/62.5, v/v); flow rate, 50 µL/min (before split). Other conditions as in Fig. 4.

**Fig. 4**A and B displays the van Deemter plots of pristine and SMA-modified epoxy-MA–DAD monoliths obtained with five alkylbenzenes as the solutes in 50% ACN/H$_2$O (v/v). As shown in Fig. 4A, plate height minimum of pristine monolith was not found above the linear velocity of 0.49 mm/s (20 µL/min, before split). Conversely, the minimum plate height (~16 µm) of modified hybrid epoxy-MA–DAD monolith could be obtained from Fig. 4B. The minimum was smaller than the plate height (~22 µm) of pristine monolith at 0.49 mm/s, to some extent, reflecting that the modification possibly contributed to the increase of column efficiency. In addition, the slopes of both plots at relatively high velocities in Fig. 4A and B were large, which indicated that the pristine monolith at 2.73 mm/s (110 µL/min, before split) (18,000 plates/m for benzene) could not keep the same efficiency as at 0.49 mm/s (27,000 plates/m for benzene). However, there is no denying that five alkylbenzenes could also be baseline-separated (Fig. S3A) on pristine epoxy-MA–DAD monolith at 2.73 mm/s using 50% ACN/H$_2$O (v/v) as the mobile phase. In addition, as shown in the van Deemter plots of pristine and ODA-modified epoxy-MA–EDMA monoliths (Fig. 4C and D), there were not the plate height minimum for two monoliths above the linear velocity of 0.50 mm/s. Comparison of the plots of two hybrid monoliths suggested that the chromatographic performance of epoxy-MA–EDMA monolith was not improved after modification with ODA.

In a word, the preparation of hybrid epoxy-MA–DAD and epoxy-MA–EDMA monoliths was based on different reaction mechanism, but they possessed similar chromatographic behaviors for the separation of alkylbenzenes. Meanwhile, they exhibited different column efficiencies. When the separations were performed on two pristine monoliths with 50% ACN/H$_2$O as the mobile
phase at the flow rate of 50 μL/min, the column efficiencies on epoxy-MA–DAD monolith were ranged in 25,000–34,000 plates/m, which were twice higher than those on epoxy-MA–EDMA monolith (12,000–13,000 plates/m). The column efficiencies of two monoliths might be related to the pore structures of the monoliths. Maybe the bicontinuous structure of hybrid epoxy-MA–DAD monolith allowed small molecules to penetrate to the adsorption sites more easily and diffuse faster than the cauliflower-like globular structure of hybrid epoxy-MA–EDMA monolith.[31,35,41] Furthermore, the chromatographic performance for the separation of alkylbenzenes on SMA-modified epoxy-MA–DAD monolith was remarkably improved (42,000–54,000 plates/m) when compared with that on pristine epoxy-MA–DAD monolith (Fig. 4A and B), while it was not obviously enhanced on ODA-modified epoxy-MA–EDMA monolith when compared with that on pristine epoxy-MA–EDMA monolith (Fig. 4C and D). The enhancement of the column efficiency of epoxy-MA–DAD monolith after modification might be ascribed to the decreased mass-transfer resistance[42].

In addition, these hybrid monoliths possessed lower column efficiencies than those hybrid monoliths previously prepared with methacrylate-containing [26] and epoxy-containing POSS [24] in our group, which could only participate in the formation of monoliths. However, epoxy-MA, containing three epoxy moieties and one methacrylate group, has potential in the fabrication of monoliths and sequential post-column modification. So epoxy-MA is an interesting monomer for its tailorability.

