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Photoinduced thiol–ene polymerization reaction for fast preparation of macroporous hybrid monoliths and their application in capillary liquid chromatography†

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Hybrid monoliths with a macroporous structure were prepared within a few minutes *via* a photoinduced thiol–ene polymerization reaction, the surfaces of which showed hydrophobic character. The monolithic column demonstrated good separation performance towards alkylbenzenes, peptides, proteins and BSA tryptic digest in cLC.

Monolithic materials with continuous porous structures continue to receive widespread research interest, and have been successfully applied as catalysts, adsorbents, separation media and storage supports.[1] In particular, in the field of separation media, three typical monoliths (polymeric, inorganic silica and organic–inorganic) have been prepared by *in situ* polymerization inside fused-silica capillaries and have achieved excellent performance in the separation of small molecules and biomacromolecules.[2] Polymeric monoliths are facile to prepare, and generally exhibit wide pH tolerance but low column efficiency. The sol–gel technique is a common approach to fabricate inorganic silica monoliths which demonstrate large surface areas and column efficiencies. However, the preparation requires a multi-step process and skilled researchers to operate. To balance the preparation technique, mechanical properties and separation performance, organic–inorganic hybrid monoliths have emerged as good candidate matrices, and have been prepared by many approaches such as sol–gel, free radical polymerization, ring-opening polymerization and so on.[3] However, compared to the thermally initiated process for the above-mentioned methods, there have been fewer studies on photoinitiated polymerization, which can be almost complete within minutes rather than hours and avoids tedious studies to investigate the influence of temperature on the hybrid monolith morphology.[4]

Click reactions, such as Cu(I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition reactions (CuAAC) and thiol–ene reactions, have been widely used in polymer and dendrimer synthesis, bioconjugation and surface modification.[5] In particular, the thiol–ene reaction does not require additional transition metal catalysts and can be carried out rapidly under radical conditions or nucleophilic catalysis. Over the past decade, considerable attention has been focused on studying the relative reactivity, control and specificity of the thiol–ene reaction.[5d] There are a few studies on the use of the thiol–ene polymerization reaction for directly preparing porous monoliths. Recently, Nischang *et al.* have synthesized hybrid porous materials by a thiol–ene reaction of polyhedral oligomeric vinylsilsesquioxane (vinylPOSS) and thiol monomers.[6] Although these monoliths were also prepared in capillaries by thermally initiated polymerization, their column efficiencies and application in the analysis of complicated samples were not investigated. The significant porous structure of hybrid monoliths for high efficiency separation needs to be carefully controlled and optimized. Herein, we report several hybrid monoliths with macroporous structures prepared with two types of silicon-containing tetravinyl monomers *via* a photoinduced thiol–ene polymerization reaction within a few minutes. The obtained monolithic capillary columns exhibited good mechanical strength, solvent and thermal stability, and were successfully used to separate complicated samples in cLC.

The preparation of macroporous hybrid monoliths was shown in Scheme 1, and the detailed composition of prepolymerization mixtures was listed in Table 1. The polymerization was initiated by UV light (λ = 365 nm) in the presence of 2,2-dimethoxy-2-phenylacetophenone (DMPA, as a photoinitiator). Fourier-transform infrared spectroscopy (FT-IR) confirmed the thiol–ene polymerization reaction of the tetravinyl monomer with the multi-thiol monomer by the decrease in or almost complete disappearance of signals corresponding to the vinyl group (1592 cm^{-1}) and the thiol group (2555 cm^{-1}) (Fig. S1, ESI†). For monoliths **III** and **IV**, the obvious absorption band at 1740 cm^{-1} ($\nu_{C=O}$) suggested the introduction of the 4SH monomer. As for monolith **II**, we selected a 1.27 : 1 functional group ratio of vinyl to thiol, and a weak signal at 1592 cm^{-1} was still observed, indicating residual vinyl groups in the obtained monolith.

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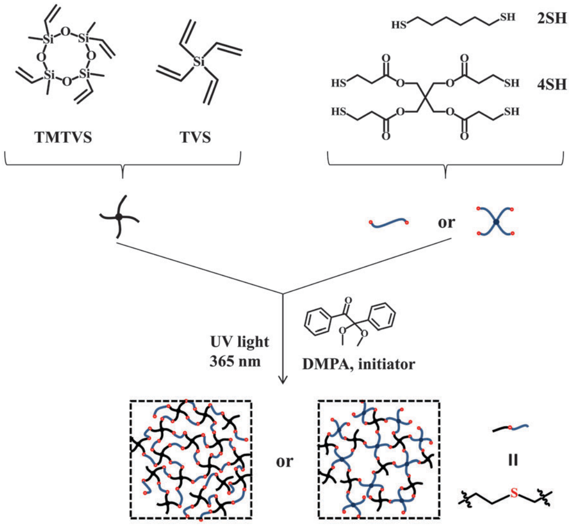

Scheme 1 Preparation of monoliths based on a photoinduced thiol–ene polymerization reaction.

