Thiol-Epoxy Click Polymerization for Preparation of Polymeric Monoliths with Well-Defined 3D Framework for Capillary Liquid Chromatography

Hui Lin,‡† Junjie Ou,†‡ Zhongshan Liu,†‡ Hongwei Wang,†‡ Jing Dong,† and Hanfa Zou*†

1Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian, 116023, China
2University of Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT: A facile approach was developed for direct preparation of organic monoliths via the alkaline-catalyzed thiol-epoxy click polymerization. Two organic monoliths were prepared by using tetraphenylolethane glycidyl ether as a multiepoxy monomer, and trimethylolpropane tris(3-mercaptopropionate) and pentaerythritol tetrakis(3-mercaptopropionate) as the multithiol monomer, respectively, in the presence of a ternary porogenic system (DMSO/PEG200/H2O). The obtained organic monoliths showed high thermal, mechanical and chemical stabilities. Benefiting from the step-growth polymerization process, two organic monoliths possessed well-defined 3D framework microstructure, and exhibited high permeabilities and column efficiencies in capillary liquid chromatography. A series of neutral, basic and acidic small molecules were used to comprehensively evaluate the separation abilities of these monoliths, and satisfactory chromatographic performance with column efficiencies ranging from 35 500 to 132 200 N/m was achieved, demonstrating good separation abilities of these organic monoliths prepared via thiol-epoxy click polymerization approach. Besides, multiple retention mechanisms, including hydrophobic, hydrophilic and π–π conjugate interactions were observed during the separation of analytes on these monoliths, which would make them promising for more extensive applications in capillary liquid chromatography.

As a state-of-the-art format of stationary phases, the monolithic columns have attracted substantial interest and been extensively applied in the field of analytical chemistry from sample pretreatment to microscale/nanoscale chromatographic separation.1–6 Compared to both silica-based and organic–inorganic hybrid monoliths, organic monoliths emerged earlier and were easily prepared, and also possessed richer chemistry as well as better pH tolerance.7,8 However, the high heterogeneity of the microstructure along with the low surface area make the chromatographic performance of polymeric monoliths far from the level of packed columns, or even other types of monolithic columns in liquid chromatography (LC), especially for the separation of small molecules.9–12 Such remarkable differences have attracted great concern in the scientific community.13 So far, many new methods have been developed to address this great challenge,14,15 such as various living radical polymerization et al.16–18 Recently, Svec and co-workers introduced hyper-cross-linking technique into the postmodification process of polymeric monoliths to dramatically increase their surface area, thereby improving the chromatographic performance.9,19–21 By contrast, although no increment of surface area was occurred, benefiting from the well-controlled skeletal structures, the monoliths prepared via epoxy-amide based ring-opening polymerization reaction also exhibited significant improvement of separation performance in analysis of small molecules.22–25 These successful precedents greatly inspired us to search new candidates in the arsenal of chemistries for convenient preparation of organic monoliths with high column efficiency in capillary liquid chromatography (cLC) separation of small molecules.

Benefiting from the excellent specificity, unrivaled efficiency and high robustness to oxygen or water, the click chemistry has revolutionized almost all fields of chemistry,26–30 and particularly, emerged as a useful tool in the arena of novel LC stationary phase exploration.31–34 Up to now, however, there are very few papers on direct preparation of monolithic columns via click polymerization, mainly focusing on the application of radical-mediated thiol–ene click chemistry.35,36 The alkaline-catalyzed thiol-epoxy click chemistry, despite the success in industrial and biomedical applications, is overlooked in the fabrication of macroporous monoliths for chromatographic application.28,37

