Rapid “one-pot” preparation of polymeric monolith via photo-initiated thiol-acrylate polymerization for capillary liquid chromatography

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Highlight:
- A highly crosslinked polymeric monolith was fast prepared with a multi-acrylate monomer as crosslinker within 5 min.
- The addition of thiol eliminated the major obstacle of oxygen inhibition in the thiol-ene (acrylate/methacrylate) polymerization.
- Poly(ODT-co-DPEPA) monolith exhibited efficient chromatographic performance for separation of alkylbenzenes and tryptic digest of proteins.

Abstract:
A facile approach was exploited for fast preparation of polymer-based monoliths in UV-transparent fused-silica capillaries via “one-pot” photo-initiated thiol-acrylate polymerization reaction of dipentaerythritolpenta-/hexaacrylate (DPEPA) and 1-octadecanethiol (ODT) in the presence of porogenic solvents (1-butanol and ethylene glycol). Due to relative insensitivity of oxygen inhibition in thiol-ene free-radical polymerization, the polymerization could be performed within 5 min. The effects of composition of prepolymerization solution on the morphology and permeability of poly(ODT-co-DPEPA) monoliths were investigated in detail by adjusting the content of monomer and binary porogen ratio. The physical properties of poly(ODT-co-DPEPA) monoliths were characterized by Fourier transform infrared spectroscopy (FT-IR), mercury intrusion porosimetry (MIP) and nitrogen adsorption/desorption measurement. The evaluation of chromatographic performance was carried out by capillary liquid chromatography (cLC). The results indicated that the poly(ODT-co-DPEPA) monolith was homogeneous and permeable, and also possessed a typical reversed-phase retention mechanism in cLC with high efficiency.
1. Introduction

Monolithic column, also called as rod column and continuous bed, has emerged since the late 1980s and early 1990s, which was generally classified into three categories, silica-based monoliths, organic-inorganic hybrid monoliths as well as polymer-based monoliths [1–3]. Macroporous organic monoliths have developed rapidly as a major chromatographic technology over the past decades, due to several advantages over both silica-based monoliths and organic-inorganic hybrid monoliths [4]. For example, the most obvious superiorities of polymer-based monoliths are fast throughput separation on account of the presence of macropores, low backpressure, wide application range of pH and ease of fabrication with a single step of in situ synthesis [5–7]. Another well-known point, polymer-based monoliths can be used for a variety of chromatographic mode through thermal or photo-initiated modes [13,14]. The latter was a good alternative as various polymer-based monoliths could be rapid prepared in a specific position of a UV-transparent capillary or a microchannel [15,16].

Although polymer-based monoliths were still widely applied into bioseparation of macromolecules and sample pretreatment, they usually exhibit relatively low mechanical stability and low column efficiency for small molecules, which also limits their application for proteomics, metabolomics and so on [10,17–19]. In order to enhance column efficiency for the isocratic separation of small molecules, several attempts have been made to achieve large surface areas by introducing nanoparticles, increasing the content of functional group, or preparing hypercross-linked monolith [5,19,20]. Thiol–ene click chemistry has widely emerged as a useful tool in the preparation of chromatographic stationary phases as a result of its excellent specificity, unrivaled efficiency and high insensitivity to oxygen or water [21–23]. What counts is that additional transition metal catalysts are not necessary in the thiol–ene reaction [24]. Over the past decade, the relative reactivity, controlling, and specificity of thiol–ene reaction have been considered [25]. However, there are only a few papers on directly preparing porous monoliths and functionalizing chromatographic media via thiol–ene polymerization reaction. Nischang’s group has prepared several hybrid monolithic materials employing polyhedral oligomeric vinylsilsequioxanes (vinylPOSS) and multifunctional thiols via radical-mediated thiol–ene click polymerization [26]. Our group successfully synthesized a highly crosslinked hybrid monolith with a multi-acrylate monomer (acyrlopophyly polyhedral oligomeric silsesquioxanes) as crosslinker via photo-initiated thiol-acrylate polymerization within 5 min [27]. These inspired us to prepare highly efficient organic monolith using radical-mediated thiol–ene click polymerization reaction.

