Preparation of Hybrid Monolithic Columns via “One-Pot” Photoinitiated Thiol–Acrylate Polymerization for Retention-Independent Performance in Capillary Liquid Chromatography

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Supporting Information

ABSTRACT: A novel “one-pot” approach was developed for ultrarapid preparation of various hybrid monolithic columns in UV-transparent fused-silica capillaries via photoinitiated thiol–acrylate polymerization of an acrylopropyl polyhedral oligomeric silsesquioxane (acryl-POSS) and a monothiol monomer (1-octadecanethiol or sodium 3-mercapto-1-propanesulfonate) within 5 min, in which the acrylate not only homopolymerizes, but also couples with the thiol. This unique combination of two types of free-radical reaction mechanisms offers a simple way to fabricate various acrylate-based hybrid monoliths. The physical characterization, including scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, and thermal gravimetric analysis was performed. The results indicated that the monothiol monomers were successfully incorporated into acryl-POSS-based hybrid monoliths. The column efficiencies for alkylbenzenes on the C18-functionalized hybrid monolithic column reached to 60 000−73 500 plates/m at the velocity of 0.33 mm/s in capillary liquid chromatography, which was far higher than that of previously reported POSS-based columns prepared via thermal-initiated free-radical polymerization without adding any thiol monomers. By plotting the plate height (H) of the alkylbenzenes versus the linear velocity (u) of the mobile phase, the results revealed a retention-independent efficient performance of small molecules in the isocratic elution. These results indicated that more homogeneous hybrid monoliths formed via photoinitiated thiol–acrylate polymerization; particularly, the use of the multifunctional cross-linker possibly prevented the generation of gel-like micropores, reducing mass transfer resistance (C-term). Another sulfonate-containing hybrid monolithic column also exhibited hydrophobicity and ion-exchange mechanism, and the dynamic binding capacity was calculated as 71.1 ng/cm (75 μm i.d.).

Macroporous organic monoliths emerged in the 1990s and possess a unique structure and exhibit many advantages, such as easy fabrication process, varieties of functionality, wide pH tolerance, and good permeability.1–3 Various organic monoliths including the polymethacrylates, polyacrylamides, and polystyrenes have been successfully fabricated and applied for the separation science, especially for separation of large biomolecules.4–10 Unfortunately, the swelling in organic solvents could lead to the change of pore structure and decrease of mechanical stability.11,12 Additionally, compared to silica-based monoliths, organic monoliths usually exhibit low column efficiency for the separation of small molecules.13,14 Great efforts have been made to improve the mechanical stability and separation efficiency of organic monoliths.15–21 As a kind of organic–inorganic hybrid nanocomposite, polyhedral oligomeric silsesquioxanes (POSS) developed at the...
end of the last century are really nanostructural chemicals with sizes from 1 to 3 nm in diameter. It has been proved that the incorporation of POSS into polymeric networks can result in significant improvement in a variety of physical and mechanical properties due to the reinforcement at the molecular level and the inorganic framework’s ceramic-like properties.24−26 On the basis of the excellent property of POSS, our group26−28 has adopted a multimethacrylate POSS (POSS-MA) monomer to prepare several hybrid monolithic columns via thermal-initiated free-radical polymerization. The preparation process was as simple as that of organic monoliths. As expected, these POSS-based hybrid monoliths exhibited better mechanical and pH stabilities by comparison with the common polymethacrylate monoliths. However, the relatively low column efficiencies (below 50 000 N/m) of those hybrid monoliths were obtained in capillary liquid chromatography (cLC).29 Fortunately, other POSS-based monoliths were successfully fabricated via epoxy−amine ring-opening polymerization29−32 and phosphine-catalyzed thiol−methacrylate click polymerization.33 Surprisingly, the resulting monoliths exhibited the highest column efficiency of 195 000 N/m for butylbenzene in reversed-phase (RP) mode.33 These results greatly inspired us to search for a novel chemistry for fabrication of monoliths with satisfactory chromatographic efficiency.

All the above-mentioned approaches for preparation of POSS-based hybrid monoliths were performed at appropriate temperature, which would be finished in several hours. However, there are only a few reports on the preparation of POSS-based hybrid monoliths via photoinitiated polymerization, which could be almost completed in minutes rather than in hours and avoided considering the effect of polymerization temperature on monolith morphology. Alves and Nischang34 have synthesized hybrid porous materials by thermal/photoinitiated thiol−ene reaction of polyhedral oligomeric vinylsilsequioxane (vinylPOSS) with multi thioli monomers. Although these POSS-based monoliths were also prepared in the capillaries, their column efficiencies in cLC were not reported.