Herein, a novel approach was developed for direct preparation of polymeric monolithic columns via thiol-epoxy click polymerization reaction by using tetraphenylolethane glycidyl ether (TPEGE) and a multithiol monomer, trimethylolpropane tris(3-mercaptopropionate) as the multithiol monomer, respectively, in the presence of a ternary porogenic system (DMSO/PEG200/H2O). The obtained organic monoliths showed high thermal, mechanical and chemical stabilities. Benefiting from the step-growth polymerization process, two organic monoliths possessed well-defined 3D framework microstructure, and exhibited high permeabilities and column efficiencies in capillary liquid chromatography. A series of neutral, basic and acidic small molecules were used to comprehensively evaluate the separation abilities of these monoliths, and satisfactory chromatographic performance with column efficiencies ranging from 35 500 to 132 200 N/m was achieved, demonstrating good separation abilities of these organic monoliths prepared via thiol-epoxy click polymerization approach. Besides, multiple retention mechanisms, including hydrophobic, hydrophilic and π–π conjugate interactions were observed during the separation of analytes on these monoliths, which would make them promising for more extensive applications in capillary liquid chromatography.

Received: January 2, 2015
Accepted: February 13, 2015
Published: February 13, 2015

DOI: 10.1021/acs.analchem.5b00006
Anal. Chem. 2015, 87, 3476–3483
lolo propane tris(3-mercaptopropionate) (TPTM) or pentaerythritol tetras(3-mercaptopropionate) (PTM), as precursors (Figure 1). Systematic characterizations of two organic monoliths, including SEM images, FT-IR spectra, pore size measurement, thermal gravimetric analysis and nitrogen adsorption/desorption measurement were carried out. Besides, the separation capability of these polymeric monoliths was assessed comprehensively by separating various samples, and high column efficiency was achieved for almost all types of analytes in cLC.

### EXPERIMENTAL SECTION

#### Chemicals and Reagents.
TPEGE, TPTM, PTM, 3-glycidoxypropyltrimethoxysilane (GPTMS), and poly(ethylene glycol) (PEG, Mn = 200 and 10,000) were obtained from Aldrich (Milwaukee, WI, USA). Trypsin was purchased from Promega (Madison, WI, USA). Bovine serum albumin (BSA), EPA 610 (including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)-anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene), thiourea, alkylbenzenes, trifluoroacetic acid (TFA) and other standard compounds, such as polycyclic aromatic hydrocarbon (PAHs), phenols, anilines and polystyrenes (MW = 800, 4,000, 13,200, 35,000, 50,000, 90,000, 280,000, 900,000) were obtained from Sigma (St Louis, MO, USA). Dithiothreitol (DTT) and iodoacetamide (IAA) were products of Sino-American Biotechnology Corporation (Beijing, China). The fused-silica capillaries with dimensions of 50 and 75 μm i.d. and 365 μm o.d. were supplied by the Refine Chromatography Ltd. (Hebei, China). HPLC-grade acetonitrile (ACN) was used for preparation of mobile phases. The water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA, USA). Ethanol, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), KOH, NaOH, HCl, and other chemical reagents were all of analytical grade.

#### Pretreatment of Fused-Silica Capillary.
Prior to preparation of monolithic columns, the inner wall of fused-silica capillary was pretreated and rinsed with 1.0 mol/L NaOH for 4 h, water for 30 min, 1.0 mol/L HCl for 14 h, and water for another 30 min, successively, and then dried by a nitrogen stream at room temperature. After that, a solution of GPTMS/methanol (50%, v/v) was used for modification of the capillary inner wall with epoxy groups. Then, the capillary was filled with GPTMS/methanol solution, sealed with rubbers at both ends and submerged in a water bath at 50 °C for 12 h. Finally, the capillary was rinsed with methanol to flush out the residual reagents, and dried again with a stream of nitrogen gas.

#### Preparation of Monoliths via Thiol–Epoxy Click Chemistry.
For the preparation of monoliths, a multi-epoxy monomer (TPEGE), a multi-thiol monomer (TPTM or PTM) and the pore size regulator PEG 10 000 were first dissolved in the porogenic solvents (DMSO, PEG 200 and H2O) to form a homogeneous solution, then a amount of catalyst KOH was added. After fully mixing and degassing by 20 min sonication, the prepolymerization mixture was injected into the pretreated capillary with a syringe. The filled capillary was then sealed with rubber stoppers and immersed in a water bath at appropriate temperature for 4 h. After polymerization, the monolithic column was rinsed with methanol to flush out the unreacted residual.