More recently, our group prepared a porous organic monolith by using pentaerythritol diacrylate monostearate (PEDAS) and trimethylolpropane tris(3-mercaptopropionate) (TPTM) via photo-initiated thiol-acrylate polymerization, in which the acrylate homopolymerized and copolymerized with the thiol simultaneously [28]. The morphology of poly(PEDAS-co-TPTM) monolith was remarkably changed by comparing with that of poly(PEDAS) monolith, contributing to great improvement of both permeability and separation efficiency for small molecules in cLC. Herein, we further prepared a porous organic monolith via “one-pot” photo-initiated thiol-acrylate polymerization using dipentaerythritolpenta-/hexaacrylate (DPEPA) and 1-octadecanethiol (ODT) (Scheme 1) as the precursors. The resulting poly(ODT-co-DPEPA) monolith was characterized and evaluated in cLC, and also applied for biological sample analysis with cLC-MS.

2. Experimental

2.1. Chemicals and materials

DPEPA, ODT (C_{18}H_{37}SH), 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS), 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-phenylacetophenone (DMPA) was purchased from Acros Organics (New Jersey, USA). Tetrahydrofuran (THF), methanol and acetonitrile (ACN) were HPLC-grade and acquired from Yuwang Group (Shandong, China). Lysozyme (chicken egg white), bovine serum albumin (BSA), myoglobin (horse heart), ovalbumin and α-casein were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Sino-American Biotechnology Corporation (Beijing, China). Thiourea, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, 1-butanol and ethylene glycol were of analytical grade, and obtained from Tianjin Kermel Chemical Plant (Tianjin, China). Deionized water was prepared with a Milli-Q system (Millipore, MA, USA). The flexible fused-silica capillary (UV-transparent coating) with inner diameter of 75 μm was purchased from Reafine Chromatography Ltd. (Hebei, China). HiTrap DEAE Sepharose FF (5 mL) was purchased from GE Healthcare Bio-Sciences AB.

2.2. Preparation of polymeric monolith via photo-initiated polymerization

Prior to preparing a polymeric monolithic column, the inner surface of the capillary was rinsed with 0.1 mol L⁻¹ NaOH (2 h), then flushed with water, followed by 0.1 mol L⁻¹ HCl (2 h), water (1 h) and methanol (2 h) successively, according to our previous report [29]. Then the capillary was adequately filled with γ-MAPS-methanol solution (50/50, v/v), and both ends were sealed by rubbers and placed in the water bath at 50°C for 12 h. Finally, the capillary was washed with methanol to flush out residual reagents and dried under a stream of nitrogen flowing, as described in a previous report [30].

The prepolymerization solution was prepared by dissolving appropriate amounts of DPEPA (30 mg), ODT (0–20 mg) and DMPA (1 μL, 16%, w/w, DMPA/1-butanol) in binary porogenic solvents, which consisted of 1-butanol (110–125 μL) and ethylene glycol (15–30 μL) (as listed in Table 1). Then the mixture was sonicated for 5 min to form a homogeneous solution. Whereafter the prepolymerization solution was introduced into the pretreated capillary with a syringe. With both ends sealed
with silicon rubbers, the capillary was placed in a UV-curing instrument (XL-1500A, λ = 365 nm, Spectronics Corporation, New York, USA) for 300 s. After irradiation, the monolith was washed with methanol to remove the residual reagents. Finally, both ends of the monolith were kept in the water for further usage. At the same time, the remaining prepolymerization solution in the centrifuge tube was also polymerized under UV exposure to form bulk monolithic material. Bulk monolithic material was cut into small pieces, ground using mortar and pestle, then flushed with ethanol three times, in the end, dried in a vacuum at 50 °C for one day.

2.3. Instruments and methods

Fourier-transformed infrared spectroscopy (FT-IR) characterization was carried out on Thermo Nicolet 380 spectrometer with KBr pellets (Nicolet, Wisconsin, USA). SEM images were obtained by using a JEM JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). Pore size distribution was measured by mercury intrusion porosimetry (MIP) on a PoreMaster GT-60 (Quantachrome, Boynton Beach, U.S.A.). 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, U.S.A.). Amygdalus pedunculata Pall. protein was purified by AKTAprime plus (GE Healthcare, U.S.A.). Tryptic digest of proteins samples were analyzed by an LTQ-Orbitrap XL mass spectrometer (Thermo Finnigan, San Francisco, CA, USA).