Herein, a simple “one-pot” approach was successfully developed for fast preparation of hybrid monoliths with various functionalization via photoinitiated thiol−acrylate polymerization reaction, in which the acrylate not only homopolymerizes, but also couples with the thiol. An acrylopropyl-POSS (cage mixture) (acryl-POSS) monomer and a monothiol compound, such as 1-octadecanethiol (ODT) or sodium 3-mercaptop-1-propanesulfonate, were selected as the precursors, respectively, and two kinds of hybrid monoliths were quickly fabricated within 5 min. The physical characterization, including scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, and thermal gravimetric analysis, and chromatographic evaluation of two hybrid monoliths were carried out.

■ EXPERIMENTAL SECTION

Chemicals and Materials. The monomer of acryl-POSS is a hybrid molecule with an inorganic silsesquioxane at the core and organic acrylopropyl groups attached at the corners of the cage, which was obtained from Hybrid Plastics, Inc. (Hattiesburg, MS, U.S.A.). ODT (C18H37SH), sodium 3-mercaptop-1-propanesulfonate, methacryloxypropyl trimethoxysilane (γ-MAPS) (298%), EPA610, 2,5-dihydroxy benzoic acid (DHB), and polystyrenes (Mw = 800, 4000, 13 200, 35 000, 50 000, 90 000, 280 000, and 900 000) were purchased from Sigma (St. Louis, MO, U.S.A.) and used directly without further purification. 1-Butanol and ethylene glycol were from J&K Scientific Ltd. (Beijing, China). 2,2-Dimethoxy-2-phenylacetophene (DMPA, 99%) was purchased from Acros Organics (New Jersey, U.S.A.). Lysozyme (chicken egg white), bovine serum albumin (BSA), myoglobin (horse heart), ovalbumin, α-casein, and RNase A were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Cytochrome c (bovine heart) was obtained from Aladdin (Shanghai, China). Dithiothreitol (DTT) and iodoacetamide (IAA) were products of Sino-American Biotechnology Corporation (Beijing, China). Thiourea, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, phloroglucinol, pyrocatechol, p-cresol, 2,6-dimethylphenol, 2,4-dichlorophenol, caffeine, carbamazepine, 2,4-dinitroaniline, 4-aminobiphenyl, 2,6-dichloro-4-nitroaniline, and other standard analytes were of analytical grade and were obtained from Tianjin Kermel Chemical Plant (Tianjin, China). The flexible fused-silica capillary (UV-transparent coating) with inner dimension of 75 μm was purchased from Polymicro Technologies (Phoenix, AZ, U.S.A.). HPLC-grade acetonitrile (ACN) was from Yuwang Group (Shandong, China) and 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, U.S.A.).

“One-Pot” Preparation of the C18-Functionalized Hybrid Monolithic Column (Monolith I). Prior to preparation of hybrid monolith, the capillary was pretreated with γ-MAPS to immobilize a layer of methacrylate groups into its inner wall. In brief, the capillary was rinsed sequentially with 0.1 mol/L NaOH (2 h), H2O (1 h), 0.1 mol/L HCl (2 h), H2O (1 h), and methanol (1 h). The washed capillary was filled with γ-MAPS/methanol (50%, v/v) for 0.5 h and then sealed with rubber septa at both ends and submerged in a water bath at 50 °C for 16 h. Finally, the capillary was rinsed with methanol to flush out the residual reagent and dried under nitrogen flow.

A multiacrylate monomer (acryl-POSS) and a monothiol (ODT) were selected as the precursors to prepare hybrid monolith I as shown in Scheme 1. The detailed composition of polymerization solutions is given in Table 1. In brief, a polymerization solution composed of acryl-POSS (32.3 mg, 0.024 mmol), 1-butanol and ethylene glycol, and DMPA was homogenized by sonication for 5 min and then sealed with rubber septa at both ends and introduced into the pretreated capillary. After sealing both ends with rubber septa, the capillary was irradiated by UV light (λ = 365 nm, 120 mJ/cm²) for 5 min. The resulting monolithic column was then flushed with methanol to remove residuals. The rest of the polymerization solution in the vial was also cured under UV light to form bulk monolithic material, which was rinsed with ethanol three times, cut into small pieces, ground using a mortar and pestle, and then dried in a vacuum at 50 °C for 24 h.

