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Facile construction of macroporous hybrid monoliths *via* thiol-methacrylate Michael addition click reaction for capillary liquid chromatography

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ABSTRACT

A facile approach based on thiol-methacrylate Michael addition click reaction was developed for construction of porous hybrid monolithic materials. Three hybrid monoliths were prepared *via* thiol-methacrylate click polymerization by using methacrylate-polyhedral oligomeric silsesquioxane (POSS) (cage mixture, $n = 8$, 10, 12, POSS-MA) and three multi-thiol crosslinkers, 1,6-hexanedithiol (HDT), trimethylolpropane tris(3-mercaptopropionate) (TPTM) and pentaerythritol tetrakis(3-mercaptopropionate) (PTM), respectively, in the presence of porogenic solvents (*n*-propanol and PEG 200) and a catalyst (dimethylphenylphosphine, DMPP). The obtained monoliths possessed high thermal and chemical stabilities. Besides, they all exhibited high column efficiencies and excellent separation abilities in capillary liquid chromatography (cLC). The highest column efficiency could reach *ca.* 195,000 N/m for butylbenzene on the monolith prepared with POSS-MA and TPTM (monolith POSS-TPTM) in reversed-phase (RP) mode at 0.64 mm/s. Good chromatographic performance were all achieved in the separations of polycyclic aromatic hydrocarbons (PAHs), phenols, anilines, EPA 610 as well as bovine serum albumin (BSA) digest. The high column efficiencies in the range of 51,400–117,000 N/m (achieved on the monolith POSS-PTM in RP mode) convincingly demonstrated the high separation abilities of these thiol-methacrylate based hybrid monoliths. All the results demonstrated the feasibility of the phosphines catalyzed thiol-methacrylate Michael addition click reaction in fabrication of monolithic columns with high efficiency for cLC applications.

1. Introduction

Monolithic columns have been widely applied in microscale and nanoscale chromatographic separation as well as sample pretreatment arena [1–7]. As a typical and novel state-of-the-art format, the organic-silica hybrid monoliths, somewhat combining merits of both organic and silica monoliths, such as high permeability, pH tolerance and mechanical strength, have proved to overcome some of the inherent drawbacks of other two types of monoliths, such as the solvent instability of the organic polymeric monoliths and the pH sensitivity of the inorganic silica-based monoliths [8,9]. So far, the organic-silica hybrid monoliths have held an impressively strong position in all chromatographic methods, not only for separation fields such as capillary liquid chromatography (cLC) and capillary electrochromatography (CEC) *etc.* [4,10,11], but also as the basis for enzyme immobilization [12], or for sample preparation such as solid-phase extraction (SPE) or solid-phase micro-extraction (SPME) [13], *etc.* However, the preparation approaches of hybrid monoliths currently are mainly based on the tedious and condition-sensitive sol–gel process, which greatly encumber their development and applications. Thus, it is still a matter of great concern to develop more facile and easier methods for the fabrication of hybrid monoliths.

The polyhedral oligomeric silsesquioxane (POSS) reagents have been successfully used to replace traditional alkoxysilanes for the preparation of hybrid monoliths *via* free radical polymerization, in which the preparation procedures were simplified as easy as those of common polymeric monoliths [14–16]. However, the relatively low column efficiencies of those hybrid monoliths (the highest one still below 50,000 N/m in capillary liquid chromatography (cLC)) [14–16] made it still a great challenge to achieve high performance

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chromatographic separation. In order to address the challenge, ring-opening polymerization has been adopted to facilely fabricate hybrid monoliths by using octaglycidyldimethylsilyl POSS (POSS-epoxy) and diamines [17,18]. Such POSS-epoxy-diamine hybrid monoliths possessed well-defined 3D skeleton, and exhibited high column efficiency (highest *ca.* 140,000 N/m) in cLC. The success of epoxy-amine ring-opening polymerization in the preparation of hybrid monoliths greatly inspired us that introducing new chemistry to replace the traditional free radical polymerization may possibly translate the various methacrylate monomers into monoliths with high column efficiency.

Due to the high yields and simple reaction conditions, "click" chemistry, such as Cu(I)-catalyzed 1,3-dipolar azide-alkyne cycloaddition reaction (CuAAC) and thiol-click reaction, has been highlighted and widely applied in new compounds synthesis, polymer preparation, surface modification, hydrogel formation and so on [19–25]. Particularly, click chemistry has become a very useful and popular tool to modify both particle beads and monolithic matrix for the preparation of novel LC stationary phases [26–33]. However, so far, there are very few reports on the direct construction of macroporous monoliths *via* click chemistry. More recently, Nischang and coworkers have synthesized several hybrid monolithic materials from vinyl-containing POSS and five multi-thiols *via* radical-mediated thiol-ene click chemistry [34]. However, such a radical-mediated thiol-vinyl reaction could not be applied for preparation of monoliths using methacryl-containing reagents, as the methacrylate groups exhibit low reactivity with thiols in radical-mediated system, in which a serious self-polymerization of methacrylate is preferred [35].