The permeability (B0) can be calculated according to Darcy’s law by the equation \( B_0 = \frac{F L}{(\eta r^2 \Delta P)} \), where \( F \) (m² s⁻¹) is the flow rate of mobile phase, \( \eta \) is the viscosity of mobile phase (0.38 \times 10⁻² Pa s for ACN), \( L \) and \( r \) (m) are effective length and inner radius of the column, respectively, \( \Delta P \) (Pa) is the pressure drop across the column. The data of \( \Delta P \) and \( F \) were obtained on an ACQUITY Ultra-Performance LC (Waters, USA). The flow rates of mobile phase were set at 0.1–1.5 μL min⁻¹.

The cLC experiments were performed on LC system combining with an Agilent 1100 micropump, a 7725i injector with a 20 μL sample loop, and a K-2501 UV detector (Knauer, Berlin, Germany). UV detector wavelength was set at 214 nm. The mobile phase was composed of ACN/water. Samples were injected through an injection valve with an internal 2 μL sample loop. A tee-piece was used as a splitter, one end of the monolithic column was connected with the tee-piece, and the other end was connected to a 15 cm long blank capillary (50 μm i.d.). The outlet of the monolithic columns was connected with a Teflon tube to an empty fused-silica capillary (50 μm i.d.), where a detection window was made by removing a 2 mm length of the polyimide coating in a position of 5.5 cm from the separation monolithic column outlet. The retention factor (k) was defined as \( (t_r-t_0)/t_0 \), where \( t_r \) and \( t_0 \) represent the retention times of the analytes and the void time marker, respectively. The actual flow rate through monolithic column was calculated by collecting the mobile phase with a centrifuge tube for 20 min, and then measuring its weight. The chromatographic data were collected at room temperature and analyzed using the software program HW-2000 from Qianpu Software (Shanghai, China).

2.4. Preparation of the tryptic digest of proteins and cLC-MS/MS analysis

Amygdalus pedunculata Pall. is a deciduous shrub of Rosaceae and Amygdalus. Mature seeds of Amygdalus pedunculata Pall. was collected from shrubs at Yulin, Shaanxi Province, China. After peeling shell and crushing, soft kernels were homogenized with phosphate buffer solution (PBS), and then stored under 4 °C. The slurry was centrifuged at 4000 r min⁻¹ for 30 min to remove the upper fat and precipitation. The crude solution was fractionated by 40–60% (w/w) saturation of \((\text{NH}_4)_2\text{SO}_4\), placed in 4 °C refrigerator after 3 h, then centrifuged at 10000 r min⁻¹ for 20 min, the sediment was desalted by dialysis. The extract was separated by ion exchange chromatography through 500 μL sample loop, using HiTrap DEAE Sepharose FF (5 mL) installed on AKTAprime plus. Mobile phase A was 0.02 mol L⁻¹ PBS (pH 7.4), and mobile phase B

<table>
<thead>
<tr>
<th>Column</th>
<th>ODT (mg)</th>
<th>ODT content in total monomers (w/w, %)</th>
<th>1-Butanol (μL)</th>
<th>Ethylene glycol (μL)</th>
<th>Permeability (10⁻⁴ m²)</th>
</tr>
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<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>30</td>
<td>0.36</td>
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<tr>
<td>II</td>
<td>8</td>
<td>21.1</td>
<td>110</td>
<td>30</td>
<td>1.42</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>21.1</td>
<td>125</td>
<td>15</td>
<td>0.77</td>
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<tr>
<td>IV</td>
<td>12</td>
<td>28.6</td>
<td>110</td>
<td>30</td>
<td>2.05</td>
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<tr>
<td>V</td>
<td>16</td>
<td>34.8</td>
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<td>16</td>
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<td>VII</td>
<td>16</td>
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<td>VIII</td>
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Table 1. Detailed composition of polymerization mixtures and the permeability of polymeric monolithic columns.

The prepolymerization mixture contains the monomer of 30 mg DPEPA and the photoinitiator of 1.0 μL 10% DMPA (w/w, DMPA/1-butanol).
was 0.02 mol L\(^{-1}\) PBS containing 0.8 mol L\(^{-1}\) NaCl (pH 7.4). Flow rate was set to 1.0 mL min\(^{-1}\). A stepwise gradient was developed by consisting of 0–15 min at 0% mobile phase B, 15–40 min at 15% mobile phase B, 40–65 min at 25% mobile phase B, 65–90 min at 35% mobile phase B. Four fractions (0% mobile phase B, 15% mobile phase B, 25% mobile phase B and 35% mobile phase B) were collected. After ultrafiltration, concentration and freeze drying of target fraction (15% mobile phase B), the protein powder was collected and named Amygdalus pedunculata Pall. proteins.