“Two-Step” Preparation of the C18-Functionalized Hybrid Monolithic Column (Monoliths II and III). A “two-step” approach was also adopted to prepare C18-functionalized hybrid monolith as shown in Scheme 1. First, hybrid monolith II was fabricated in the capillary by using acryl-POSS as the sole precursor via photoinitiated free-radical polymerization. Briefly, as listed in Table 1, a polymerization solution containing acryl-POSS (32.3 mg, 0.024 mmol), 1-butanol and ethylene glycol, and DMPA was homogenized by sonication for 5 min and then introduced into the γ-MAPS-pretreated capillary. After sealing both ends with rubber septa, the capillary was irradiated by UV light (λ = 365 nm, 120 mJ/cm²) for 5 min. The resulting
monolithic column was then flushed with methanol to remove residuals.

To modify the above-mentioned monolith, an ethanol solution containing 5.0% (w/v) ODT and 0.3% (w/v) DMPA was flushed through the hybrid monolith. Then the reaction was performed by UV light irradiation for 5 min. Finally, hybrid monolith III was obtained by washing with methanol to remove the residuals.

**“One-Pot” Preparation of the Sulfonate-Containing Hybrid Monolithic Column (Monolith IV).** Similarly, another sulfonate-containing hybrid monolithic column (assembled as monolith IV) was also prepared with the “one-pot” process by using a monothiol compound of sodium 3-mercaptop-1-propanesulfonate to replace ODT. Ten microliters water was added into the porogenic solvents (7.1%, v/v) to dissolve the thiol compound.

**Instruments and Methods.** The polymerization was irradiated in UV cross-linkers (XL-1500A, Spectronics Corporation, New York, U.S.A.). FT-IR characterization was carried out on a Thermo Nicolet 380 spectrometer (Nicolet, Wisconsin, U.S.A.). The microscopic morphology of the monolithic materials was obtained by SEM (JEOL JSM-5600, Tokyo, Japan). Pore size distribution was measured by mercury intrusion porosimetry (MIP) on a PoreMaster GT-60 (Quantachrome Instrument Corporation, 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.). Thermogravimetric (TG) data were collected on Pyris 1 TGA (PerkinElmer, U.S.A.).

The permeability was calculated according to Darcy’s law by the equation $B = Fq/(πr^2\Delta P)$, where $F$ (m$^2$/s) is the flow rate of mobile phase, $\eta$ is the viscosity of mobile phase (0.90 × 10$^{-3}$ Pa s for ACN/H$_2$O = 40/60, v/v), $L$ and $r$ (m) are effective length and inner diameter of the column, respectively, $\Delta P$ (Pa) is the pressure drop of column. The data of $\Delta P$ and $F$ were obtained on an ACQUITY UltraPerformance LC (Waters, U.S.A.). The flow rates of mobile phase were set at 0.1–4.0 $\mu$L/min.

The cLC experiments were performed on LC system coupled with an Agilent 1100 microchip, a 7725i detector with a 20 $\mu$L sample loop, and a K-2501 UV detector (Knauer, Berlin, Germany). A T-union connector was used as a splitter, with one end connected to a blank capillary (150 cm × 50 $\mu$m i.d.) and the other connected to the monolithic column. The detection window was made by removing the polyimide coating of the fused-silica capillary tube with 50 $\mu$m i.d. in a position 5.0 cm from the separation monolithic column outlet. The real flow rate through monolithic column was measured by using a blank capillary (10 cm × 75 $\mu$m i.d.) connected to the monolithic column. It was calculated according to the equation, $\mu = \pi r^2 L/t$, where $L$ and $r$ (m) are the length and inner diameter of the blank capillary, respectively, and $t$ is the time that mobile phase (through monolithic column) flowed through the blank capillary. All chromatographic data were collected and analyzed using the software program HW-2000 from Qianpu Software (Shanghai, China).