In this study, a facile approach for direct construction of a series of macroporous hybrid monoliths was firstly developed by using the multi-methacrylate POSS and multi-thiols as precursor *via* phosphines catalyzed thiol-methacrylate Michael addition click reaction. Three hybrid monoliths were conveniently synthesized by slightly adjusting the feed recipes. Systematical characterization of the monoliths, such as SEM and FT-IR, *etc.* were performed. Comprehensively chromatographic assessments and applications of these monoliths were also carried out by cLC separation of polycyclic aromatic hydrocarbons (PAHs), phenols, anilines, EPA 610 and bovine serum albumin (BSA) digest.

2. Materials and methods

2.1. Chemicals and reagents

Methacrylate-polyhedral oligomeric silsesquioxane (POSS) (cage mixture, $n = 8$, 10, 12, POSS-MA), trimethylolpropane tris(3-mercaptopropionate) (TPTM), pentaerythritol tetrakis(3-mercaptopropionate) (PTM), stearyl methacrylate (SMA), 3-methacryloxypropyltrimethoxysilane (γ-MAPS) and poly(ethylene glycol) (PEG, $M_n = 200$) were purchased from Aldrich (Milwaukee, WI, USA). 1,6-Hexanedithiol (HDT), dimethylphenylphosphine (DMPP) and potassium hydroxide (KOH) were obtained from J&K Scientific (Beijing, China). 1,2-Epoxyoctadecane (C18-epoxy) was supplied by Alfa Aesar (Ward Hill, MA, USA). Trypsin was purchased from Promega (Madison, WI, USA). Bovine serum albumin (BSA), EPA 610, thiourea, alkylbenzenes and other standard compounds were supplied by 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, 75 and 200 μm i.d. and 365 μm o.d. were obtained from Refine Chromatography Ltd. (Yongnian, Hebei, China). C18-particles (5 μm, 120 Å pore) were purchased from DAISO (Osaka, Japan). 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). Methanol, *n*-propanol, NaOH, HCl and other chemical reagents were all of analytical grade.

2.2. Pretreatment of fused-silica capillary

Before the preparation of monolithic columns, the inner wall of fused-silica capillary was activated. First, the capillary was rinsed by 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, to modify the capillary inner wall with double bonds, the capillary was filled with a mixture of γ-MAPS/methanol (50%, v/v), sealed with rubbers at both ends and submerged in water bath at 50 °C for 12 h. Finally, the capillary was rinsed with methanol and flush out the residual reagents, and dried again with a stream of nitrogen gas.

2.3. Preparation of hybrid monoliths via thiol-methacrylate click chemistry

The prepolymerization solutions were prepared by mixing POSS-MA, a multi-thiol (HDT or TPTM or PTM), porogenic solvents (*n*-propanol and PEG 200) and catalyst (DMPP) with appropriate feed recipes, as listed in Table 1 and Table S1 (SI). After 5 min of sonication at 0 °C for mixing and degassing, the prepolymerization mixtures were injected into the modified capillaries with a syringe. The filled capillaries were then sealed with rubber stoppers and reacted in water baths at appropriate temperature for 2 h. After that, the capillaries were flushed with methanol to remove the unreacted residual. For the preparation of bulk monolithic materials, the polymerization reaction was taken place in centrifuge tubes. The centrifuge tubes filled with the prepolymerization mixtures were placed in water baths under appropriate temperature for 2 h. After polymerization, the bulk monoliths were cut into smaller pieces, extracted with ethanol overnight in a Soxhlet apparatus and dried in a vacuum.

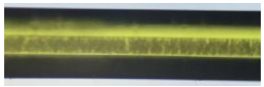
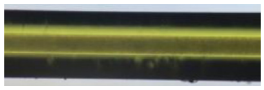
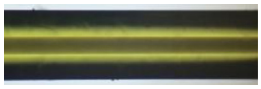
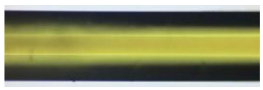
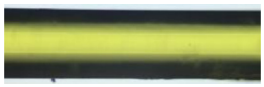
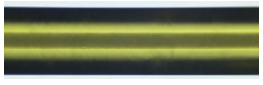
2.4. Post-modification of hybrid monolithic column

To modify the hybrid monolithic column, a *n*-propanol solution containing 0.1 mol/L C18-epoxy and 0.01 mol/L KOH was pumped through the column at room temperature overnight. Then the modified column was washed with ethanol to remove the unreacted residual.

2.5. Capillary liquid chromatography

The chromatographic evaluation of monolithic columns was performed on a LC system equipped with an Agilent 1100 (Hewlett-Packard) micropump, a UV detector (K-2501, Knauer, Germany) and a 7725i injector with a 20 μL sample loop. In order to avoid the sample overloading, the partial injection was adopted in this study. Data was collected at 214 nm or 254 nm, and processed by a chromatography workstation (Beijing Cailu Scientific Instrument Ltd., Beijing, China). 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 columns was connected with a Teflon tube to an empty fused-silica capillary (75 μm i.d. and 365 μm o.d.). 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.