3.4. Separation of polar analytes on two kinds of hybrid monoliths

The polar phenols and basic compounds were used to investigate the selectivity of these hybrid monoliths. The separations of these analytes on hybrid epoxy-MA–DAD monolith were shown in Fig. 5. As presented in Fig. 5A and B, baseline separations of six phenols including hydroquinone, pyrocatechol, phenol, p-cresol, 2,6-xylenol and 4-tert-butyl-phenol were performed on both pristine and modified hybrid epoxy-MA–DAD monoliths by using 40% ACN/H2O (v/v) as mobile phase. The retention of phenols was based on their hydrophobicity. The column efficiencies of phenols on pristine monolith altered from 18,000 to 37,000 plates/m, while they transformed from 18,000 to 44,000 plates/m on SMA-modified monolith. As for analysis of seven basic compounds, carbamazepine and barbitone could be baseline-separated on SMA-modified monolith by using 37.5% ACN/H2O (v/v) as mobile phase, while they were not baseline-separated on the pristine one (see Fig. 5C and D). The increase of hydrophobicity after SMA modification may contribute to the baseline separation of carbamazepine and barbitone. Additionally, the column efficiencies of basic compounds on SMA-modified monolith ranged from 18,000 to 35,000 plates/m, while they varied from 12,000 to 34,000 plates/m on pristine monolith. Similar to hybrid epoxy-MA–DAD monolith, hybrid epoxy-MA–EDMA monolith also had selectivity for the separations of polar analytes (shown in Fig. 6). Six phenols and seven basic compounds were also adopted to evaluate the chromatographic performance of pristine and modified epoxy-MA–EDMA monolith. All phenols were well separated on both pristine and modified monoliths using 40%ACN/H2O (v/v) as mobile phase, and all basic compounds but 1,4-phenylenediamine and 2,4-diaminotoluene, were baseline separated using 37.5% ACN/H2O (v/v) as mobile phase. It was revealed from Fig. 6 that pristine and ODA-modified hybrid epoxy-MA–EDMA monoliths had similar resolution when they were applied in the separations of six phenols or seven basic compounds.
During the formation of epoxy-MA–DAD monolithic matrix, amino and hydroxyl groups were produced after the ring-opening polymerization of epoxy-MA and DAD. These functional groups on the surface of the monoliths might make it possible to be applied in hydrophilic interaction chromatography (HILIC). Basic compounds might be retained with hydrogen-bonding interaction. However, similar to the hybrid monolith in our previous work, which was also fabricated via ring-opening polymerization using an epoxy-containing POSS and poly(ethyleneimine) (PEI) as precursor [43], epoxy-MA–DAD monolith might possess hydrophobic property. It could be deduced that the separation of basic compounds on epoxy-MA–DAD monolith might be based on both reversed-phase mechanism and hydrogen-bonding interaction using 37.5% ACN/H2O (v/v) as mobile phase. On the other hand, epoxy-MA–EDMA monolithic matrix was prepared by free radical polymerization of epoxy-MA and EDMA, without generating or introducing extra polar groups such as amino or hydroxyl group. It was concluded that the separation of basic compounds on epoxy-MA–EDMA monolith was mainly based on reversed-phase mechanism.

4. Conclusions

Two kinds of hybrid monoliths, epoxy-MA–DAD and epoxy-MA–EDMA monoliths, were successfully prepared with epoxy-MA as the functional monomer via ring-opening polymerization and free radical polymerization, respectively. Compared with the preparation of epoxy-MA–DAD monolith, the preparation of epoxy-MA–EDMA monolith was time-consuming and susceptible to oxygen. In addition, due to the different phase separation mechanism, two hybrid monoliths exhibited different morphology, and bicontinuous and globular structures could be vividly described for the features of two resulting monoliths, respectively. As a result, their chromatographic performance for the separation of alkylbenzenes was remarkably different. Column efficiencies for alkylbenzenes on hybrid epoxy-MA–DAD monolith were higher than those on hybrid epoxy-MA–EDMA monolith, while the retention of alkylbenzene using epoxy-MA–EDMA monoliths was stronger than epoxy-MA–DAD monoliths. Taking the preparation process and column efficiency into consideration, it could be deduced that the hybrid epoxy-MA–DAD monolith had the advantages, such as fast preparation and high efficiency, over the preparation of epoxy-MA–EDMA monolith. Furthermore, owing to different remaining functional groups on two hybrid monoliths, epoxy-MA–DAD and epoxy-MA–EDMA monoliths could be easily modified via different derivative approaches. It offered us a good option to tailor-make various hybrid monoliths for a myriad of chromatographic application.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.09.072.

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