A novel polymeric monolith prepared with multi-acrylate crosslinker for retention-independent efficient separation of small molecules in capillary liquid chromatography

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HIGHLIGHTS

- A crosslinker with multiple acrylate groups was first used for preparing polymeric monoliths.
- The poly(LMA-co-DPEPA) monoliths exhibited the column efficiencies of 111,000–165,000 N m⁻¹ for alkylbenzenes in cLC.
- Highly crosslinked monoliths also exhibited retention-independent efficient chromatographic performance.

GRAPHICAL ABSTRACT

ABSTRACT

Low column efficiency for small molecules in reversed-phase chromatography is a major problem commonly encountered in polymer-based monoliths. Herein, a novel highly crosslinked porous polymeric monolith was in situ prepared by using a multi-acrylate monomer, dipentaerythritol penta-/hexa-acrylate (DPEPA), as crosslinker, which copolymerized with lauryl methacrylate (LMA) as functional monomer in a UV-transparent fused-silica capillary via photo-initiated free-radical polymerization within 5 min. The mechanical stability and permeability of the resulting poly(LMA-co-DPEPA) monolith were characterized in detail. One series of highly crosslinked poly(LMA-co-DPEPA) columns were prepared with relatively higher content of crosslinker (63.3%) in the precursor. Although they exhibited lower permeability, high column efficiency for alkylbenzenes was acquired in cLC, and the minimum plate height (column B) was in

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1. Introduction

In recent years, monolithic columns have developed rapidly in preparation, characterization and application in separation of small molecules and macromolecules due to their excellent chromatographic properties [1,2], such as facile preparation, fast separation at high flow rate, varieties of functionality and good permeability [3,4]. Generally speaking, based on the nature chemistry of the monolithic matrix, monolithic columns can be divided into three types, including silica-based monolith, organic–inorganic hybrid monolith and polymer-based monolith. Silica monolith provides good mechanical stability, permeability and high column efficiency for fast separation of small molecules [5,6]. However, functionalization of the surface of silica monolith is labor-intensive and time-consuming, limiting its development [7,8]. Although the organic–inorganic hybrid monolith presents some outstanding features such as facility of preparation, less shrinkage, good mechanical stability and high surface area, the lacking types of commercial organic-trialkoxyxilanes also limits the development of organic–inorganic hybrid monolith [9–11].

Compared with silica-based monolith and organic–inorganic hybrid monolith, polymer-based monolith, including polymeric acrylamide, polycrylamide and polystyrene monoliths [12–14], shows significant advantages, such as good chemical stability, simple preparation and variety of functionality [15,16]. On account of the diversity of functional monomers, polymer-based monolith has been widely applied to several chromatographic modes including reversed-phase liquid chromatography (RPLC) [17], hydrophilic interaction chromatography (HILIC) [18] and ion-exchange chromatography (IEC) [19] etc. However, it presents low column efficiency as well as retention-dependent column performance for small molecules especially in the isocratic elution because of its less mesopores and lower specific surface areas [20]. The presence of micropores will be harmful to the separation efficiency since they allow small molecules to permeate in the gel structure and enhance chromatographic dispersion due to the stagnant mass transfer resistance [21,22]. Additionally, polymer-based monolith will shrink or swell when the mobile phase contains high content of organic solvents. For these reasons, improving the chromatographic efficiency and mechanical stability of polymer-based monolith is still a research focus [20,23–25].

It is well known that crosslinker plays a significant role in the preparation of polymeric monolith. The size, polarity, functional groups and content of crosslinker in polymerization mixture could affect the size of pores, permeability and column efficiency. More numbers of functional groups of crosslinker participated in the reaction, and higher crosslinking density was obtained. Then the separation efficiency of monolithic column for small molecules significantly improved with the increase in the number of functional groups [2,26]. Commercially available N,N-methylenebisacrylamide, divinylbenzene and ethylene dimethacrylate are generally selected as crosslinker to prepare various kind of monolithic columns [1,27–29]. Several attempts to optimize the capillary monolith have led to columns affording the column efficiencies of 35,000–50,000 N m⁻¹ for benzene [24,25,27–32]. Recently, Lee and co-workers [20] prepared a highly crosslinked polymeric monolith with 1,6-hexanediol dimethacrylate (HDDMA) as crosslinker, and the resulting monolithic column demonstrated the column efficiency up to 86,000 N m⁻¹.