**Preparation of the Tryptic Digest of Four Proteins and Capillary Liquid Chromatography–Tandem Mass Spectrometry Analysis.** The tryptic digestion of four proteins (BSA, myoglobin, ovalbumin, and α-casein) and capillary liquid chromatography–tandem mass spectrometry (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 $\mu$m i.d.), and the subsequent separation was carried out on a hybrid monolith (monolith I or III) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of

**Table 1. Detailed Composition of Prepolymerization Mixtures and the Permeability of Hybrid Monoliths**

<table>
<thead>
<tr>
<th>hybrid monolith</th>
<th>ODT (mg)</th>
<th>porogenic solvents ((\mu$L)/(\mu$L))</th>
<th>total monomer content (w/v, %)</th>
<th>permeability (10^{-14}$ m$^2$</th>
<th>specific surface area (m$^2$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>14</td>
<td>115/25</td>
<td>33.1</td>
<td>1.3</td>
<td>178.9</td>
</tr>
<tr>
<td>I-2</td>
<td>14</td>
<td>112/28</td>
<td>33.1</td>
<td>1.8</td>
<td>38.2</td>
</tr>
<tr>
<td>I-3</td>
<td>14</td>
<td>110/30</td>
<td>33.1</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>II-1</td>
<td>0</td>
<td>115/25</td>
<td>23.1</td>
<td>1.2</td>
<td></td>
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<tr>
<td>II-2</td>
<td>0</td>
<td>112/28</td>
<td>23.1</td>
<td>1.4</td>
<td></td>
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<tr>
<td>II-3</td>
<td>0</td>
<td>110/30</td>
<td>23.1</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)The prepolymerization solution also contained the monomer of 32.3 mg of acryl-POSS and the photoinitiator of 1.5 $\mu$L of 10% DMPA (w/v, DMPA/1-butanol). The capillary was irradiated in UV light (wavelength 365 nm, 120 mJ/cm$^2$) for 5 min. \(^b\)The porogenic solvents were the mixture of 1-butanol and ethylene glycol (v/v).
capillary. (Detailed experimental procedures are provided in the Supporting Information.)

### RESULTS AND DISCUSSION

**Preparation of C18-Functionalized Hybrid Monolithic Columns.** The “one-pot” preparation of C18-functionalized hybrid monolith (monolith I) was carried out according to the process shown in Scheme 1, and the composition in pre-polymerization mixtures is listed in Table 1. It is well-known that the porogenic system plays a vitally important role in the formation of monolithic materials. We finally selected a mixture of 1-butanol and ethylene glycol as the porogenic system after a series of experiments. As shown in Table 1, the volume ratio of 1-butanol to ethylene glycol had an effect on the permeabilities of two kinds of hybrid monoliths (I and II). It could be seen that the permeability of monolith I was increased from 1.3 to 2.2 × 10\(^{-14}\) m\(^2\) with an increase of ethylene glycol in the porogenic system, which served as the macroporogenic solvent (poor solvent) in porogenic system. Meanwhile, the permeability of monolith II was also increased from 1.2 to 1.5 × 10\(^{-14}\) m\(^2\) with the volume of ethylene glycol. By comparison of two monoliths I-2 and II-2 prepared with the same porogenic system, the total precursor content in the pre-polymerization solution for monolith I-2 was reached to 33.1% (w/v), which was remarkably higher than that for monolith II-2 (23.1% w/v); however, the permeability of monolith I-2 (1.8 × 10\(^{-14}\) m\(^2\)) was higher than that of monolith II-2 (1.4 × 10\(^{-14}\) m\(^2\)). These results indicated that the thiol monomer, ODT, causes the chain transfer, affecting the degree of polymerization and the pore size of the hybrid monolith.

As expected, the acryl-POSS containing several acrylate groups homopolymerized for the formation of monolith II. It is easily understood that homopolymerization of acrylate is inevitable to form monolith I in the “one-pot” process. The molar ratio of thiol to acrylate groups in the pre-polymerization solution for monolith I was about 1/4, so the thiol–acrylate addition reaction also proceeds. It seems that “one-pot” process proceeds largely through the homopolymerization at early stages of the reaction followed by a more like thiol–ene coupling reaction at the end. As a matter of fact, thiol–acrylate precursors undergo rapid polymerization processes because the acrylate couples with the thiol (thiol addition to the acrylate bond followed by the hydrogen abstraction of thiol hydrogen) and homopolymerizes (acrylate group propagation).\(^{36,37}\) As shown in Scheme S1, two types of reaction mechanisms would simultaneously occur when using DMPA as photoinitiator.\(^{38}\)

Although the rate constant for the hydrogen abstraction of the thiol group by the carbon-centered radical on the acrylate radical chain end to the reaction is lower than that for acrylate homopolymerization, the monothiol ODT could be simultaneously integrated into the POSS-based monolith via “one-pot” thiol–acrylate reaction. Two reaction mechanisms could be convincingly proved by the formation of monolith II and following postmodification to prepare monolith III via “two-step” approach.