The tryptic digestions of four standard proteins (BSA, myoglobin, ovalbumin and \(\alpha\)-casein) and Amygdalus pedunculata Pall. proteins and the cLC-MS/MS analysis were performed according to the procedures previously reported by us with minor modification [21]. The sample trapping was achieved with a homemade C18-particle-packed trap column (4.0 cm in length \times 200 \mu m i.d.), and the subsequent separation was carried out on a polymeric monolithic column (30.0 cm in length \times 75 \mu m i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of capillary.

2.5. Database searching

The RAW files obtained by Xcalibur 2.1 were converted to *.MGF by Proteome Discoverer (v1.2.0.208, Thermo, San Jose, CA) and searched with Mascot (version 2.3.0, Matrix Science, London, UK). The BSA, myoglobin, ovalbumin, \(\alpha\)-casein and Amygdalus pedunculata Pall. proteins and the cLC-MS/MS analysis were performed according to the procedures previously reported by us with minor modification [21]. The sample trapping was achieved with a homemade C18-particle-packed trap column (4.0 cm in length \times 200 \mu m i.d.), and the subsequent separation was carried out on a polymeric monolithic column (30.0 cm in length \times 75 \mu m i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of capillary.

3. Results and discussion

3.1. Optimization of preparation conditions of polymeric monolith

The approach of “one-pot” thiol-acrylate polymerization reaction was successfully developed to fabricate the POSS-containing hybrid monoliths, in which the acrylates not only homopolymerized, but also coupled with the thiol [27]. In this case, we tried to prepare a polymeric monolith by this approach. Through many experiments for the selection of monomers and porogenic systems, such as n-propanol/ethylene glycol, n-hexanol/ethylene glycol and so on, we eventually chose DPEPA, which has 5 or 6 acrylate groups, as crosslinker, ODT as functional monomer, and the mixture of 1-butanol and ethylene glycol as porogenic solvents to prepare the polymeric monolithic columns, as illustrated in Scheme 1. Similarly, the amount of ODT had effect on the polymerization rate of DPEPA, even affecting the phase separation. It was also observed that the white solid emerged in the vial after 35 s UV irradiation for preparation of bulk poly(DPEPA) monolith without adding ODT (column I, Table 1), indicating the occurrence of phase separation. However, when 8 mg ODT was added in the prepolymerization solution (column II), the phase separation would emerge after UV irradiation for 15 s. We also tried to add more ODT (20 mg) into the prepolymerization solution to fabricate poly(ODT-co-DPEPA) monolith (column IX), but the prepolymerization solution could not be introduced into capillary after the operation of sonication to form a homogeneous solution, possibly due to the emergence of thiol-acrylate polymerization reaction in the room even without any UV irradiation. This phenomenon indicated that the formation of thioether bond could make a significant contribution to eliminate oxygen inhibition in thiol-ene photo-initiated free-radical polymerization [31,32]. From the viewpoint of reaction mechanism, as for column I, the homopolymerization of DPEPA occurred without adding ODT. As for column II, the molar ratio of thiol/acrylate was about 1/12 when adding 8 mg ODT. It could be deduced that a part of DPEPA homopolymerized, and the rest of DPEPA would take part in coupling with ODT. When the amount of ODT increased to 20 mg (column IX) (molar ratio of thiol/acrylate, 1/5), it could be deduced that more alkyl thiols were convert to thiol radicals which participated in thiol-acrylate click reaction with increasing the amount of ODT, thereby less DPEPA self-homopolymerized and finally accelerated the phase separation. This phenomenon has been observed in thiol-ene photopolymerization, as the chain-transfer hydrogen-abstraction process is the slow step for thiol—acrylate, thiol/allyl ether and thiol-alkene copolymerizations [32].