In this study, we first selected dipentaerythritol penta-/hexa-acrylate (DPEPA) as crosslinker, which has five or six acrylate groups to participate in the polymerization for the formation of

\[
\text{RO} + \text{H}_2\text{C}_3\text{OCH}_2\text{CH}_7 + \text{hv} \rightarrow \text{C}_2\text{H}_5 + \text{C}_12\text{H}_{25} + \text{C}_12\text{H}_{25} + \text{C}_12\text{H}_{25}
\]

highly crosslinked polymeric monolith (Scheme 1). This novel polymeric monolith was fast fabricated by copolymerization of DPEPA and lauryl methacrylate (LMA) as functional monomer via photo-initiated polymerization reaction. The optimal highly crosslinked polymeric monoliths allowed for the separation of alkylenzenes with a retention-independent efficient performance. The resulting poly(LMA-co-DPEPA) monolith was characterized by Fourier-transformed infrared spectrum (FT-IR), scanning electron microscopy (SEM) and mercury intrusion porosimetry (MIP), etc. The chromatographic performance was also evaluated by separation of phenols, basic compounds and intact proteins by capillary liquid chromatography (cLC).

2. Experimental

2.1. Chemicals and materials

DPEPA (contains ≤650 ppm MEHQ as inhibitor), LMA (contains 500 ppm MEHQ as inhibitor, technical grade), 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS ≥98%), formic acid (FA, for mass spectrometry, ~98%) and polystyrene standards (Mw = 800, 4000, 13,200, 50,000, 90,000, 280,000 and 900,000) were purchased from Sigma (St. Louis, MO, USA), and used directly without further purification. 2,2-Dimethoxy-2-phenylacetophene (DMPA, 99%) was gotten from Acros Organics (New Jersey, USA), and dissolved in hexyl alcohol (10%, w/v) prior to use. Lysozyme (chicken egg white), bovine serum albumin (BSA), myoglobin (horse heart) and RNase A were obtained from Sigma–Aldrich (St. Louis, MO, USA). Cytochrome c (bovine heart) was from Aladdin (Shanghai, China). Tetrahydrofuran (THF), methanol and acetonitrile (ACN) were HPLC-grade and acquired from Yuyang Group (Shandong, China). Thiourea, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, phloroglucinol, pyrocatechol, para-cresol, 2,6-dimethyl phenol, 2,4-dichlorophenol, caffeine, carbamazepine, 2,4-dinitroaniline, 4-aminobiphenyl and 2,6-dichloro-4-nitroaniline were of analytical grade, and obtained from Tianjin Kermel Chemical Plant (Tianjin, China). Deionized water was prepared with a Milli-Q system (Milli-pore, MA, USA). The UV-transparent fused-silica capillary with 75 μm i.d. and 365 μm o.d. was the product of Reafine Chromatography Ltd. (Hebei, China).

2.2. Preparation of polymeric monolith via photo-initiated polymerization

Prior to the preparation of a polymeric monolithic column, the inner wall of fused-silica capillary was pretreated and modified with a layer of methacrylate groups. Briefly, the capillary was first rinsed with 0.1 mol L⁻¹ NaOH for 1 h, then washed with water till pH 7, followed by 0.1 mol L⁻¹ HCl for 5 h, and finally rinsed with water and methanol. After that the capillary was dried by nitrogen stream at room temperature. Then the capillary was filled with a solution of γ-MAPS and methanol (50/50, v/v) with a syringe and kept in the water bath at 50 °C overnight with both ends sealed with silicon rubber. Finally, the capillary was rinsed with methanol to flush out the residual reagents and dried with a stream of nitrogen gas.