Table 1 Detailed composition of prepolymerization mixtures and the properties of the resulting hybrid monoliths

Monolith[a]	Vinyl/mg	Thiol/mg	Porogenic solvents[b]	Contact angle	k^d
I	25.0	21.3	80/100	134°	3.5
II	15.5	26.8	150/30[c]	131°	2.7
III	32.4	35.0	100/140	120°	1.8
IV	17.0	45.0	103/137	121°	0.9

[a] Monoliths: **I**-(TMTVS + 2SH), **II**-(TVS + 2SH), **III**-(TMTVS + 4SH), **IV**-(TVS + 4SH). [b] DEGDE/1-propanol (v/v). [c] 1-Propanol was replaced with tetrahydrofuran for **II**. [d] Retention factor: butylbenzene as the model analyte and thiourea as the void time marker, acetonitrile/water (60/40, v/v) as the mobile phase. TMTVS: 2,4,6,8-tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane; TVS: tetravinylsilane; 4SH: pentaerythritol tetrakis(3-mercapto-propionate); 2SH: 1,6-hexanedithiol; DEGDE: diethylene glycol diethyl ether.

After removal of residuals and drying of the opaque bulk monoliths, scanning electron microscopy (SEM) micrographs showed macroporous structures with pores of around 1 μm (Fig. 1a–d and Fig. S2, ESI†). However, for monolith **IV**, a nanoglobular aggregated morphology was obtained, while a continuous reticular skeleton was observed in monolith **II**. The difference in morphology may result from the phase separation process. The 4SH monomer is more reactive than the 2SH monomer, which may result in a high local degree of polymerization and form crosslinked nanoglobular polymers separating from solvents.[7] Additionally, in the present work, the porogenic solvent DEGDE acted as a good solvent for the preparation of monoliths **I**, **III** and **IV**, but a poor solvent for monolith **II**. Therefore, 1-propanol was replaced with tetrahydrofuran (THF) to tailor smaller pore sizes, and the permeability of monolith **II** decreased with increasing THF content (Fig. S3, ESI†). What's more, a good linear relationship between flow rate and back pressure drop was maintained even at high pressure, suggesting a great mechanical strength. PEG200 was also selected as a phase separator, and a monolith of similar morphology but with a smaller pore size was obtained by the replacement of 1-propanol (Fig. S2, ESI†). However, the monolith with the smaller pore size tended to shrink while being gradually dried.

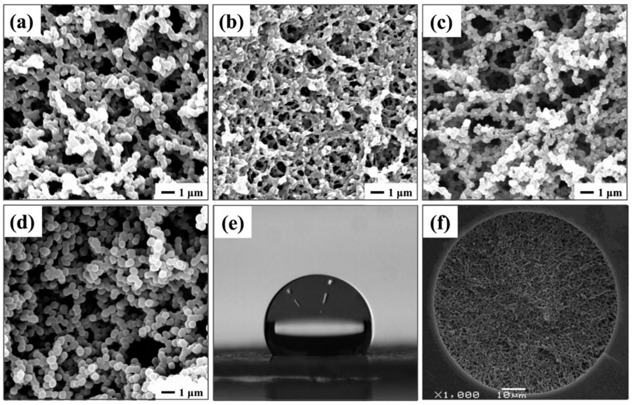

Fig. 1 SEM micrographs of hybrid monoliths **I** (a), **II** (b), **III** (c) and **IV** (d); (e) water contact angle of the surface of monolith **IV**; (f) hybrid monolith **II** in a UV-transparent capillary.

The thermal stability of the four monoliths was investigated by thermogravimetry (TG) (Fig. S4, ESI†). An endothermic mass loss began at 300 °C and continued up to 500 °C due to the pyrolysis of organic moieties under an air atmosphere. The hypothesis that the final solid residue was silicon dioxide was adopted to calculate the silicon content in the obtained monoliths. The result indicated that the calculated values were lower than the theoretical values (Table S1, ESI†). Such a difference may be related to the removal of unreacted tetravinyl monomer. The information of carbon loading was also measured by elemental analysis (Table S2, ESI†). Nevertheless, silicon was introduced into the monoliths, which possibly contributes to enhance the mechanical strength of the monolithic columns.