It was observed that the white solid emerged in the vial after 30 s of UV irradiation for preparation of bulk hybrid monolith II, demonstrating the phase separation took place. However, phase separation for preparation of bulk hybrid monolith I occurred only after 10 s of UV irradiation. We have tried to add more ODT in the pre-polymerization solution to fabricate hybrid monoliths, but it was difficult to fill the pre-polymerization solution into the capillary, possibly due to the occurrence of thiol–acrylate polymerization reaction in the room even without any UV irradiation. This phenomenon could be attributed to an important advantage of relative insensitivity to oxygen inhibition in thiol–ene photoinitiated free-radical polymerization.\(^{36,39}\) As shown in Scheme S1, the peroxo radicals are formed by the reaction of carbon-centered propagation radicals with molecular oxygen, facilitating the hydrogen abstraction of a thiol hydrogen to form the thyl radicals, and then the main propagation steps continue. As a result, the polymerization occurs in air almost as rapidly as in an inert atmosphere. The major obstacle of oxygen inhibition in traditional free-radical polymerization is essentially eliminated in thiol–ene (acrylate/methacrylate) polymerization.

**Characterization of C18-Functionalized Hybrid Monoliths Prepared with “One-Pot” and “Two-Step” Approaches.** Figure 1 presents the FT-IR spectra of acryl-POSS monomer, monoliths I, II, and III, respectively. It could be observed from Figure 1a that the peak signals at 1728 and 1636 cm\(^{-1}\) implied the presence of \(\alpha,\beta\)-unsaturated carbonyl (C=O) and the stretching vibration of C=C bond in the acryl-POSS monomer, while the peak signal at 1728 cm\(^{-1}\) was changed to 1738 cm\(^{-1}\), and the intensity of the peak at 1636 cm\(^{-1}\) was remarkably decreased in Figure 1b. This result indicated that free-radical polymerization of the acrylate groups in acryl-POSS was carried out in the formation of monolith II; meanwhile, part of the unreacted acrylate groups still existed. The peak at 1636 cm\(^{-1}\) was slightly changed to 1634 cm\(^{-1}\), and its intensity was further decreased in Figure 1c, confirming the successful postmodification via thiol–acrylate coupling reaction between unreacted acrylate groups and ODT for the preparation of monolith III. It could be observed from Figure 1d that the peak at 1728 cm\(^{-1}\) was clearly changed to 1738 cm\(^{-1}\), and the peak signal at 1636 cm\(^{-1}\) was almost disappeared. These results forcefully indicated that both homopolymerization reaction and thiol–ene coupling reaction simultaneously occurred in the “one-pot” process.

It could be found from the thermogravimetric analysis, Figure S1, that no significant weight loss occurred until the temperature reached to 350 °C, and the pyrolysis continued up to 650 °C for hybrid monoliths I, II, and III, indicating better thermostability than those of hybrid monoliths reported previously. The residue for monolith I was only 36.19%, and lower than those for monoliths II (48.61%) and III (45.29%). This result also proved that more ODT was incorporated into...
monolith I in the “one-pot” approach, while ODT could only react with acrylate sites located in the surface of monolith II in the “two-step” approach.

SEM images showed that two kinds of C18-hybrid monolithic matrixes were well-attached to the inner walls of the γ-MAPS-pretreated capillaries without any disconnection (Figure 2, Figures S2 and S3). Particularly, monolith I contained much more macro through-pores around 1 μm, while monolith II presented the cauliflower-like globular structure intertwined with typical micrometer-sized pores. Their specific surface areas were measured in the dry state and calculated as 178.9 (monolith I) and 38.2 (monolith II) m² g⁻¹ according to nitrogen adsorption/desorption isotherm, indicating that the mesopores possibly existed in monolith I, while there was a lack of micropores and mesopores in monolith II. The size exclusion chromatography using THF as mobile phase also provided the porosity of the two hybrid monoliths, as shown in Figure S4, parts a and b. The total porosity of monolith I was measured at 86.1%, while the total porosity of monolith II was only 62.3%, indicating better permeability for monolith I than that for monolith II. However, the pore size distribution for monolith I looked much wider than that for monolith II (Figure S4, parts c and d).