The prepolymerization solution consisting of functional monomer, crosslinker, initiator and porogenic solvents was mixed in a vial to form a homogeneous solution (the detail composition of different prepolymerization solution is listed in Table 1). Then the prepolymerization solution was introduced into the pretreated capillary with a syringe. After both ends were sealed with silicon rubber, the capillary was put in the UV crosslinkers (XL-1500A, λ = 365 nm, Spectronics Corporation, New York, USA) at 365 nm for 300 s. The capillary was then flushed with methanol to remove the residual reagents. Finally, both ends of the capillary was kept in the water for usage.

The rest of prepolymerization solution in the vial was also cured in the UV crosslinkers at 365 nm for 300 s to form bulk monolithic material, which would be rinsed with ethanol three times, cut into small pieces, grinded using mortar and pestle, and then dried in a vacuum at 50 °C for 2 days.

2.3. Structural characterization of the monolith

The microscopic morphology of polymeric monoliths was obtained by SEM (JEOL JSM-5600, Tokyo, Japan). FT-IR characterization was carried out on Thermo Nicolet 380 spectrometer using KBr pellets (Nicolet, Wisconsin, USA). The specific surface area was calculated from nitrogen adsorption/desorption measurements of dry bulk monoliths using a Quadrasorb SI surface area analyzer (QuantaChrome, Boynton Beach, USA). Pore size distribution was measured by MIP on a PoreMaster GT-60 (QuantaChromze Instrument Corporation, USA).

2.4. Chromatographic characterization of the monolith

The HPLC experiment was performed on an LC system coupled with an Agilent 1100 micro pump, a 7725i injector with a 20 μL sample loop and a UV detector (K-2501, Knaur, Berlin, Germany), in which the detection window was made by removing the polimide coating of fused-silica capillary tube with 50 μm i.d. in a position 5.0 cm from the separation monolithic column outlet, and the detection wavelength was set at 214 nm. A T-union connector was used as a splitter, with one end connected to the capillary monolithic column and the other end to a blank capillary (50 μm i.d. and 150 cm in length). The flow rates of pump were set at 50–230 μL min⁻¹, the actual flow rates in the monolithic column were 80–380 nL min⁻¹, resulting in split ratio about 1/600. All chromatographic data were collected and analyzed using the software program HW-2000 from Qianpu Software (Shanghai, China).

The permeability was calculated according to Darcy’s law [33] by the equation: \( \eta \frac{Fl}{r^2 \Delta P} \), where \( F \) (m² s⁻¹) is the flow rate of mobile phase, \( \eta \) is the viscosity of mobile phase (0.38 × 10⁻³ Pa s for ACN), \( L \) and \( r \) (m) are effective length and inner diameter of the column, \( \Delta P \) (Pa) is the pressure drop of column. The data of \( \Delta P \) and

<table>
<thead>
<tr>
<th>Column</th>
<th>DPEPA (mg)</th>
<th>LMA (mg)</th>
<th>Crosslinker content (w/w, %)</th>
<th>Hexyl alcohol (μL)</th>
<th>Ethylene glycol (μL)</th>
<th>Permeability (10⁻¹⁴ m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>12.4</td>
<td>73.8</td>
<td>140</td>
<td>20</td>
<td>0.83</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>17.4</td>
<td>63.3</td>
<td>140</td>
<td>20</td>
<td>0.83</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>17.4</td>
<td>63.3</td>
<td>130</td>
<td>30</td>
<td>0.90</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>17.4</td>
<td>63.3</td>
<td>120</td>
<td>40</td>
<td>0.92</td>
</tr>
<tr>
<td>E</td>
<td>25</td>
<td>22.4</td>
<td>52.7</td>
<td>140</td>
<td>20</td>
<td>1.30</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>22.4</td>
<td>52.7</td>
<td>130</td>
<td>30</td>
<td>1.45</td>
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<tr>
<td>G</td>
<td>25</td>
<td>22.4</td>
<td>52.7</td>
<td>120</td>
<td>40</td>
<td>1.65</td>
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<tr>
<td>H</td>
<td>20</td>
<td>27.4</td>
<td>42.2</td>
<td>140</td>
<td>20</td>
<td>2.13</td>
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</tbody>
</table>

Polymerization mixture contains 1.5 μL 10% DMPA (w/w, DMPA/hexyl alcohol).
were obtained on an ACQUITY UltraPerformance LC (Waters, USA). The flow rates of mobile phase were set at 0.1–1.5 μL min⁻¹.