For the bulk material, the prepolymerization mixture was placed in centrifuge tubes and reacted in a water bath at the same temperature for 4 h. After polymerization, the bulk monolith was cut into smaller pieces, extracted with DMSO and ethanol successively overnight in a Soxhlet apparatus and dried in a vacuum.

#### Instrumentation.
The chromatographic evaluation of monolithic columns was performed using an LC system equipped with an Agilent 1100 (Hewlett-Packard) micropump and a UV detector (K-2501, Knauer, Germany). Data were collected at 214 or 254 nm, and processed by a chromatography workstation (Beijing Cailu Scientific Instrument Ltd., Beijing, China). A 7725i injector with a 20 μl sample loop was used. A T-union connector served as a splitter with one end connected to the capillary monolithic column and the other end to a blank capillary (95 cm long, 50 μm i.d. and 365 μm o.d.). The outlet of the monolithic column was connected with a Teflon tube to an empty fused-silica capillary (75 μm i.d. and 365 μm o.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.

SEM images were obtained by using a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). FT-IR was measured with a Bruker Tensor 27 FT-IR spectrometer (Bruker Daltonics, Ettlingen, Germany). Pore size measurement was carried out on an Autopore IV 9500 (Micromeritics, Norcross, USA). Thermal gravimetric analysis was performed on a Setsys 16/18 (Setaram, Caluire, France). Nitrogen adsorption/desorption measurement of dry bulk monolith was carried out on a Quadrant Shimadzu instrument (Quantachrome, Boynton Beach, USA).

#### Preparation of BSA Tryptic Digest and cLC-MS Analysis.
The tryptic digestion of BSA and cLC-MS/MS analysis were performed according to procedures previously reported by us with minor modification. The sample trapping was achieved with a homemade C18-particle-packed trap.
column (4.0 cm length × 200 μm i.d.), and the subsequent separation was carried out on a thiol-epoxy click chemistry based monolith (34.0 cm length × 75 μm i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of capillary. (Detailed experimental procedures are provided in the Supporting Information.)

■ RESULTS AND DISCUSSION

Preparation of Organic Monoliths via Thiol–Epoxy Click Chemistry. The preparation of organic monoliths was performed according to the procedure and feed recipes shown in Figure 1 and Table 1, respectively. For simplifying the optimization process, a nearly equimolar amount of epoxy and thiol groups was first adopted. In order to search a suitable porogenic system, several candidates, such as DMSO/ethanol, DMSO/H2O, DMSO/PEG 200 and DMSO/PEG 200/H2O, were examined. Finally, the ternary porogenic system, DMSO/PEG 200/H2O, was selected due to the appropriate solubility and porogenic ability. Since the component of porogenic system has significant influence on the morphology of monoliths, the content of each solvent was carefully optimized. As shown in Table 1, for monolith TPEGE-PTM, it was found that the permeability significantly decreased from 11.57 to 5.40 × 10⁻¹⁴ m² (which calculated according to the Darcy’s Law of permeability, B₀ = FηL/(πr²ΔP), F: = linear velocity of mobile phase; η: = dynamic viscosity of mobile phase; L: = effective column length; r: = inner radius of column; ΔP: pressure drop across the column, measured at room temperature.

![Image](https://via.placeholder.com/150)