Table 1
Effects of synthesis parameters on the formation of POSS-PTM hybrid monoliths[a]

Monolith	*n*-Propanol[b] (wt%)	PEG 200[c] (wt%)	Catalyst[d,e] (wt%)	Temp (°C)	Optical microscope image	Permeability[f] ($\times 10^{-14}$ m^2)
A	60	36	4	50		18.20
B	54	42	4	45		1.97
C	54	42	4	50		1.01
D	54	42	4	55		Too hard to pump through
E	48	48	4	50		Too hard to pump through
F	56	42	2	50		0.37
G	52	42	6	50		3.12

[a] The amounts of POSS-MA and PTM were kept at 35 mg and 25 mg, respectively.
[b] Weight percentage of *n*-propanol in the porogenic solvents.
[c] Weight percentage of PEG 200 in the porogenic solvents.
[d] Weight percentage of the catalyst in the porogenic solvents.
[e] The catalyst was DMPP/*n*-propanol solution (0.7 mol/L).
[f] Permeability, $B_0 = F\eta L/(\pi r^2 \Delta P)$, 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 [56], measured under room temperature.

2.6. Other instruments for characterization

SEM images were obtained by using a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). FT-IR spectra were measured with a Bruker Tensor 27 FT-IR spectrometer (Bruker Daltonics, Ettlingen, Germany). Thermal gravimetric analysis was carried out on a Setsys 16/18 (Setaram, Caluire, France). Water contact angles were measured with an OCA20 contact angle system (Dataphysics, Germany) at ambient temperature. Nitrogen adsorption/desorption measurements of dry bulk monoliths were performed on a Quadrasorb SI surface area analyzer and pore size analyzer (Quantachrome, Boynton Beach, USA). Pore size measurement was carried out on a PoreMasre GT-60 (Quantachrome, Boynton Beach, USA).

2.7. Preparation of BSA tryptic digest and cLC–MS/MS analysis

The tryptic digest of BSA and cLC–MS/MS analysis were performed according to procedures previously reported by us with minor modifications [36]. The sample trapping was achieved with a homemade C18-particle-packed trap column (4 cm length × 200 μm i.d.), and the subsequent separation was carried out on either a thiol-methacrylate click chemistry based hybrid monolith (30 cm length × 75 μm i.d.) or a C18-particle-packed column (15 cm length × 75 μm i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of capillary [37]. (Detailed experimental procedures are provided in the Supporting Information.)

3. Results and discussion

3.1. Preparation of hybrid monoliths via thiol-methacrylate click chemistry

The multi-methacrylate POSS (POSS-MA) has been successfully adopted to prepare hybrid monoliths *via* free radical polymerization [14–16,38]. However, the faster homopolymerization of methacrylate groups make it unpractical to direct construction of hybrid monoliths from POSS-MA and multi-thiols *via* radical-mediated thiol-methacrylate reaction. What's more, the radical-mediated thiol-methacrylate reaction even cannot be considered as a click-type reaction for polymer-polymer conjugation chemistry due to the formation of significant side products [25,39]. Oppositely, the phosphines catalyzed thiol-methacrylate Michael addition reaction is more feasible as which yields complete conversion in a few minutes under mild conditions [40]. Such reaction is, therefore, of interest in our effort to direct synthesis of hybrid monoliths from POSS-MA and multi-thiols.

As shown in Fig. 1, three hybrid monoliths were successfully synthesized by using POSS-MA and three multi-thiol monomers (HDT, TPTM and PTM) as precursors, respectively, *via* thiol-methacrylate Michael addition click reaction. As the component of

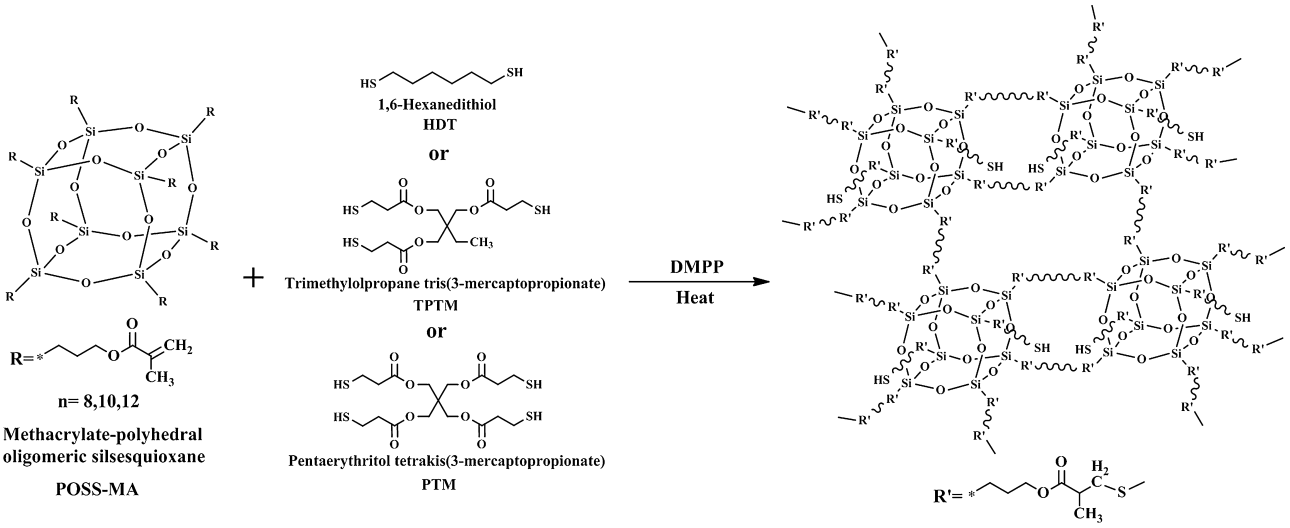


Fig. 1. Schematic preparation of hybrid monoliths *via* thiol-methacrylate Michael addition click reaction.