3. Results and discussion

3.1. Preparation of polymeric monoliths

The preparation of polymeric monoliths is illustrated in Scheme 1. As expected, the morphology and permeability of the polymeric monolithic columns were obviously controlled by the composition of prepolymerization solution, which was investigated in detail. We selected DPEPA as crosslinker and LMA as functional monomer to prepare the polymeric monolithic columns via photo-initiated free-radical polymerization in a UV-transparent capillary. Meanwhile, the mixture of hexyl alcohol and ethylene glycol was selected as porogenic solvents, and the ratio of porogenic solvents was optimized to achieve the polymeric monolithic columns with well-defined porous structure. The percentage of total monomers in polymerization mixture was kept at 25.8% (wt%). The polymerization mixture was directly illuminated with UV radiation (λ = 365 nm, 120 mJ cm⁻²) in the presence of photo-initiator (1.5 μL, 10% DMPA, wt%). It could be observed that white solid appeared in the vials after illuminating for 50 s, indicating rapid polymerization with photo initiation mode.

As shown in Table 1, a series of polymeric monolithic columns were synthesized with different contents of crosslinker and functional monomer. It was found that the permeability was significantly increased from 0.83 to 2.13 × 10⁻¹⁴ m² when the content of DPEPA (in the total monomers, w/w%) decreased from 63.3% to 42.2%, which were prepared with the same porogenic solvents (columns A, B, E, and H, Table 1). It was worth noting that higher content of crosslinker in the precursor inclined to form microporous structure, resulting in lower permeability. The SEM micrographs for columns A, B and E (Fig. 1) also proved that larger macropores emerged with a decrease of DPEPA content. What is worse, high content of DPEPA (column A) would lead to monolith matrix detaching from the inner wall of fused-silica capillary (Fig. 1a and d).

The composition of porogenic solvents (hexyl alcohol and ethylene glycol) is another important factor affecting the formation of poly(LMA-co-DPEPA) monolithic columns. As for the columns B, C and D shown in Table 1, which were prepared with relatively higher content of crosslinker (63.3%), the permeability slightly increased from 0.83 to 0.92 × 10⁻¹⁴ m² when the proportion of ethylene glycol was increased from 12.5% to 25.0% (v/v). Meanwhile, as for the columns E–G, which were prepared with relatively lower content of crosslinker (52.7%), the permeability remarkably increased from 1.30 to 1.63 × 10⁻¹⁴ m² when the proportion of ethylene glycol was increased from 12.5% to 25.0% (v/v). As a result, the ethylene glycol served as macroporogenic solvent (poor solvent), while hexyl alcohol as microporogenic solvent (good solvent). Additionally, it proved that the proportion of porogenic solvents had a little influence on the permeability of highly crosslinked polymeric monoliths fabricated with high content of crosslinker.

Considering both separation efficiency and permeability, columns B and E (Table 1) were employed for further characterization and evaluation by cLC in the following experiments. The repeatability and reproducibility of poly(LMA-co-DPEPA) columns (columns B and E) were also characterized by measuring the relative standard deviations (RSDs) of the retention factor (k) of benzene (thiourea as the void time maker) under the mobile phase of 60% ACN. Two columns exhibited good repeatability by cLC separation. The RSDs of run-to-run repeatability, column-to-column and batch-to-batch reproducibility were all less than 0.2%, 1.3%, 2.1% (column B, n = 5) and 1.1%, 3.2%, 3.9% (column E, n = 5), respectively. The results demonstrated that the repeatability and reproducibility of polymeric monolithic columns were acceptable.