The surface properties of monoliths have a significant effect on the separation mechanism, and their hydrophilic/hydrophobic characters were determined by their water contact angle. The values ranged from 120° to 134° (Table 1), which suggested the four monoliths exhibited hydrophobicity to some extent. So we prepared the monoliths in vinyltrimethoxysilane pretreated UV-transparent capillaries (otherwise void formation was observed near the inner wall; Fig. S5, ESI†) for reversed-phase liquid chromatography (RPLC). Alkylbenzenes were selected as probes. By comparing the retention factor of butylbenzene (Table 1) under the same chromatographic conditions, monoliths **I** and **II** were demonstrated to be much more hydrophobic than monoliths **III** and **IV**, due to the distinct hydrophobicity of the 2SH and 4SH monomers, which was consistent with the water contact angle measurements. Additionally, monolith **II** was used to further investigate the chromatographic retention behavior, on which the alkylbenzenes were successfully separated with good peak shapes. The well-defined porous structure facilitated the high column efficiency (*ca.* 120 000–160 000 plates per m) (Fig. 1f and 2a, b). Interestingly, the monolith has a small surface area of 3.8 $m^2\ g^{-1}$, indicating that hardly any micropores or mesopores existed, which was similar to that found in our previous work.[3e] When the acetonitrile content in the mobile phase was increased up to 80%, baseline separation of alkylbenzenes was still achieved

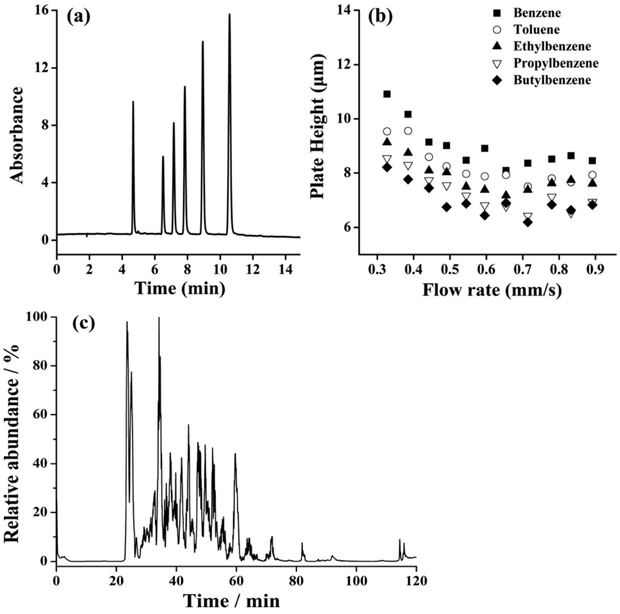

Fig. 2 (a) Separation of alkylbenzenes by cLC. Elution order: thiourea, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene. (b) Dependence of the plate height of analytes on the linear velocity of the mobile phase. (c) Base peak chromatogram of tryptic digest by cLC-MS/MS analysis. Experimental conditions: monolith **II** with a column length of 25 cm × 75 µm i.d.; for (a) and (b), mobile phase: acetonitrile/water (v/v, 70/30), flow rate: 160 µL min^{-1} (before split) and detection wavelength: 214 nm; for (c) mobile phase A 0.1% aqueous formic acid, B 0.1% formic acid in acetonitrile, gradient: 5% to 35% B over 90 min, flow rate: 100 µL min^{-1} (before split).

on monolith **II** (Fig. S6, ESI†). These results indicated that monolith **II** would be a good reversed-phase separation material. To further demonstrate its applicability, separations of peptides and proteins were also carried out, and satisfactory results were obtained (Fig. S7 and S8, ESI†). Additionally, a BSA digest was also used to evaluate the separation ability of the hybrid monolithic column by cLC-MS/MS (Fig. 2c). These results showed the potential of hybrid monoliths in the analysis of macrobiomolecules and complex biosamples.

In conclusion, several hybrid monoliths have been synthesized by a photoinduced thiol–ene polymerization reaction in a few minutes. The porous structure is controllable by changing the ratio and type of porogenic solvents. The resulting monoliths showed good separation ability for alkylbenzenes, peptides, proteins and protein digest by a reversed-phase mechanism. Furthermore, rapid photopolymerization avoided the tedious work required to optimize the temperature conditions compared with thermally initiated processes, and the obtained monolith with residual thiol or vinyl groups provides a facile route for post-modification with various applications.

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