The relationship between flow rate and back-pressure drop of hybrid monoliths I and II was also measured. As shown in Figure S5, the good linear relationship (R = 0.999) between back-pressure and flow rate was obtained. Two monoliths could undergo a high pressure over 45.0 MPa without any deterioration on the framework, indicating satisfactory mechanical strength. In addition, the permeabilities were calculated at 1.8 and 1.4 × 10⁻¹⁴ m² for the columns I-2 and II-2 (Table 1), respectively, indicating good permeability. As a result, columns I-2 and II-2 were selected for following cLC evaluation, and the C18-modified column II-2 (assigned as monolith III-2) was also measured in cLC. In order to investigate the pH stability, monolith I-2 was flushed by 60% ACN with different pH values for more than 100 h. The retention factor (k) was then monitored by cLC, and the result is shown in Figure S6. All the retention factors remained above 90% after flushing by the solution with different pH values (pH = 1, 2, and 10), indicating that the monolith had good pH stability. However, the monolithic matrix was flushed out from the capillary after flushing by the solution at pH 11 for 60 h. It may be due to the fact that the linkage between the matrix and capillary wall, the Si–O–Si–C bonds formed via γ-MAPS and silanol, was destroyed under such strong basic ambiance.26

![Figure 2. SEM images of hybrid monoliths (a and b) I-2 and (c and d) II-2. Magnification: panels a and c, 5000×; panels b and d, 10 000×.](image)

![Figure 3. (a and c) Separation of alkylbenzenes and (b and d) dependence of the plate height of alkylbenzenes on the linear velocity of the mobile phase on hybrid monoliths (a and b) I-2 and (c and d) II-2 in cLC, respectively. The analytes: (1) thiourea, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene, and (6) butylbenzene. Experimental conditions: columns dimensions for panels a and b, 21 cm × 75 μm i.d., for panels c and d, 22 cm × 75 μm i.d.; mobile phase, ACN/H₂O (60/40, v/v); flow rates for panel a, 170 μL/min (before split), 64 nL/min (after split), for panel c, 170 μL/min (before split), 41 nL/min (after split); detection wavelength, 214 nm.](image)
addition, significant decrease of the retention factor and column efficiency of monolith I-2 were not observed after keeping both ends of the column in water for 3 months, which could remain 86% and 85%, respectively. This result indicated that the hybrid monolith had high stability.

The repeatability and reproducibility of monolith I-2 were also characterized by measuring the relative standard deviations (RSDs) of the retention factor of toluene as test compound (thiourea as void time marker) under the mobile phase of 60% ACN. The RSDs of run-to-run, column-to-column, and batch-to-batch tests were less than 2.5%, 2.7%, and 3.3% (n = 5), respectively. The results demonstrated that the repeatability and reproducibility of hybrid monolith were acceptable.

**Chromatographic Evaluation and Application of Two C18-Functionalized Hybrid Monolithic Columns Prepared with “One-Pot” and “Two-Step” Approaches.** The chromatographic assessment of three monolithic columns was carried out in reversed-phase liquid chromatography (RPLC). Five alkylbenzenes were baseline-separated on monolith I with the mobile phase of ACN/H2O (60/40, v/v) within 8 min, and the peaks were all symmetric (Figure 3a). The highest column efficiencies for five alkylbenzenes reached to 60 000–73 500 plates/m (corresponding to 13.6–16.5 μm of plate height) at the velocity of 0.33 mm/s (Figure 3b). As shown in Figure 3c and Figure S7, five alkylbenzenes were also baseline-separated on monoliths II and III under the same chromatographic conditions, indicating a typical RP retention mechanism in monolith II due to the hydrophobicity of acryl-POSS. Particularly, the highest efficiencies for five alkylbenzenes on monolith II reached to 84 600–102 700 plates/m (corresponding to 9.7–11.8 μm of plate height) at the velocity of 0.84 mm/s (Figure 3d). The column efficiencies on two acryl-POSS-based columns prepared via photoinitiated thiol–acylate polymerization with both “one-pot” and “two-step” modes were far higher than those of previously reported POSS-MA-based columns prepared via thermal-initiated free-radical polymerization.  

The difference in column efficiency of monoliths I and II was mainly attributed to their distinction in morphology and pore size distribution, which was vividly reflected in the Van Deemter curves of alkylbenzenes on monoliths I and II (Table 2). The values of the eddy diffusion term (A-term) for monolith I ranging from 3.77 to 6.48 were slightly smaller than those for monolith II ranging from 5.15 to 7.62, while the values of mass transfer resistance (C-term) for monolith I in the range of 13.1–15.9 were remarkably higher than those for monolith II in the range of 2.52–5.89. It is well-known that the A-term is the main contributor of the peak broadening, especially in the separation of small molecules. Herein, the relatively small values of the A-term were much smaller than those of traditional organic monoliths and silica-based monoliths in LC, implying the limitation of eddy diffusion. Furthermore, the values of the C-term were also lower (<20.0 ms) than those of methacrylate-based monoliths. Compared to monolith I, the smaller values of the C-term for monolith II 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). As a result, due to the combination of two effects, higher column efficiencies were achieved on monolith II in cLC separation of small molecules. It is deduced that the morphology and pore size distribution properties of photo-initiated polyacrylate networks would be altered by the addition of thiol-containing monomers in the “one-pot” mode.