**Table 1. Effects of Synthesis Parameters on the Formation of TPEGE-PTM Monoliths**

<table>
<thead>
<tr>
<th>Monolith</th>
<th>DMSO b (wt%)</th>
<th>PEG200 c (wt%)</th>
<th>H2O d (wt%)</th>
<th>PEG10000 e (wt%)</th>
<th>KOH f (wt%)</th>
<th>Optical microscope image</th>
<th>Permeability h (× 10⁻¹⁴ m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>66.0</td>
<td>20.0</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
<td></td>
<td>11.57</td>
</tr>
<tr>
<td>B</td>
<td>68.5</td>
<td>17.5</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
<td>Too hard to pump through</td>
<td>5.40</td>
</tr>
<tr>
<td>C</td>
<td>77.0</td>
<td>9.0</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
<td>Too hard to pump through</td>
<td>19.00</td>
</tr>
<tr>
<td>D</td>
<td>70.0</td>
<td>18.0</td>
<td>6.0</td>
<td>4.0</td>
<td>2.0</td>
<td>Too hard to pump through</td>
<td>19.58</td>
</tr>
<tr>
<td>E</td>
<td>67.5</td>
<td>17.0</td>
<td>9.5</td>
<td>4.0</td>
<td>2.0</td>
<td></td>
<td>15.54</td>
</tr>
<tr>
<td>F</td>
<td>70.0</td>
<td>18.0</td>
<td>8.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>67.5</td>
<td>17.0</td>
<td>9.0</td>
<td>5.5</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>68.5</td>
<td>17.5</td>
<td>7.0</td>
<td>4.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>68.5</td>
<td>17.5</td>
<td>7.0</td>
<td>4.0</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aThe amounts of TPEGE, PTM, and porogenic system were kept at 40, 33, and 262 mg, respectively. Reaction temperature: 50 °C. bWeight percentage of DMSO in the porogenic solvents. cWeight percentage of PEG 200 in the porogenic solvents. dWeight percentage of H2O in the porogenic solvents. eWeight percentage of PEG 10,000 in the porogenic solvents. fWeight percentage of KOH solution in the porogenic solvents. gThe concentration of the catalyst KOH solution: 0.25 mol/L. hPermeability, B₀ = FηL/(πr²ΔP), F: = linear velocity of mobile phase; η: = dynamic viscosity of mobile phase; L: = effective column length; r: = inner radius of column; ΔP: pressure drop across the column, measured at room temperature. iThe amounts of TPEGE-TPTM, and porogenic system were kept at 40, 33, and 262 mg, respectively. Reaction temperature: 50 °C. jWeight percentage of DMSO in the porogenic solvents. kWeight percentage of PEG 200 in the porogenic solvents. lWeight percentage of PEG 10,000 in the porogenic solvents. mThe concentration of the catalyst KOH solution: 0.25 mol/L. nPermeability, B₀ = FηL/(πr²ΔP), F: = linear velocity of mobile phase; η: = dynamic viscosity of mobile phase; L: = effective column length; r: = inner radius of column; ΔP: pressure drop across the column, measured at room temperature.

Similarly, the feed recipes of monolith TPEGE-TPTM were also optimized. The monolith (TPEGE-TPTM) prepared with TPEGE (40 mg), TPTM (32.5 mg) in the presence of porogenic system DMSO/PEG 200/H2O/PEG 10,000/KOH was further increased to 77% (wt %) (or the amount of PEG 200 was further decreased to 9% (wt %)), the column could not even be pumped through (as column C). Thus, the DMSO acted as the microporogenic solvent (good solvent) in the porogenic system, while the PEG 200 served as the macroporogenic solvent (poor solvent). The H2O had a similar effect as the PEG 200 on the final column morphology. As seen from the columns B, D, and E, the permeability of the monoliths increased when the amount of H2O was increased. In contrast, the PEG 10,000 had an opposite porogenic effect to the PEG 200. Increasing the content of PEG 10,000 would lead to a decrease of the permeability of the monoliths (as columns B, F, and G). Herein, the KOH solution (0.25 mol/L) was selected as the catalyst, and the amount of KOH also had significant influence on the column morphology. Increasing the amount of KOH would accelerate the thiol-epoxy click polymerization reaction, leading to a more compact monolith (as columns B, H, and I). In addition, the effect of preparation temperature was also investigated. Higher preparation temperature would not only accelerate the reaction process, but also improve the solubility of porogenic system, thus resulting in a decrease of permeability (data not shown). Finally, the preparation temperature was determined at 50 °C. After comprehensive optimization of these parameters, the column B was employed in the following experiments.
(66.0/19.0/7.5/5.5/2.0, wt %, 267 mg in total) at 60 °C was employed for further experiments.