prepolymerization mixture and the reaction condition would have significant influences on the morphology of hybrid monoliths, a systematic optimization of these parameters was performed. Due to the high efficiency and conversion of thiol-click reaction, a nearly equimolar amount of methacrylates and thiols was first adopted to prepare the hybrid monolith POSS-PTM. Then, we examined several kinds of porogenic systems, including *n*-propanol/1,4-butanediol, toluene/dodecanol and cyclohexaneol/decanol, *etc.* Finally, the mixture of *n*-propanol/PEG 200 was selected due to its favorable solubility, and a series of columns were fabricated, as shown in Table 1. It could be found that the permeability was significantly decreased from 18.20 to 1.01×10^{-14} m^2 as the content of PEG 200 in the porogenic mixture (*n*-propanol/PEG 200) was increased from 36 to 42% (wt%) (as columns A and C). If the amount of PEG 200 was further increased to 48% (wt%), the column could not even be pumped through (as column E). Thus, the PEG 200 acted as the good solvent in the porogenic system, correspondingly the *n*-propanol served as the poor solvent. Due to the fast reaction rate of thiol-methacrylate Michael addition reaction initiated by phosphines [25,41], the amount of the catalyst, DMPP was investigated carefully. As seen from the columns F, B and G (Table 1), the permeability was slightly increased from 0.37 to 3.12×10^{-14} m^2 when the content of DMPP increased. It is possibly because that a larger feeding amount of DMPP would lead to a faster polymerization rate, generating many oligomers. The large amount of oligomers would further initiate an earlier phase separation process, finally forming larger through pores (macropores) in the monolith. Although a higher temperature would also accelerate the reaction rate, it has an opposite effect on the morphology of monolith. Increasing the temperature (from 45 to 55 °C) would result in the remarkable decrease of the permeabilities (columns B–D in Table 1). It is because the higher temperature would improve the solubility of the porogenic system, and the phase separation process would be later emerged. As a result, the oligomers would be further covalently linked to form highly crosslinked polymers, finally forming a more compact monolith. After comprehensive consideration of the influences of these parameters, the column B in Table 1 was employed in the following experiments.

Similar to the preparation of monolith POSS-PTM, the feed recipes of monoliths POSS-HDT and POSS-TPTM were also optimized. As summarized in Table S1 (SI), the preparation conditions were employed to prepare these kinds of hybrid monoliths for further experiments.

3.2. Characterization of three hybrid monoliths

The cross-sectional morphologies of three hybrid monolithic columns were shown in Fig. 2. It could be found that all monolithic matrices were tightly anchored to the inner wall of capillaries without any disconnection, and the macropores of 1–2 μm and a skeleton of ~1 μm in diameter were also observed (Fig. 2 and Fig. S1, SI). Particularly, the morphlogies of these thiol-methacrylate click chemistry based hybrid monoliths were remarkably distinct from the cauliflower-like microglobules of other POSS-MA based hybrid monoliths prepared *via* free radical polymerization, but similar to the bicontinuous-like skeletons typical of silica-based monoliths prepared by sol–gel chemistry.

Fig. S2 in Supporting Information indicates the FT-IR spectrum of the hybrid monolith POSS-PTM. The increase of peak at 697 cm^{-1} (assigned to the carbon sulfur bonds) and the decrease (or almost disappearance) of peaks at 1637 and 2569 cm^{-1} (assigned to the stretching vibrations of —C=C— and S—H groups, respectively) demonstrated the occurrence of thiol-methacrylate Michael addition click reaction. Besides, the remarkable decrease (almost disappearance) of the characteristic absorption peaks of methacrylate and thiol groups revealed nearly total consumption of these two functional groups, further confirming the high reactivity of thiol-methacrylate Michael addition reaction. In addition, the effluent of monolith POSS-PTM was also analyzed by MALDI-TOF. As shown in Fig. S3 (SI), it could be found that some oligomers with molecular mass less than 3000 Da were detected. The significant mass difference of 488.66 Da, which was corresponded to the PTM monomer, further convincingly demonstrated the occurrence of thiol-methacrylate click reaction.

Generally, a higher crosslinking degree could enable the monolith better thermostability. It could be found from the thermogravimetric analysis (Fig. S4, SI) that no significant weight loss was occured until the temperature reached 320 °C, and the pyrolysis continued up to 650 °C for hybrid monolith POSS-PTM, indicating better thermostability than those of hybrid monoliths reported previously [17,42–44]. The high thermostability may be ascribed to the high efficiency and conversion of thiol-methacrylate Michael addition click reaction, generating highly crosslinked monoliths.