As shown in Figure 3, it is also observed that the efficiency for the strongly retained compounds (such as butylbenzene) was slightly higher than those of weakly retained compounds (such as benzene). This result demonstrated that the two hybrid monoliths revealed a retention-independent performance of small molecules in the isocratic mode. As shown in Table 2, it could be seen that the C-terms of butylbenzene were lower than those of benzene on the two hybrid monoliths. This low mass transfer resistance is possibly attributed to the lack of micropores (the low specific surface areas indicated the lack of micropores in the dry state). It can be deduced that the employment of multifunctional cross-linker (acryl-POSS) prevents the hybrid monoliths from generation of gel-like micropores via free-radical polymerization, which reduced the permeation of small molecules in the gel-like structure and the mass transfer resistance, and finally improved the efficiency of the strongly retained compounds.

By comparison of the retention factors of alkylbenzenes on hybrid monoliths II and III (Figure 3c and Figure S7), the latter exhibited more hydrophobic nature than the former, further indicating that the ODT was successfully postmodified on the surface of monolith II. Figure S8 presents the effect of ACN content in mobile phases on the retention factors of alkylbenzenes on three POSS-based monolithic columns, indicating the RP separation mechanism. The plots of logarithmic retention factors of alkylbenzenes versus the number of saturated carbon atoms in the alkyl chain of alkylbenzenes were all linear, and the methylene selectivities of the three monolithic columns could be calculated, as given in Table S1. It is found that hybrid monolith I exhibited the strongest hydrophobicity among the three monoliths, whose methylene selectivities were determined to be 1.63 and 1.55 at 50% and 55% ACN in mobile phases, respectively. Although the postmodification could increase the hydrophobicity of monolith II, the hydrophobicity of monolith III, whose methylene selectivities were calculated to be 1.46 and 1.39 at 50% and 55% ACN in mobile phases, respectively, was still lower than that of monolith I. These results further proved that more ODT could be incorporated into monolith I in the “one-pot” approach, while ODT could only react with acrylate groups located on the surface of monolith II during the “two-step” process.

### Table 2. Van Deemter Coefficients Measured with Alkylbenzenes for Monoliths I-2 and II-2 in cLC

<table>
<thead>
<tr>
<th>analytes</th>
<th>I-2</th>
<th>II-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>4.49</td>
<td>5.29</td>
</tr>
<tr>
<td>toluene</td>
<td>5.09</td>
<td>5.15</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>6.48</td>
<td>6.67</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>3.77</td>
<td>6.40</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>4.81</td>
<td>7.62</td>
</tr>
</tbody>
</table>
Due to better permeability and stronger hydrophobicity of the C18-functionalized hybrid monolith I prepared with the “one-pot” process, it would be a good RP separation material. The separation of small molecules such as basic and phenolic compounds was performed, and satisfactory results were obtained (Figure S9). Additionally, EPA610 and a mixture of proteins were also separated on hybrid monolith I with gradient mode in cLC (Figure S10). Additionally, the separation of highly complicated samples was also performed on two hybrid monoliths. As illustrated in Figure 4, the tryptic digest of four proteins (BSA, myoglobin, ovalbumin, and α-casein) on hybrid monoliths I-2 (a) and III-2 (b) in cLC−MS/MS analysis. Experimental conditions: column dimensions were (a) 43 cm × 100 μm i.d., (b) 32 cm × 100 μm i.d.; mobile phase A, 0.1% formic acid in water, mobile phase B, 0.1% formic acid in ACN; the gradient was 5−35% B in 85 min, 35−90% in 3 min, retained 90% B for 10 min; flow rate, 60 μL/min. Protein sequence coverage: (a) BSA (75%), myoglobin (68%), ovalbumin (65%), α-casein (39%); (b) BSA (68%), myoglobin (63%), ovalbumin (61%), α-casein (39%).