It is well-known that the composition of porogens had a great effect on the porous morphology and permeability of polymeric monolithic columns, which was investigated in detail. As shown in Table 1, a series of polymeric monolithic columns were synthesized with different contents of monomer. Concerning about columns I, II, IV and V, which were prepared with the same porogenic solvents (as shown in Table 1), the permeability remarkably increased from 0.36 to 2.27 \times 10^{-14} m^{2}/s when the content of ODT (in the total monomers, w/w%) increased from 0 to 34.8% (w/w%). The results demonstrated that more ODT reacted with acrylate group of DPEPA and decreased the amount of acrylate group to participate in the homopolymerization, which concomitantly affected the crosslinking of microporous structure. It was also proved that the content of crosslinker is another important factor affecting the porous morphology and permeability. Furthermore, it could be observed that the permeability of columns V, VI, VII and VIII decreased from 2.27 to 1.65 \times 10^{-14} m^{2}/s with an increase of 1-butanol content in porogenic system, which were fabricated with the same content of ODT. So it is concluded that 1-butanol served as the microporogenic solvent (good solvent) in porogenic system, while ethylene glycol served as macroporogenic solvent (poor solvent) in porogenic system.

Considering both morphology and permeability, we finally selected columns III and VIII for further cLC experiments. The reproducibility of polymeric monolith was evaluated through the relative standard deviation (RSD) for the retention factor (k) of tolune as model analyte (thiourea as the marker of void time). The RSDs of run-to-run, column-to-column and batch-to-batch reproducibility were 0.67%, 2.89%, 5.57% (column III, \(n\) = 5) and 0.26%, 3.00%, 7.89% (column VIII, \(n\) = 5), respectively. All results indicated the good reproducibility of poly(ODT-co-DPEPA) monolith.

3.2. Characterization of polymeric monoliths

SEM images (Fig. 1) showed the morphology of poly(ODT-co-DPEPA) monolithic columns (III and VIII). It could be observed that monolithic matrix tightly attached to the inner walls of capillaries without any disconnection. Meanwhile, these two monoliths contained a few macro pores around 1 \mu m, which could permit rapid flow through the column and provide low back pressure. Fig. 2 presented the pore size distribution for poly(ODT-co-DPEPA) monoliths by mercury intrusion porosimetry, further indicating the existence of macropores (>1 \mu m diameter).

Subsequently, the specific surface areas of poly(ODT-co-DPEPA) monoliths were calculated as 12.1 (column III) and 11.1 (column VIII) m^{2}/g on the basis of nitrogen adsorption/desorption isotherm (BET), respectively, which is a good indication of the absence of mesopores as measured in the dry state [33]. These values did not exceed those of typical hybrid monolithic columns, partly implying a rich of macropores and lack of micropores in two monoliths [27,34]. The porosity of two monoliths was provided by size exclusion chromatography in Fig. 3. The total porosity and the
The mechanical stability was calculated via the backpressure measurement by connecting a 20 cm × 75 μm i.d. polymeric monolith to a NanoLC pump using ACN as the mobile phase, and the result was shown in Fig. S1 (Supporting Information). Linear relationships of polymeric monolithic columns (column III and VIII) between back pressure and flow rate ($R_1 = 0.995$ and $R_2 = 0.999$) clearly implied that the poly(ODT-co-DPEPA) monoliths possessed good mechanical stability.

Fig. 4 shows the FT-IR spectrum of DPEPA crosslinker, ODT external porosity of column III were measured at 69.1% and 50.2%, respectively, while the total porosity and the external porosity of column VIII were larger, and reached 75.6% and 59.6%. Besides, the large porosity would lead to a high permeability (e.g. 1.65 × 10^{-14} m² for column VIII).

The mechanical stability was calculated via the backpressure measurement by connecting an 20 cm × 75 μm i.d. polymeric monolith to a NanoLC pump using ACN as the mobile phase, and the result was shown in Fig. S1 (Supporting Information). Linear relationships of polymeric monolithic columns (column III and VIII) between back pressure and flow rate ($R_1 = 0.995$ and $R_2 = 0.999$) clearly implied that the poly(ODT-co-DPEPA) monoliths possessed good mechanical stability.