3.3. Chromatographic evaluation of hybrid monoliths in cLC

Currently, monolithic columns have gained substantial interest in flow-through system [45,46]. Herein, by utilizing the macro

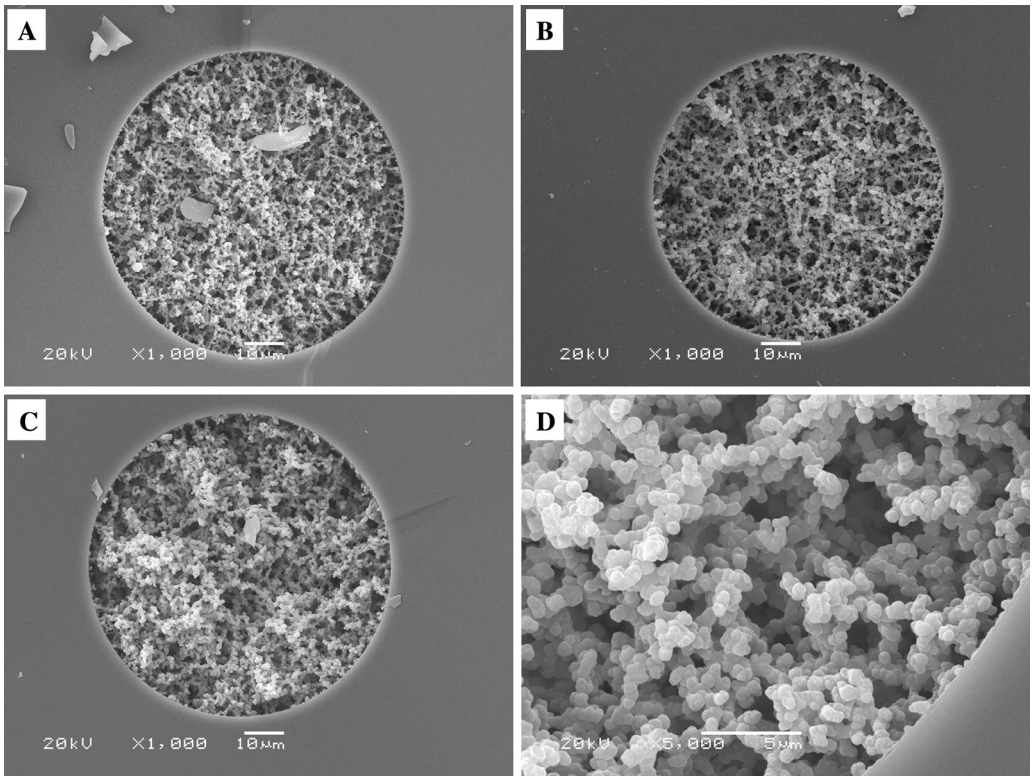


Fig. 2. The cross-sections of the thiol-methacrylate click chemistry based hybrid monolithic columns (A) POSS-HDT, (B) POSS-TPTM and (C) and (D) POSS-PTM. Magnification: (A)–(C), 1000×; (D), 5000×.

through-pores and hydrophobic surfaces, the resulting hybrid monoliths were applied for reversed phase (RP) cLC. Taking the aforementioned hybrid monolith POSS-PTM as an example, five alkylbenzenes were successfully baseline-separated according to their hydrophobicities in the mobile phase of ACN/water (60/40, v/v) (as shown in Fig. 3A), revealing a typical RP retention mechanism. The highest efficiency *ca.* 142,000 N/m was achieved for butylbenzene at 0.82 mm/s (as shown in Fig. 3B). Highly efficient separations of alkylbenzenes were also performed on other hybrid monoliths, POSS-HDT and POSS-TPTM (Figs. S5 and S6, SI). It is worth noting that the highest column efficiency of *ca.* 190,000 N/m for butylbenzene was achieved on hybrid monolith POSS-TPTM at 0.64 mm/s (as shown in Fig. S6, SI). These good chromatographic performances demonstrated the potential of thiol-methacrylate Michael addition click reaction in the preparation of monoliths with high column efficiency.

It is well known that the column efficiency depends on the microstructure of the monolithic columns. As observed from the SEM images (Fig. 2 and Fig. S1, SI), all monolithic columns possess uniform through-pores of 1–2 μm and narrow skeleton of ~1 μm. The large through-pore/skeleton size ratio would result in a high permeability (*e.g.* 1.01×10^{-14} m^2 for monolith POSS-PTM) and a large external porosity (*e.g.* 71.6% for monolith POSS-PTM), enabling fast mass transfer due to the convection during the separation process [2,47]. Besides, the thin skeletons would provide a shorter diffusion distance and greatly accelerate the mass transfer, leading to a lower C_s term [1,46,48–52]. The combination of these two effects finally resulted in a small *C* term and improved