Characterization of the Organic Monoliths. The occurrence of thiol-epoxy click polymerization reaction was convincingly demonstrated by FT-IR spectra (Supporting Information Figure 1), as the characteristic absorption peaks of epoxy and S–H groups at 725–910 and 2570 cm⁻¹, respectively, were significantly decreased and almost disappeared. Meanwhile, the remarkable changes of these peaks also implied a nearly complete consumption of two functional groups, indicating the high efficiency of this thiol-epoxy click polymerization reaction based approach. Besides, the high conversion of the epoxy and thiol groups resulted in a high degree of cross-linking, and further endowed the monoliths high thermal stabilities. As shown in Supporting Information Figure 2, no significant weight loss was observed until the temperature above 300 °C, and the pyrolysis continued up to 600 °C for the monolith TPEGE-PTM. Such a high heat resistance is even better than some hybrid monoliths.²³,³⁹

The SEM images revealed that the monolithic matrices with reticular framework were tightly anchored to the inner walls of the capillaries without any disconnection, and the macropores of ~1 μm were homogeneously separated by the narrow framework (~0.5 μm) (Figure 2). Particularly, their morphologies were distinct from the cauliflower-like microglobules of traditional organic monoliths prepared via free radical polymerization, and were similar to those of monoliths synthesized via epoxy-amine based ring-opening polymerization or controllable living radical polymerizations.¹⁸,²²–²⁴ The high regularity of microstructure was also demonstrated by the narrow pore size distribution (Supporting Information Figure 3). Such well-controlled 3D framework microstructure was possibly attributed to the step-growth mechanism of thiol-epoxy click polymerization. As mentioned above, the epoxy and thiol groups were stoichiometric fed and almost equally consumed, indicating that a step-growth polymerization process is favored during the formation of organic monoliths.³⁰,³⁵ Distinct from the free radical polymerization, the mild step-growth polymerization would induce spinodal decomposition (SD) during the thiol-epoxy-click polymerization process, resulting in high conversions of the functional groups at the gel point and largely uniform, homogeneous networks,³⁰,⁴⁰ and finally formed a highly ordered bicontinuous skeleton.

The relationship between flow rate and the back-pressure drop of monolith TPEGE-PTM was also measured. As shown in Supporting Information Figure 4, the good linear relationship (R = 0.9968) between the back-pressure and flow rate was obtained. The monolith even could undergo a high pressure over 42.0 MPa without any deterioration on the framework, indicating satisfactory mechanical strength. In addition, the permeability was calculated at 5.40 × 10⁻¹⁴ m² (as seen the column B in Table 1), indicating a good permeability of the monolith.

Swelling propensity (SP) is another important parameter for evaluation of the stability of organic monoliths, which has a significant influence on the resolution and column efficiency.⁴¹ The SP factor can be calculated with the equation SP = (p(solvent) – p(water))/p(water) by measuring the pressure drop for water and for an organic solvent,⁴² where p is the ratio of the mobile phase pressure drop and the corresponding viscosity. Closer to zero of SP factor, smaller effect of shrinkage/swelling of monolith in a given solvent. First, as a commonly used mobile phase additive, ACN was used to test the SP of monolith TPEGE-PTM. The SP factor of 0.5

Figure 2. Cross-section of the polymeric monolithic columns prepared via thiol-epoxy click polymerization. Magnification: 1000x (top), 5000x (bottom).
indicated a high organic solvent tolerance of the monolith. Surprisingly, almost no swelling effect was observed when the monolith was immersed into the weakly polar solvents such as methanol and THF (the SP factors were very close to zero). Although the reason for this phenomenon is still unknown, there was no doubt that these organic monoliths possess better organic solvent tolerance than common poly(methacrylate) organic monoliths.

**Chromatographic Evaluation of the Organic Monoliths in cLC.** Owing to the satisfactory mechanical and chemical stabilities, as well as the highly regular microstructure, these organic monoliths were applied for separation of analytes in cLC. As shown in Figure 3A, 5 alkylbenzenes were well-separated under the mobile phase of ACN/water (60/40, v/v) according to their hydrophobicity on monolith TPEGE-PTM, indicating a typical reversed-phase (RP) retention mechanism (Supporting Information Figure 5). It should be pointed that the monolith TPEGE-PTM exhibited high column efficiencies for alkylbenzenes, and the highest one could reach ~132 000 N/m (for benzene, Figure 3B). Comparable high column efficiencies were also achieved on another monolith TPEGE-TPTM (the highest ~128 000 N/m for propylbenzene, Supporting Information Figure 6). Such high column efficiencies could be ascribed to the high homogeneous microstructure of these monoliths.