Fig. 4 shows the FT-IR spectrum of DPEPA crosslinker, ODT
monomer, monoliths III and VIII, respectively. As shown in Fig. 4a, absorbance peaks emerged at 1100–1200 cm\(^{-1}\) due to the presence of C—O—C bond, and the apparent strong peaks observed at 1700–1750 cm\(^{-1}\) was assigned to the stretching vibration of C=O bond, while the broad band at 1500–1675 cm\(^{-1}\) was the stretching vibration of C=C bond. The peak signals at 1730 cm\(^{-1}\) implied the presence of \(\alpha,\beta\)-unsaturated C=O bond in the DPEPA, while the peak signal at 1730 cm\(^{-1}\) was changed to 1739 cm\(^{-1}\) (monolith III, Fig. 4c) and 1741 cm\(^{-1}\) (monolith VIII, Fig. 4d). Meanwhile, the intensity of the peak at 1635 cm\(^{-1}\) (C=C bond) was remarkably decreased in poly(ODT-co-DPEPA) monolith (Fig. 4c and d). Moreover, there is insignificant difference between the FT-IR spectra of monoliths III and VIII. These results indicated that free-radical polymerization reaction successfully took place, but a portion of unreacted C=C bonds still existed.

3.3. Chromatographic evaluation of poly(ODT-co-DPEPA) monoliths in cLC

The chromatographic assessment of two monoliths (columns III and VIII) was investigated by cLC for the separation of alkylbenzenes as probes. Fig. 5a and b showed that the baseline separation of 5 alkylbenzenes were obtained on columns III and VIII by using ACN/H\(_2\)O (60/40, v/v) as the mobile phase at the flow rate of 160 and 200 \(\mu\)L min\(^{-1}\) (before split), respectively. The actual flow rate after splitting was 230 nL min\(^{-1}\) (column III) and 440 nL min\(^{-1}\) (column VIII), respectively. Five alkylbenzenes were eluted in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene according to the hydrophobicity, indicating typical reversed-phase mode in cLC. Although all peaks were successfully baseline-separated with narrow peak widths, the peaks were slightly tailing. One reason for this phenomenon is related to the nonuniform microstructure of monoliths. To some extent, another reason was possibly related to the off-column detection mode in our case. There was a 5-cm-long empty fused-silica capillary between the poly(ODT-co-DPEPA) monolith and detection window, which produced large dead volume and further caused the peak tailing.

The relationships between the flow rate from 40 to 220 \(\mu\)L min\(^{-1}\) (before split) and the plate height for alkylbenzenes on two poly(ODT-co-DPEPA) columns were shown in Fig. 5c and d. The highest column efficiencies of two columns reached 62,000–75,000 N m\(^{-1}\) at a velocity of 0.35 mm s\(^{-1}\) and 56,000–61,000 N m\(^{-1}\) at a velocity of 0.56 mm/s, respectively, corresponding to 13.39–16.08 \(\mu\)m and 16.32–17.66 \(\mu\)m of plate height. Comparing the chromatographic efficiency, there was no significant difference between two columns at relatively low velocity. Due to the limitation of the maximum backpressure for our LC system, the maximum velocity for column VIII could reach 1.57 mm/s, which was great higher than that of column III (0.97 mm/s). What is more, column VIII exhibited higher permeability than column III. It is implied that column VIII could make relatively high rapid and efficient separation of small molecules.

The effect of the ratio of ACN/water on retention factors \((k)\) of alkylbenzenes was depicted in Fig. S2a and b. It showed that the \(k\) values decreased following the increase of ACN content ranging from 50 to 75% as expected, revealing typical reversed-phase retention mechanism on poly(ODT-co-DPEPA) columns. The logaiithm of the retention factors linearly increased with an increase of the number of alkyl groups. Therefore, the hydrophobicity of polymeric monolithic columns could be characterized using the following equation [35,36]:

\[
\log k = \log a + b[\text{ACN}] + \log \beta,
\]

where \(n\) is carbon number of homologous compounds, \(\beta\) is retention factor surmised to \(n = 0\), and the methylene selectivity \((a_{\text{CH2}})\) usually characterizes the hydrophobicity of a given chromatographic system. The \(a_{\text{CH2}}\) values of two poly(ODT-co-DPEPA) columns were calculated under 50%, 55%, 60%, 65%, 70% and 75% ACN in the mobile phase, namely of 1.41, 1.36, 1.30, 1.26, 1.24 and 1.21 (column III), 1.48, 1.41, 1.36, 1.33, 1.29 and 1.27 (column VIII), respectively. The column VIII had higher methylene selectivity value \((a_{\text{CH2}})\) and stronger hydrophobic property, demonstrating that the amount of ODT could effect on the hydrophobicity of poly(ODT-co-DPEPA) monolithic columns.