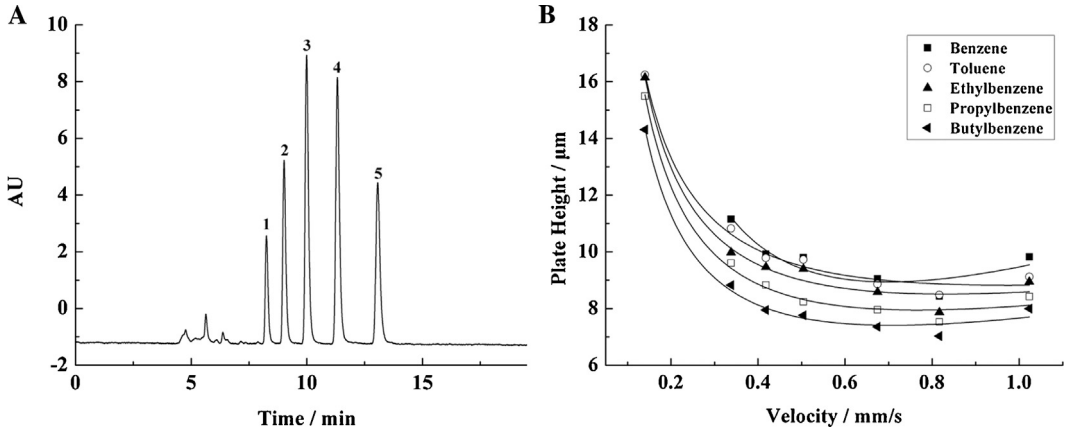


Fig. 3. (A) Separation of alkylbenzenes and (B) dependence of plate heights on the linear velocity of mobile phase on the hybrid monolithic column POSS-PTM in cLC. Analytes: (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene; Experimental conditions: column dimension, 24.2 cm × 75 μm i.d.; mobile phase, ACN/water (60/40, v/v); flow rate, 220 nL/min (after split); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

Table 2
van Deemter coefficients obtained after fitting the plot of HTEP for alkylbenzenes on hybrid monolith POSS-PTM.

	A/μm	B/μm^2 s^{-1}	C/ms
Benzene	0.72	2877.64	5.87
Toluene	5.90	1424.52	1.50
Ethylbenzene	4.79	1546.77	2.24
Propylbenzene	3.81	1589.08	2.69
Butylbenzene	3.15	1509.20	3.00

the chromatographic performances of these hybrid monoliths. This consequence was demonstrated by the plot of HTEP for alkylbenzenes on monolith POSS-PTM (Table 2). The low *C* terms ranged from 1.5 to 5.9 ms indicated a good communication between the stationary phase and analytes. Unfortunately, due to the serious shrink of these bulk monoliths in dry state, the macropore size measurement (Fig. S7, SI) could not accurately reflect the real microstructure. However, the low surface areas (all < 10 m^2/g, *e.g.* 9.8 m^2/g for hybrid monolith POSS-PTM) would partly imply a rich of macropores and a lack of mesopores in these monoliths. Thus, it was supposed that the microstructures were more homogeneous in these monoliths than those in other POSS-MA-based monoliths prepared *via* free radical polymerization. The homogeneous microstructure may ascribe to the ordered polymerization manner of thiol-methacrylate click chemistry. In view of the nearly equal consumption of methacrylate and thiol groups, it could be deduced that a step-growth polymerization was favored in the formation of these monoliths [34,53–55], which presented several significant advantages over the classical free radical polymerization. Particularly, the step-growth polymerization favors the formation of multiple low-molecular-weight species prior to gelation, resulting in high conversions at the gel point and largely uniform, homogeneous networks [25,55]. The hybrid thiol-methacrylate based monoliths with better chromatographic performance in the flow-through system were thereby obtained.

As shown in Fig. S8 (SI), the measured back-pressure was increased linearly ($R > 0.999$) even over 42.0 MPa with an increase of flow rate, indicating the potential of these monoliths in fast cLC separation at high pressure. In addition, the run-to-run repeatability and day-to-day reproducibility for column POSS-PTM were evaluated through the relative standard deviation (RSD) for the retention factor (k) of benzene as the model analyte (thiourea as the void time marker), reached 1.89% ($n = 15$) and 1.95% ($n = 5$), respectively, indicating good stability and reproducibility. Meanwhile, significant decrease of column efficiency or clear column deterioration was not observed on monolith POSS-PTM even after continuously using for several weeks, and the retention factor and column efficiency could remain about 93% and 90%, respectively (benzene as the test compound), which further demonstrated the high robustness and stabilities of these monoliths.

3.4. Pre- and post-modification of hybrid monoliths

The high versatility of this thiol-methacrylate Michael addition click reaction based approach enables the preparation of monoliths with tailor-made chemical and physical properties. The hybrid monolith could also be prepared by using ternary precursors (POSS-MA, PTM and SMA (5 μL)). As shown in Fig. S9 (SI), five alkylbenzenes were also baseline-separated on the resulting hybrid POSS-PTM-SMA column in the mobile phase of ACN/water (60/40, v/v) at the flow rate of 320 nL min^{-1} (after split), and the column efficiency of butylbenzene reached *ca.* 142,400 N/m. The good chromatographic performance reflected a well maintenance of uniform microstructure of the hybrid monolith prepared with ternary precursors. By comparison of two hybrid monoliths, POSS-PTM-SMA and POSS-PTM, the retention factors of alkylbenzenes on the monolith POSS-PTM-SMA was slightly higher than those on the monolith POSS-PTM (Fig. S10, SI). Meanwhile, the water contact angle of the monolith POSS-PTM-SMA was also higher than that on the monolith POSS-PTM (Fig. S11, SI). Moreover, the thermal stability of the monolith POSS-PTM-SMA was also measured. By comparison of the ceramic yield, it could be found that the ceramic yield for the hybrid monolith POSS-PTM-SMA was lowered than that of monolith POSS-PTM (Fig. S12, SI). These results indicated that the functional monomer, SMA, was simultaneously introduced in the formation of hybrid monolith.