Although the nitrogen adsorption/desorption measurement implied low surface areas of these monoliths (e.g., 2.25 and 1.75 m²/g for monoliths TPEGE-PTM and TPEGE-TPTM, respectively), which is possibly unfavorable for improving the separation performance. The size exclusion chromatography in THF afforded total porosity of monolith TPEGE-PTM at 86.2%, while the volume of through-pores was calculated at 57.6% (as shown in Supporting Information Figure 7). This significant pore volume was beneficial to the separation of small molecules. Besides, the large through-pore/framework size ratio as well as the high ordered microstructure would enable fast mass transfer as the convection is dominant during the separation process.43−45 Meanwhile, the thin framework would also effectively reduce the diffusion distance and greatly accelerate the mass transfer,13,46−50 thus leading to a smaller C-term in van Deemter equation. What’s more, the high ordered microstructure could also significantly limit the eddy diffusion (resulting in a smaller A-term in van Deemter equation).23,43,47 Such effects were vividly reflected in the van Deemter curves of alkylbenzenes on monolith TPEGE-PTM (Figure 3B and Table 2). The values of A, B, and C-terms were much smaller than those of other traditional organic monoliths in LC, and even comparable to those of silica-based and hybrid monoliths or some particulate-packed columns in capillary electrophromatography (CEC).52,53 It is well-known that the eddy diffusion term is the main contributor of the band broadening, especially in the separation of small molecules.54,55 Herein, the relatively small values of A-term ranged from 0.981 to 2.619, implying the limitation of eddy diffusion. Such small A-terms were comparable to those of columns packed with core−shell and totally porous particles, whose typical values were 0.9 and 1.5, respectively.56 Furthermore, the small values of C-term also indicated a better communication between the stationary phase and analytes (the mass transfer was faster as the molecules were transported almost solely by convection instead of diffusion). Thus, ascribing to the combination of these effects, high column efficiencies were achieved on these organic monoliths in cLC separation of small molecules.

It should be mentioned that the elution order of thiourea and toluene on monolith TPEGE-PTM was reversed when the ACN content in mobile phase was higher than 85% (v/v) (Supporting Information Figure 8), exhibiting a hydrophilic interaction chromatography (HILIC) retention mechanism. This phenomenon may be attributed to the generation of hydroxy groups on the surface of the matrix via thiol-epoxy click reaction (Figure 1). The multiple retention mechanisms of these monoliths could provide more choices of separation modes in analysis of some polar compounds, and is beneficial to more extensive cLC application.

**Applications of the Organic Monoliths in cLC.** For an overall assessment of the separation capability of these polymeric monoliths in analysis of small molecules, various kinds of analytes including neutral and polar, basic and acidic, simple and complicated mixtures were separated on monolith TPEGE-PTM. As shown in Supporting Information Figure 9, 5 polycyclic aromatic hydrocarbons (PAHs) were well-separated with the mobile phase of ACN/water (75/25, v/v), and column

---

**Figure 3.** Separation of (A) alkylbenzenes and (B) dependence of plate heights of them on the linear velocity of mobile phase on monolith TPEGE-PTM in cLC. Experimental conditions: column dimension, 28.0 cm × 75 μm i.d.; flow rate for (A), 240 nL/min; mobile phase, ACN/water (60/40, v/v); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