In order to prove the incorporation of sulfonate group in hybrid monolith IV, a breakthrough curve was measured. A solution of dipeptide Gly-Tyr (238.2 Da) (0.5 mg/mL), which bears a 2− charge at pH 2.3, was used to determine the dynamic binding capacity of monoliths IV and II-2 (13 cm × 75 μm i.d.). As shown in Figure 5c, the dynamic binding capacity of monolith IV was calculated as 71.1 ng/cm, which bears a 2− charge at pH 2.3, was used to determine the dynamic binding capacity of monolith IV and II-2 (13 cm × 75 μm i.d.). As shown in Figure 5c, the dynamic binding capacity of monolith IV was calculated as 71.1 ng/cm. Meanwhile, a sharp increase in the baseline could be observed when the column was saturated (Figure 5b), and the dynamic binding capacity of monolith IV was calculated as 71.1 ng/cm, which was about 3 times as many as that of monolith II-2. These results indicated that the sulfonate group was integrated into hybrid monolith IV, which exhibited ion-exchange mechanism. As shown in Figure S13, five alkylbenzenes could be baseline-separated on monolith IV by using the mobile phase of ACN/H2O (50/50, v/v). It is due to the hydrophobicity of the POSS unit in hybrid monolith IV, indicating a typical RP retention mechanism. However, the methylene selectivities of monolith IV were determined to be 1.37 and 1.32 at 50% and 55% ACN in the mobile phase, respectively (Table S1), and lower than those of monolith II, whose methylene selectivities were 1.39 and 1.34 at 50% and 55% ACN in mobile phases, respectively. These results further demonstrated the successful incorporation of hydrophilic thiol of 3-mercaptopropanesulfonate into monolith II-2 was calculated as 22.9 ng/cm. Meanwhile, a sharp increase in the baseline could be observed when the column was saturated (Figure 5b), and the dynamic binding capacity of monolith IV was calculated as 71.1 ng/cm, which was about 3 times as many as that of monolith II-2. These results indicated that the sulfonate group was integrated into hybrid monolith IV, which exhibited ion-exchange mechanism. As shown in Figure S13, five alkylbenzenes could be baseline-separated on monolith IV by using the mobile phase of ACN/H2O (50/50, v/v). It is due to the hydrophobicity of the POSS unit in hybrid monolith IV, indicating a typical RP retention mechanism. However, the methylene selectivities of monolith IV were determined to be 1.37 and 1.32 at 50% and 55% ACN in the mobile phase, respectively (Table S1), and lower than those of monolith II, whose methylene selectivities were 1.39 and 1.34 at 50% and 55% ACN in mobile phases, respectively. These results further demonstrated the successful incorporation of hydrophilic thiol of 3-mercaptopropanesulfonate into...
acryl-POSS-based monolith via the “one-pot” approach, and this monolith actually exhibited a reversed-phase and ion-exchange mixed-mode retention mechanism.

## CONCLUSION

We have developed a novel “one-pot” approach for rapid preparation of various POSS-containing monolithic columns in a UV-transparent fused-silica capillary via photoinitiated thiol–acyrly polymerization. The resulting hybrid monoliths exhibited good permeability and separation ability for small molecules and proteins in RPLC. In the “one-pot” process, the acrylate monomer not only homopolymerizes, but also couples with the thiol-containing monomer, indicating several advantages over the “two-step” approach. First, reducing the procedures would facilitate enhancement of preparation reproducibility of monolithic columns. Second, the polymerization rate in the “one-pot” approach was much higher than that of the formation of polyacrylate monoliths in traditional photoinitiated free-radical polymerization. It is attributed to the addition of thiol into the “one-pot” thiol–acrylate polymerization, and the major obstacle of oxygen inhibition is essentially eliminated in the thiol–ene (acrylate/methacrylate) polymerization. As a result, ultrarapid photoinitiated polymerization is almost completed in minutes rather than in hours and avoids the tedious works to optimize the preparation conditions; particularly, it is not necessary to consider the effect of temperature on the morphology of monoliths compared with thermal-initiated polymerization. In a word, this unique combination of two types of free-radical polymerization mechanisms offers a novel way to directly fabricate various acrylate-based monolithic materials. It is anticipated that thiol–ene (acrylate/methacrylate) polymerization will be further applied as a useful tool for widespread applications in the area of material science, especially for the synthesis of novel stationary phases in chromatographic research.

## ASSOCIATED CONTENT

1. Supporting Information
   The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01707.
   Experimental procedures, reaction mechanism, and additional figures and table (PDF)

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Notes
The authors declare no competing financial interest.

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