Although thiol-methacrylate Michael addition click reaction is highly efficient, it is deduced that part of thiol groups would be possibly exposed in the surface (Fig. 1). For verification of this hypothesis, the hybrid monolith POSS-PTM was further modified with C18-epoxy *via* thiol-epoxy click reaction. The significant increase of retention factors of five alkylbenzenes on the C18-modified monolith POSS-PTM well corroborated the hypothesis (Fig. S13, SI).

3.5. Application of hybrid monoliths in cLC

For an overall evaluation of the potential of these monoliths in chromatographic application, separations of polycyclic aromatic hydrocarbons (PAHs), phenols, and anilines were carried out. As shown in Fig. 4A, the PAHs were baseline-separated according to their hydrophobicity on the hybrid monolith POSS-PTM with the mobile phase of ACN/water (60/40, v/v), and the column efficiencies were ranged from 94,200 to 114,000 N/m (in the order from naphthalene to *p*-terphenyl). Besides, six phenols were also separated with the column efficiencies ranged from 55,500 to 81,400 N/m (in the order from hydroquinone to 4-*tert*-butylphenol) (Fig. 4B). Particularly, two positional isomers, pyrocatechol and hydroquinone, were baseline-separated, demonstrating the potential of the hybrid monolith (POSS-PTM) in separation of positional isomers. Additionally, six anilines, benzidine, 2,4-diaminotoluene, 4-aminobiphenyl, 2,4-dinitroaniline, 2-nitroaniline and 2,6-dichloro-4-nitroaniline, were well-separated on the hybrid monolith POSS-PTM without any peak tailing (Fig. 4C). The column efficiencies in the range of 51,400–117,000 N/m were achieved for these basic compounds, and increased with their elution order. (the column efficiencies increased in the order of benzidine, 2,4-diaminotoluene, 4-aminobiphenyl, 2,4-dinitroaniline, 2-nitroaniline and 2,6-dichloro-4-nitroaniline). All these results demonstrated the strong separation ability of hybrid monolith POSS-PTM for various kinds of small molecules.

For further assessment of the separation abilities of these monoliths, the analysis of two complex samples, EPA 610 and BSA digest was subsequently performed. As shown in Fig. 5A, 16 priority pollution PAHs were well-separated on hybrid monolith POSS-PTM in gradient mode including 12 baseline-separated ones and four partially separated ones, and the peak capacity was calculated at 36. For BSA digest, the hybrid column (POSS-PTM) also showed good separation ability when coupling into a cLC–MS/MS system (as shown in Fig. 5B). Based on the database search of the chromatogram, 69 unique peptides were positively identified with protein sequence coverage of 67.8%, which was comparable to the results obtained on a C18-particle-packed column, where 64 unique peptides were positively identified with protein sequence coverage of 66.9% (Fig. S14, SI). What's more, better separation performance on the hybrid monolith could be further improved by using a longer column due to its good permeability and lower back-pressure. All these results demonstrated the potential of these thiol-methacrylate click chemistry based hybrid monoliths in the separation of highly complicated environmental and biological samples.