**Table 2. van Deemter Coefficients Measured with Alkylbenzenes for Monolith TPEGE-PTM in cLC**

<table>
<thead>
<tr>
<th>compounds</th>
<th>eddy dispersion, A (μm)</th>
<th>longitudinal diffusion, B (×10⁵ μm²/s)</th>
<th>mass transfer resistance, C (×10⁻⁵ s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>2.619</td>
<td>1.700</td>
<td>4.240</td>
</tr>
<tr>
<td>toluene</td>
<td>1.857</td>
<td>1.848</td>
<td>5.550</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>1.715</td>
<td>1.749</td>
<td>6.280</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>1.534</td>
<td>1.753</td>
<td>6.510</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>0.981</td>
<td>1.808</td>
<td>7.100</td>
</tr>
</tbody>
</table>
efficiencies were in the range of 100 800−112 000 N/m. Particularly, the elution order of 4,4′-dimethylbiphenyl and acenaphthene was reversed by varying the ACN content in mobile phase (Supporting Information Figure 10). It is implied that these monoliths could provide π−π conjugate interaction besides the RP retention mechanism in separation of analytes with π electron, attributing to the existence of a lot of benzene rings (as shown in Figure 1).

Figure 4A showed the separation chromatogram of six phenols, and the column efficiencies were in the range of 86 600−107 700 N/m. The baseline-separation of the two positional isomers (hydroquinone and pyrocatechol) demonstrated the possibly potential of these polymeric monoliths in analysis of some positional isomers.

Generally, it is difficult to separate the basic compounds on C18 silica particles packed LC columns as the phenomenon of peak tailing is emerged. However, such phenomenon was not observed on the monolith TPEGE-PTM. As shown in Figure 4B, 5 anilines were baseline-separated with good peak shapes, achieving column efficiencies in the range of 58 900−114 100 N/m. Additional, satisfactory chromatographic performance was also obtained in the analysis of five alkaline drugs, and the column efficiencies were ranged from 35 500 to 71 300 N/m (Figure 4C), demonstrating the potential applications of these organic monoliths in pharmaceutical research.

Satisfactory separation of acidic compounds was also achieved, as 5 benzoic acid analogues were well-separated with column efficiencies in the range of 81 300−107 700 N/m (Figure 4D). Compared to the monoliths synthesized via epoxy-amide based ring-opening polymerization, these thiol−epoxy click chemistry based monoliths are more suitable for the separation of charged analytes, as they were lack of ionizable groups on the surface, and the electrostatic interaction between the analytes and stationary phase was not occurred. It is worth noting that no significant decrease of column efficiency or clear column deterioration was observed on monolith TPEGE-PTM even after continuously using for several weeks, even under the acidic mobile phase, demonstrating high chemical stability of the monoliths.

Additionally, the separation of highly complicated samples was also performed on monolith TPEGE-PTM. As illustrated in Figure 4A, favorable separation of EPA 610 was obtained with ten baseline-separated ones and six partly separated ones, and a column capacity of 47 was calculated. Furthermore, the BSA digest was also used to evaluate the separation ability of monolith TPEGE-PTM, and an acceptable performance was achieved (Supporting Information Figure 11), 48 unique peptides were positively identified with protein sequence coverage of 50.58%. All results convincingly demonstrated the great capability of these thiol-epoxy click chemistry based organic monoliths in highly efficient separation of small molecules.

### CONCLUSIONS

In summary, a facile approach was first developed for direct preparation of organic monoliths via thiol−epoxy click polymerization reaction. Ascribing to the high conversion of the epoxy and thiol functional groups, the resulting organic monoliths exhibited better thermal and chemical stabilities than traditional polymethacrylate(acrylate) organic monoliths prepared via free radical polymerization. Besides, benefiting from the relatively ordered phase-separating process resulted by the...
Analytical Chemistry

**REFERENCES**


**ASSOCIATED CONTENT**

Additional Information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

*Corresponding Authors*  
*Tel*: +86-411-84379576. *Fax*: +86-411-84379620. *E-mail*: junjieou@dicp.ac.cn  
*Tel*: +86-411-84379610. *Fax*: +86-411-84379620. *E-mail*: hanfazou@dicp.ac.cn.

**Notes**  
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

Financial support is gratefully acknowledged from the China State Key Basic Research Program Grant (2013CB-911203, 2012CB910601), the National Natural Sciences Foundation of China (21235006), the Creative Research Group Project of NSFC (21321064), and the Knowledge Innovation program of DICP to H.Z., as well as the National Natural Sciences Foundation of China (No. 21175133) to J.O.