3.2. Characterization of polymeric monoliths

The corresponding SEM micrographs of the poly(LMA-co-DPEPA) monolithic columns are shown in Fig. 1. The SEM images demonstrated that column B (Fig. 1b and e) and column E (Fig. 1c and f) possessed uniform porosity structure and well linked to the inner wall of capillary. However, the monolithic matrix of column A detached from the inner wall of fused-silica capillary as shown in Fig. 1a and d. It was concluded that too high content of crosslinker would cause the polymer to shrink. Compared with column B, larger size of through-pores was found in column E, which allowed the fluid to rapidly pass the capillary and provided a better

![Fig. 1. SEM micrographs of polymeric monolithic columns with (a, d) column A, (b, e) column B and (c, f) column E.](image-url)
permeability. Pore size measurement also indicated that large macropores (>1.0 μm in diameter) existed in columns B and E, as shown in Fig. 2a and b. Their specific surface areas were calculated as 27.4 (column B) and 19.8 (column E) m² g⁻¹ based on nitrogen adsorption/desorption isotherm. The size exclusion chromatography using THF as mobile phase also provided the porosity of the polymer monolithic columns as shown in Fig. 2c and d. The porosity of column B was measured at 45.2%. In contrast with column B, the porosity of column E was higher, and could reach 57.7%.

The IR spectrum of the polymeric monolith is shown in Fig. 3. The apparent peaks in the region of 1700–1750 cm⁻¹ are stretching vibration of the C=O bond. The existence of α,β-unsaturated carbonyl bond (C=O) in the structure of DPEPA and LMA is proved by a characteristic peak position at 1728 cm⁻¹ and 1722 cm⁻¹ (Fig. 3a and b), respectively. However, the stretching vibration of the C=O band of poly(LMA-co-DPEPA) monolith appeared at 1736 cm⁻¹ (Fig. 3c). Meanwhile, the characteristic peak of the stretching vibration of C=C bond of poly(LMA-co-DPEPA) monolith changed slightly from 1635 cm⁻¹ (DPEPA) and 1639 cm⁻¹ (LMA) to 1636 cm⁻¹, and its intensity was remarkably decreased. These results demonstrated that polymerization reaction successfully occurred, but a few unreacted C=C bonds still existed.

As shown in Fig. 4, the mechanical stability of polymeric monolithic columns (columns B and E) was studied via the back-pressure measurement by connecting an 18-cm-long polymeric monolithic column to a NanoLC pump with ACN as the mobile phase. The back-pressure of two columns up to 40 MPa (R₁ = 0.996 and R₂ = 0.999) increased linearly with the increase of flow rate from 0.1 to 1.8 and 0.2 to 3.0 μL min⁻¹, respectively. The results suggested that the resulting poly(LMA-co-DPEPA) monolithic columns owned satisfactory mechanical stability.

The chromatographic evaluation of two poly(LMA-co-DPEPA) monolithic columns (B and E) was performed by cLC separation of alkylbenzenes as probes. As shown in Fig. 5a and b, five alkylbenzenes were baseline-separated with good peak shapes using ACN/H₂O (60/40, v/v) as the mobile phase at 170 μL min⁻¹ (before split) (thiourea as the void time marker). The elution of alkylbenzenes was in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene according to their

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**Fig. 2.** Pore size distribution of (a) column B and (b) column E; calibration curve of column B (c) and column E (d) by size exclusion chromatography. Experimental conditions: column dimension, 20.0 cm × 75 μm i.d.; off column dimension, 5.0 cm × 75 μm i.d.; flow rates, 66.7 ml min⁻¹ (column B) and 140.1 ml min⁻¹ (column E); mobile phase, THF; injection volume, 2.5 μL in split mode; detection wavelength, 214 nm; temperature, 25 °C; solutes, benzene, polystyrenes (Mw = 800, 4000, 12,000, 50,000, 90,000, 280,000 and 900,000).