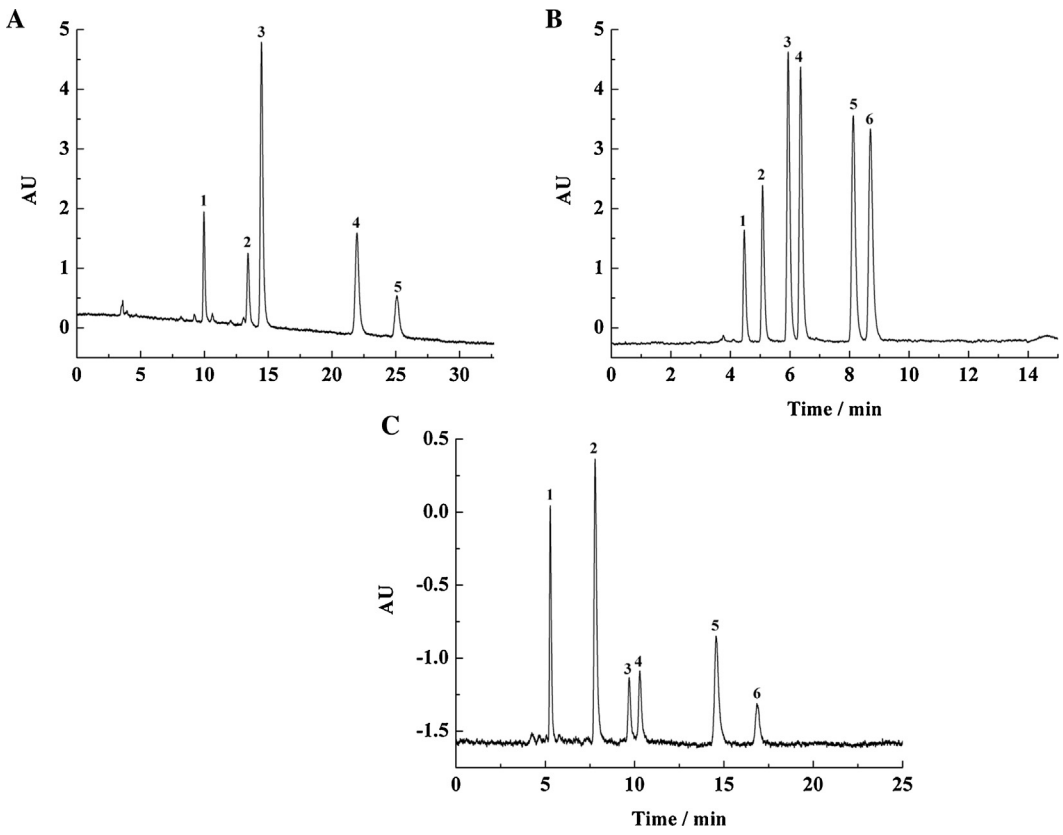


Fig. 4. Separations of (A) PAHs, (B) phenols and (C) anilines on the hybrid monolith POSS-PTM in cLC. Analytes: (A), (1) naphthalene, (2) acenaphthene, (3) 4,4′-dimethylbiphenyl, (4) pyrene, (5) p-terphenyl; (B), (1) hydroquinone, (2) pyrocatechol, (3) phenol, (4) 4-cresol, (5) 2,6-xylenol, (6) 4-tert-butylphenol; (C), (1) 2,4-diaminotoluene, (2) benzidine, (3) 2-nitroaniline, (4) 2,4-dinitroaniline, (5) 4-aminobiphenyl, (6) 2,6-dichloro-4-nitroaniline; Experimental conditions: column dimension, 24.2 cm × 75 μm i.d.; mobile phases for (A), ACN/water (60/40, v/v), for (B, C), ACN/water (50/50, v/v); flow rates for (A), 300 nL/min, for (B), 280 nL/min, for (C), 250 nL/min (after split); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

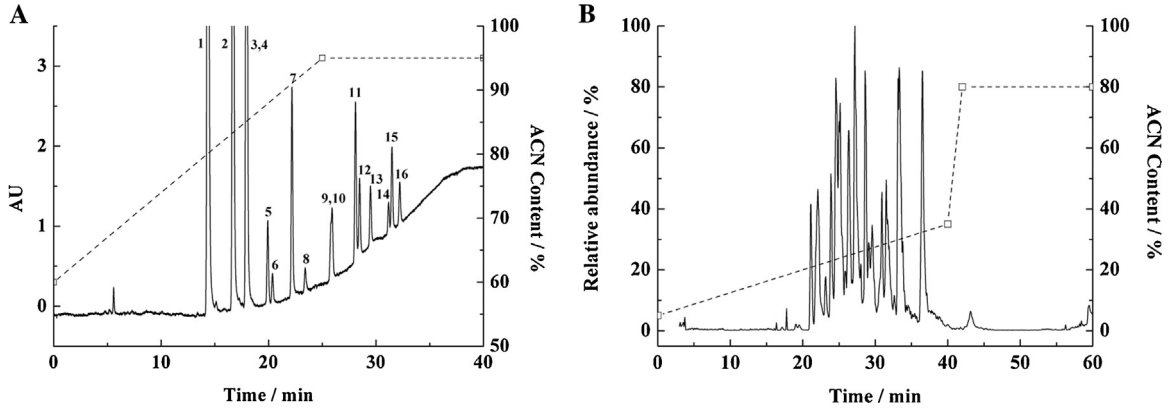


Fig. 5. Separations of (A) EPA 610 and (B) BSA digest on the hybrid monolithic column POSS-PTM in cLC and cLC–MS/MS, respectively. Analytes: (A) (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo(*a*)anthracene, (10) chrysene, (11) benzo(*b*)fluoranthene, (12) benzo(*k*)fluoranthene, (13) benzo(*a*)pyrene, (14) dibenzo(*a,h*)anthracene, (15) benzo(*g,h,i*)perylene, (16) indeno(1,2,3-*cd*)pyrene; Experimental conditions: column dimensions for (A), 24.0 cm × 75 μm i.d., for (B), 30.0 cm × 75 μm i.d.; gradients for (A), 40% water to 95% ACN in 25 min, for (B), 95% water (containing 0.1% formic acid) to 35% ACN (containing 0.1% formic acid) in 40 min, then to 80% ACN (containing 0.1% formic acid) in 2 min; flow rate, 200 nL/min (after split); injection volumes for (A), 2.5 μL in split mode, for (B), 0.5 μg; detection wavelength for (A), 254 nm.

4. Conclusions

In summary, we have demonstrated a facile approach for fabrication of a series of hybrid monoliths from POSS-MA and multifunctional thiols *via* DMPP catalyzed thiol-methacrylate Michael addition click reaction. The obtained hybrid monoliths not only possessed high chemical stability, but also exhibited excellent separation efficiency in the flow-through system. Analytes from simple small molecules including alkylbenzenes, PAHs, phenols and anilines to complex samples such as EPA 610 and BSA digest were all well separated with high column efficiencies, the highest one even can reach *ca.* 190,000 N/m (for butylbenzene). Besides, the high flexibility of this thiol-methacrylate Michael addition click reaction based approach as well as the high surface tailorability of monoliths raised the feasibility to prepare monolithic materials with diverse chemical properties *via* pre- or post-modification.

Further assessment of the versatility of this novel approach as well as other thiol-click chemistries in preparation of diverse monolithic materials for a myriad of application is currently explored and would be published in the near future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.12.031.

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