The van Deemter curves for poly(ODT-co-DPEPA) monolithic columns vividly reflected their distinction in column efficiency, morphology and pore size distribution. According to van Deemter equation: \(H = A + B/u + Cu\), where \(u\) is the mean velocity of the mobile phase, \(A\)-term represents the eddy diffusion term which depends on homogeneities of chromatographic bed, \(B\)-term is longitudinal dispersion coefficient decreasing with the increase of molecular mass, \(C\)-term is defined as resistance to mass transfer between the mobile and stationary phases [37–39]. The value of \(A\), \(B\) and \(C\) terms were listed in Table 2. Herein, the values of the \(A\) terms for two monolith were much smaller than those of traditional organic monoliths [40] and silica-based monolith [41], implying the limit of peak broadening and eddy diffusion. It could be found that the values of \(A\)-term for column III were much smaller than those of column VIII, which slightly increased from 2.063 to 3.577 \(\mu\)m with an increase of hydrophobicity of alkylbenzenes, indicating a small eddy diffusion and presumably profiting by smaller skeleton size. Compared to those methacrylate ester-based monoliths, two poly(ODT-co-DPEPA) monolithic columns had relatively smaller \(C\)-term, indicating a good communication between stationary phase and analytes [37,42,43]. However, column III showed a little larger \(C\)-term and lower permeability (0.77 \(\times\) 10\(^{-14}\) m\(^2\)) comparing with column VIII, implying a slower mass transfer. As a result of these effects, column III exhibited a slightly higher chromatographic efficiency in separating of small molecules. It can be deduced that the skeleton size and through-pore/framework size ratio have great influence on the efficient separation of small molecules.

It can also be observed from Fig. 5c and d that the efficiency for the strong-retained compounds (such as butylbenzene) was remarkably higher than those of weak-retained compounds (such as benzene), demonstrating a retention-independent efficient performance of small molecules in the isocratic elution. As shown in Table 2, it could be seen that the \(C\)-terms of butylbenzene were lower than those of benzene on two organic monoliths. These low mass transfer resistance were possibly ascribed to low specific surface area of two organic monoliths and lack of micropores. It can...
be deduced that using multifunctional cross-linker (DPEPA) prevents the organic monoliths from generating gel-like micropores via free-radical polymerization, which could reduce the permeation of small molecules in the gel-like structure and the mass transfer resistance, and further improved the chromatographic efficiency of the strongly retained compounds [44].

Porous structure is helpful to accelerate the mass transfer rate on account of remarkably reduced convection [37] and facilitates the efficient separation of small molecules. Considering the above analysis, column VIII (containing higher content of ODT) had a higher permeability (1.65 \( /C \times 10^{14} \text{ m}^2 \text{s}^{-1} \)) and a larger external porosity (59.6%), and produced rapid separation with a good column efficiencies. Hence, we chose column VIII for further separation of the tryptic digest of four standard proteins and complex plant proteins.

### 3.4. Application of polymeric monolithic columns

In order to further characterize the performance of poly(ODT-co-DPEPA) monolithic columns, the tryptic digest of four standard proteins (BSA, myoglobin, ovalbumin, and \( \alpha \)-casein) was separated on the column VIII under reversed-phase mode by cLC-MS, and the chromatogram was shown in Fig. 6a. Based upon the database search, 78 unique peptides were positively identified with high protein sequence coverage of 72.5%, 72.7%, 55.7% and 37.4%, respectively. Comparing with a C18 packed column, the chromatogram was illustrated in Fig. S3. Based on the database search of the chromatogram, 76 unique peptides were positively identified with protein sequence coverages of 75.8%, 57.1%, 28.8% and 33.6%, respectively. The separation performance on the column VIII was comparable to the C18 packed column, indicating a good separation ability of poly(ODT-co-DPEPA) monolith.

Amygdalus pedunculata Pall. is a type of sand dune-stabilizing and oil-bearing shrubs. Due to its cold and drought tolerance, deep root system, strong adaptability to a wide range of soil types and soil moisture conditions, it could be widely used as a good candidate for desert reclamation. At present, several high value-added products has been produced from Amygdalus pedunculata Pall., such as biodiesel and activated carbons [45,46]. Our research would provide the experimental basis for separation and purification of Amygdalus pedunculata Pall. proteins, which could not only improve high value utilization of Amygdalus pedunculata Pall., but also broaden the plant protein sources of raw materials. Herein, we made the first attempt to identify the proteins extracted from Amygdalus pedunculata Pall. seed by cLC-MS/MS. As shown in Fig. 6b, Amygdalus pedunculata Pall. protein sample was also digested and analyzed on column VIII by cLC-MS as described above. After controlling the FDR <1% for identification, the numbers of unique proteins and peptides were 91 and 333, respectively.