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**Fig. 3.** FT-IR spectra of (a) DPEPA (crosslinker), (b) LMA (functional monomer) and (c) polymeric monolith.

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**Fig. 4.** Back pressure against flow rate for polymeric monolithic columns (column B and E). Experimental conditions: effective length of 18.0 cm × 75 μm i.d.; mobile phase, ACN.
hydrophobicity, indicating typical reversed-phase separation mechanism. The long carbon chain of LMA contributed to the hydrophobic property of poly(LMA-co-DPEPA) monolithic columns. It can be clearly observed that the separation of alkylbenzenes on column B was performed within 13 min (Fig. 5a), while the separation of alkylbenzenes on column E was finished within 9 min (Fig. 5b).

The column efficiencies of two poly(LMA-co-DPEPA) columns were evaluated with ACN/H2O (60/40, v/v) as the mobile phase by cLC. The curves of plate height changed over flow rate in the range of 50–230 μL min⁻¹ (before split) are depicted in Fig. 5c and d. The minimum plate height of highly crosslinked column B was in the range of 6.04–9.00 μm for alkylbenzenes, corresponding to 111,000–165,000 N m⁻¹. However, column B produced the minimum plate height of 10.75–20.04 μm for alkylbenzenes, corresponding to 50,000–93,000 N m⁻¹. A large amount of LMA-based polymeric monolithic columns had been prepared in the past decades, and ethylene dimethacrylate (EDMA) was commonly selected as crosslinker [3,32,34–39]. As one kind of polymer-based monolithic columns, the resulting poly (LMA-co-EDMA) produced low column efficiencies for small molecules in the isocratic elution, on which the highest column efficiency could only reach 53,000 N m⁻¹ for alkylbenzenes by cLC. However, in our case, the highly crosslinked poly(LMA-co-DPEPA) columns exhibited higher column efficiency than those of poly(LMA-co-EDMA) monolithic columns.

The values of A, B and C terms in van Deemter equation are listed in Table 2. A-term is the eddy diffusion term that is influenced by homogeneities of chromatographic bed. B-term value represents the longitudinal diffusion, which depends on analyte diffusion coefficients and decreases with the increasing molecular mass [40]. C-term is the mass transfer term in mobile and stationary phases. As shown in Table 2, the B-term and C-term values of column E are much larger than those of column B, while the A-term of column E was lower than column B. Compared with a C18-modified silica monolith, on which the A-term value was about 3.0 μm, the A-term values of columns B and E were all lower than 3.0 μm, likely benefiting from the homogeneous structure. The C-terms of columns B and E were also lower (<20.0 ms) than those of common methacrylate-based monoliths [41,42]. The lower C-term of column B ranged from 2.52 to 6.76 ms indicated a good communication between stationary phase and analytes. However, the C-term of column E was higher than that of column B, which ranged from 6.16 to 19.99 ms. The remarkable difference in column efficiency and C-terms was possibly related to the pore structure and specific surface area. Porous structure accelerates the rate of mass transfer as a result of obviously reduced convection [41], and the specific surface area is a good indication of the presence of mesopores as measured in the dry state [43], while the images of SEM could not accurately reflect the real microstructure in the dry state. The mesopores affect the mass transfer resistance, and hence the chromatographic separation efficiency and C-term. The column E (contains lower content of crosslinker) had a higher porosity of 57.7%, but it had lower specific surface area (19.8 m²g⁻¹) and lacked mesopores. Therefore, column E produced higher plate height (lower theoretical plates per meter) and C-terms. Contrary to column E, column B (contains higher content of crosslinker) had a lower total porosity of 45.2%, but larger specific surface area (27.4 m²g⁻¹) and much more mesopores, which facilitated the efficient separation of small molecules [22].