### Table 2

<table>
<thead>
<tr>
<th>analytes</th>
<th>Column III</th>
<th>Column VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/\mu m</td>
<td>B/(10^1 \mu m^2 s^-1)</td>
</tr>
<tr>
<td>benzene</td>
<td>2.063</td>
<td>2.496</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.308</td>
<td>2.349</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>2.178</td>
<td>2.122</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>2.733</td>
<td>1.913</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>3.577</td>
<td>1.580</td>
</tr>
</tbody>
</table>

### 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: (a, c) column III and (b, d) column VIII; column dimension (a, c) 19.0 cm x 75 \( \mu \text{m} \) i.d., (b, d) 20.5 cm x 75 \( \mu \text{m} \) i.d.; mobile phase, ACN/H2O (60/40, v/v); flow rate (a) 160 \( \mu \text{L} \text{min}^{-1} \) (before split), 230 \( \mu \text{L} \text{min}^{-1} \) (actual flow rates), (b) 200 \( \mu \text{L} \text{min}^{-1} \) (before split), 440 \( \mu \text{L} \text{min}^{-1} \) (actual flow rates); detection wavelength, 214 nm.
polymerization may have good designability and expansibility in terms of alkene monomers and thiol compounds are available, thiol-ene polymerization. As a great deal of different kinds of alkene-containing functional monomer. Due to the addition of thiol into merely self-polymerized, but also coupled with the thiol–acrylate polymerization, the major obstacle of oxygen inhibition is essentially eliminated in the thiol-ene (acrylate/methacrylate) polymerization. During this process, the crosslinker (DPEPA), which has multiple acrylate groups, not merely self-polymerized, but also coupled with the thiol-containing functional monomer. Due to the addition of thiol into the “one-pot” thiol-acrylate polymerization, the major obstacle of oxygen inhibition is essentially eliminated in the thiol-ene (acrylate/methacrylate) polymerization. As a great deal of different kinds of alkene monomers and thiol compounds are available, thiol-ene polymerization may have good designability and expansibility in future monolithic materials. Meanwhile, the resulting poly(ODT-co-DPEPA) monolithic columns exhibited good permeability and strong ability for small molecules, and showed typical reversed-phase retention mechanism in cLC, signifying that the polymeric monolithic columns can be taken as a powerful tool for further application in the future such as material science, life science and analysis of biological macromolecules.

4. Conclusions

In this study, “one-pot” photo-initiated thiol-acylate polymerization of DPEPA and ODT as monomers was utilized for rapid preparation of polymeric monolithic columns. During this process, the crosslinker (DPEPA), which has multiple acrylate groups, not merely self-polymerized, but also coupled with the thiol-containing functional monomer. Due to the addition of thiol into the “one-pot” thiol-acrylate polymerization, the major obstacle of oxygen inhibition is essentially eliminated in the thiol-ene (acrylate/methacrylate) polymerization. As a great deal of different kinds of alkene monomers and thiol compounds are available, thiol-ene polymerization may have good designability and expansibility in future monolithic materials. Meanwhile, the resulting poly(ODT-co-DPEPA) monolithic columns exhibited good permeability and strong ability for small molecules, and showed typical reversed-phase retention mechanism in cLC, signifying that the polymeric monolithic columns can be taken as a powerful tool for further application in the future such as material science, life science and analysis of biological macromolecules.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.04.012.

References


Fig. 6. Separation of (a) tryptic digest of four standard proteins and (b) Amygdalus pedunculata Pall. proteins on column VIII by cLC-MS/MS. Experimental conditions: column dimension, 30.0 cm × 75 μm i.d.; injection, (a) 1.6 µg, (b) 6.0 µg; mobile phase A, 0.10% FA in water, B, 0.10% FA in ACN; flow rate, 165 μl min −1; gradient, 5–25% B in 2−75 min, 35–80% B in 95−98 min, 100% A in 108−125 min; detection wavelength, 214 nm.