Traditional porous polymeric monoliths exhibit small specific surface area reaching only a few tens of m²g⁻¹ since they lack the mesopores. Nonetheless, Urban et al. [43] prepared

![Fig. 5. (a, b) Separation of alkylbenzenes on polymeric monolithic columns by cLC and (c, d) dependence of the plate height (H) of analytes on the linear velocity (u) of mobile phase on polymeric monolithic columns. Analytes: (1) thiourea, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene and (6) butylbenzene. Experimental conditions: columns, (a, c) column B and (b, d) column E; effective length of 18.0 cm × 75 μm i.d.; mobile phase, ACN/H2O (60/40, v/v); flow rates for (a, b), 170 μL min⁻¹ (before split); detection wavelength, 214 nm.](https://example.com/fig5.png)

| Table 2 | Fitted values of A, B and C terms in van Deemter equation: \(H = A + \frac{B}{u} + Cu\). |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Analytes       | Column B       | Column E       |                |                |                |
|                | A (μm)         | B (μm²s⁻¹)     | C (ms)         | A (μm)         | B (μm²s⁻¹)     | C (ms)         |
| Benzen         | 2.40           | 1780           | 6.76           | 13.92          | 12500          | 19.99          |
| Toluene        | 2.85           | 1420           | 5.67           | 7.50           | 11000          | 14.55          |
| Ethylbenzene   | 2.13           | 1490           | 5.60           | 4.76           | 8990           | 11.98          |
| Propylbenzene  | 2.93           | 1240           | 3.46           | 0.31           | 5810           | 7.89           |
| Butylbenzene   | 0.39           | 1720           | 2.52           | 0.44           | 4450           | 6.16           |
hyper-crosslinked polymeric monoliths with large surface area (more than 600 m² g⁻¹ in dry state), showing high column efficiency for small molecules. It demonstrated that a large number of mesopores and micropores were existent. However, the specific surface areas of poly(LMA-co-DPEPA) monolith were only tens of m² g⁻¹, indicating the lack of micropores in the dry state.

As shown in Fig 5, it is also observed that the efficiencies for the strong-retained compounds (such as butylbenzene) were significantly higher than those of weak-retained compounds (such as benzene). The results demonstrated that poly(LMA-co-DPEPA) monolith revealed a retention-independent efficient performance of small molecules in the isocratic elution. As shown in Table 2, it was apparent that the C-terms of butylbenzene were lower than those of benzene on both column B and column E. This low mass transfer resistance is possibly caused by the lack of micropores. Therefore, it can be deduced that the employ of multi-functional crossliner prevents polymeric monolith from generation of gel-like micropores, which reduced the permeation of small molecules in the gel-like structure and the mass transfer resistance in the polymeric monolith [44].

The effect of ACN content on the retention factor (k) of five alkylbenzenes is shown in Fig. 6a and b. It could be found that the retention factors of five alkylbenzenes (thiourea as the void time maker) on two columns B and E decreased with an increase of ACN

Fig. 6. Relationship between retention factor (k) of alkylbenzenes and ACN content on polymeric monolithic (a) column B and (b) column E; dependence of log k on the number of carbon in alkyl chain on (c) column B and (d) column E. Experimental conditions: effective length of 18.0 cm × 75 μm i.d.; flow rate, 140 μL min⁻¹ (before split).

Fig. 7. Separations of (a and b) phenols and (c and d) basic compounds on polymeric monolithic (a and c) column B and (b and d) column E by cLC, respectively. Analytes: (a, b) (1) phloroglucinol, (2) pyrocatechol, (3) para-cresol, (4) 2,6-dimethyl phenol and (5) 2,4-dichlorophenol; (c, d) (1) caffeine, (2) carbamazepine, (3) 2,4-dinitroaniline, (4) 4-aminobiphenyl and (5) 2,6-dichloro-4-nitroaniline. Experimental conditions: effective length of 18.0 cm × 75 μm i.d.; flow rates, 140.1 μL min⁻¹ for (a, b) and 150 μL min⁻¹ for (c, d) (before split); mobile phases, ACN/H₂O (35/65, v/v) for (a, b) and ACN/H₂O (40/60, v/v) for (c, d); detection wavelength, 214 nm.
content from 45% to 70%. The trend followed the principle of reversed-phase chromatography for alkylbenzenes on the two polymeric monolithic columns. The logarithm of the retention factors linearly decreased with an increase of ACN content. The hydrophobicity of polymeric monolithic columns could be characterized by the methylene selectivity ($\alpha_{CH_2}$) using the following equation [45]:

$$\log k = n \log \alpha_{CH_2} + \log \beta,$$

where $n$ is the carbon number of alkylbenzenes (−CH$_2$−), and $\log \alpha_{CH_2}$ and $\log \beta$ are constants for a given homologous series and chromatographic system ($\beta$ is the retention factor of benzene). As shown in Fig. 6c and d, the slopes of the lines represented the logarithmic methylene selectivity of column B (the linear relationship $R > 0.996$), giving the $\alpha_{CH_2}$ values of 1.61, 1.52, 1.45, 1.40, 1.35 and 1.32 at 45, 50, 55, 60, 65 and 70% ACN in mobile phases, respectively. Meanwhile, the $\alpha_{CH_2}$ values of column E were also calculated to be 1.67, 1.57, 1.51, 1.45, 1.40 and 1.36 at 45, 50, 55, 60, 65 and 70% ACN in mobile phases, respectively. The values of column E were slightly higher than those of column B, illustrating that increasing LMA content (decreasing the crosslinker content) would enhance the hydrophobicity of the resulting polymeric monolith.

3.3. Application of polymeric monolithic columns

For further research on the selectivity of the poly(LMA-co-DPEPA) monolithic columns, the phenols and basic compounds were separated on both column B and column E by cLC. The results are shown in Fig. 7. As presented in Fig. 7a and b, baseline separation of five phenols on column B was performed within 20 min by using ACN/0.1% CH$_3$COOH (95/5, v/v) as the mobile phase, and the highest column efficiency for 2,6-dimethyl phenol was calculated at 86,000 N m$^{-1}$. Comparing with column B, the separation of these phenols on column E was also performed within 13 min, and the column efficiency for 2,6-dimethyl phenol was only 52,000 N m$^{-1}$. Similarly, 5 basic compounds were baseline-separated on two poly(LMA-co-DPEPA) monolithic columns, as shown in Fig. 7c and d. The separation of basic compounds on column B was completed within 25 min, exhibiting the column efficiencies of 39,000–67,000 N m$^{-1}$. Comparing with column B, the relative fast separation was obtained on column E within 15 min, and the column efficiencies were decreased in the range of 19,000–52,000 N m$^{-1}$. Although the column efficiencies for the phenols and basic compounds on column E were lower than those on column B, the faster separation was obtained on column E as expected. Column E was also selected to separate intact proteins by cLC with gradient elution, as shown in Fig. 8. Five intact proteins were well separated within 16 min. These results showed that potential application of such highly crosslinked polymeric monolithic columns in different chromatographic conditions for both large molecules and small molecules.

4. Conclusions

A novel highly crosslinked poly(LMA-co-DPEPA) monolithic column has been successfully synthesized by using DPEPA as crosslinker, which has multiple acrylate groups to manufacture highly crosslinked monolithic columns. Since the use of multifunctional groups crosslinker could protect polymer monolith from forming gel-like micropores and the absence of gel-like micropores reduced the mass transfer resistance, the poly(LMA-co-DPEPA) monolith exhibited the retention-independent efficient performance in the separation of small molecules. Therefore, it is deduced that the multi-functional crosslinker could facilitate to improve the chromatographic separation efficiency for small molecules in cLC. Meanwhile, various kinds of functional monomers could be copolymerized with multi-functional crosslinker reagents like DPEPA to fabricate a myriad of polymeric monoliths for different chromatographic modes, such as reversed-phase liquid chromatography (RPLC), hydrophilic interaction chromatography (HILIC) and ion-exchange chromatography (IEC).

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