



Click polymerization for preparation of monolithic columns for liquid chromatography



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ARTICLE INFO

Keywords:

Click polymerization
Monolithic column
Thiol-ene reaction
Ring-opening polymerization
Phase separation
Liquid chromatography

ABSTRACT

In recent years, polymerizations based on click reactions (thiol-ene, thiol-yne, thiol-Michael, thiol-epoxy and amine-epoxy) have been utilized to prepare monolithic columns. These polymerization systems are easily carried out under mild conditions. Either hybrid or organic monolithic columns fabricated by click polymerization demonstrated homogeneous network structures. For separation of small molecules, the column efficiencies, such as plate height with less than 10 μm , have been greatly improved comparing with organic monolithic columns prepared with free radical polymerization. In this review, we will summarize recent progress on the preparation of monolithic columns and their chromatographic performances.

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1. Introduction

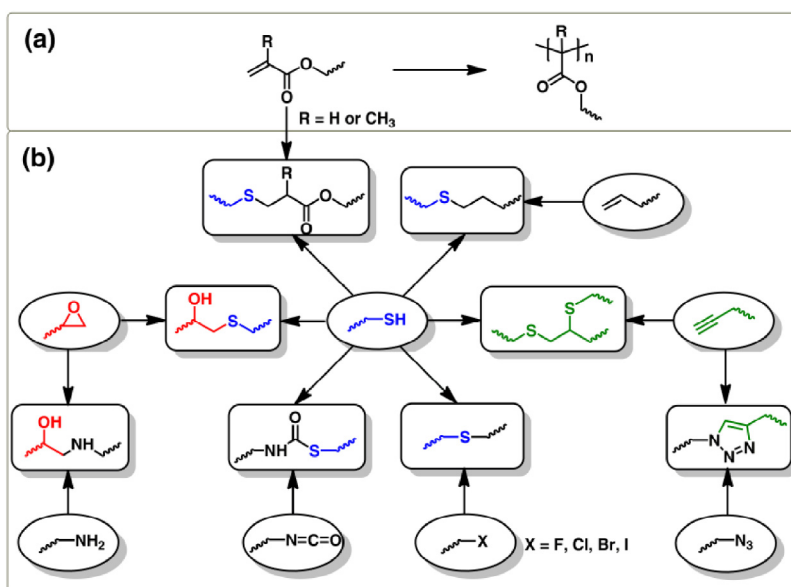
In the past two decades, monolithic columns have been rapidly developed in the aspect of preparation techniques and characterization methods, and widely applied in high performance liquid chromatography (HPLC), capillary electrochromatography (CEC) and capillary liquid chromatography (CLC) [1–28]. As for preparation techniques, the conventional free radical polymerization (Fig. 1a) is one of the most common methods comparing with sol-gel chemistry due to its facile implementation and lots of commercially

available functional monomers. However, it is still a matter of great concern to improve the separation efficiency of small molecules on polymer-based monolithic columns. Researchers have attempted to control the polymerization process by living radical polymerization, as well as to prepare hypercrosslinked monoliths with large surface area [25,29–35]. These improvements gave us some hints that homogeneous network structure and adequate surface area could facilitate the improvement the chromatographic performance for polymer-based monolithic columns.

Click reaction, which features simplicity, selectivity, efficiency and high conversion under mild conditions, has become a versatile toolbox in the applications of organic synthesis, bioconjugation, surface modification, polymer science, etc. (Fig. 1b and 2a–e) [36–42]. Particularly, the click reaction has been employed for direct construction of advanced polymers, such as linear polymers using double-functional monomers (A_2 -monomer) as schemed in Fig. 2f.

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Tang and co-workers have summarized the developments of polymerizations based on alkyne-azide and thiol-yne click reactions using the terminology with “click polymerization” in their reviews [43–45]. If one A_2 -monomer is replaced with a trifunctional monomer (B_3 -monomer), the click polymerization would generate hyperbranched polymers or three dimensional gels (Fig. 2g). It is not difficult to understand that the polymerization of multifunctional monomers like A_4 -type also produce hypercrosslinked polymers, which are insoluble and in various forms, such as gels, films and bulk materials [46–49]. As photoinitiated thiol-based reactions are rapid and high efficient, Prasath and co-workers have

utilized thiol-ene polymerization to prepare monodisperse macroporous or nonporous functional beads by a home-made microfluidic device equipped with UV light [50]. Compared with traditional synthesis methods, click polymerization as a novel technique demonstrates great potential for preparation of novel materials with high crosslink density.

Recently, click polymerization was adopted to fabricate monolithic columns according to the experience-based prescription that the prepolymerization mixture was composed of monomers, porogenic solvents and an initiator if necessary. These polymerization systems are controllable and easily carried out under mild

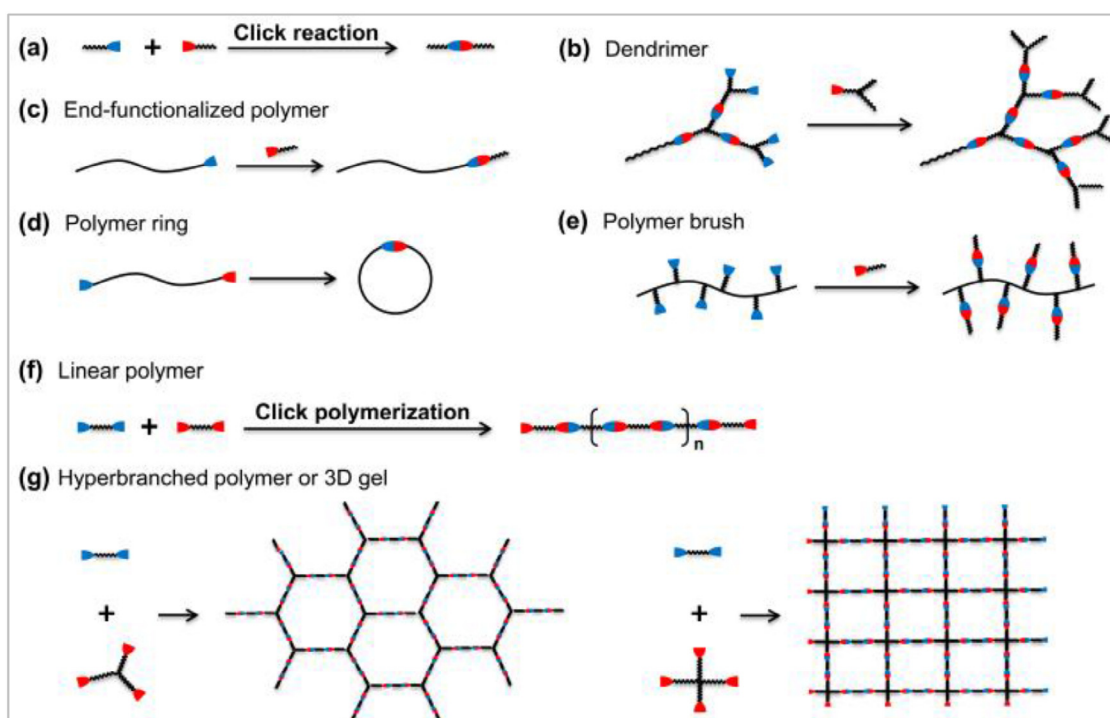


Fig. 2. Examples of macromolecular structures synthesized by click (polymerization) reactions.

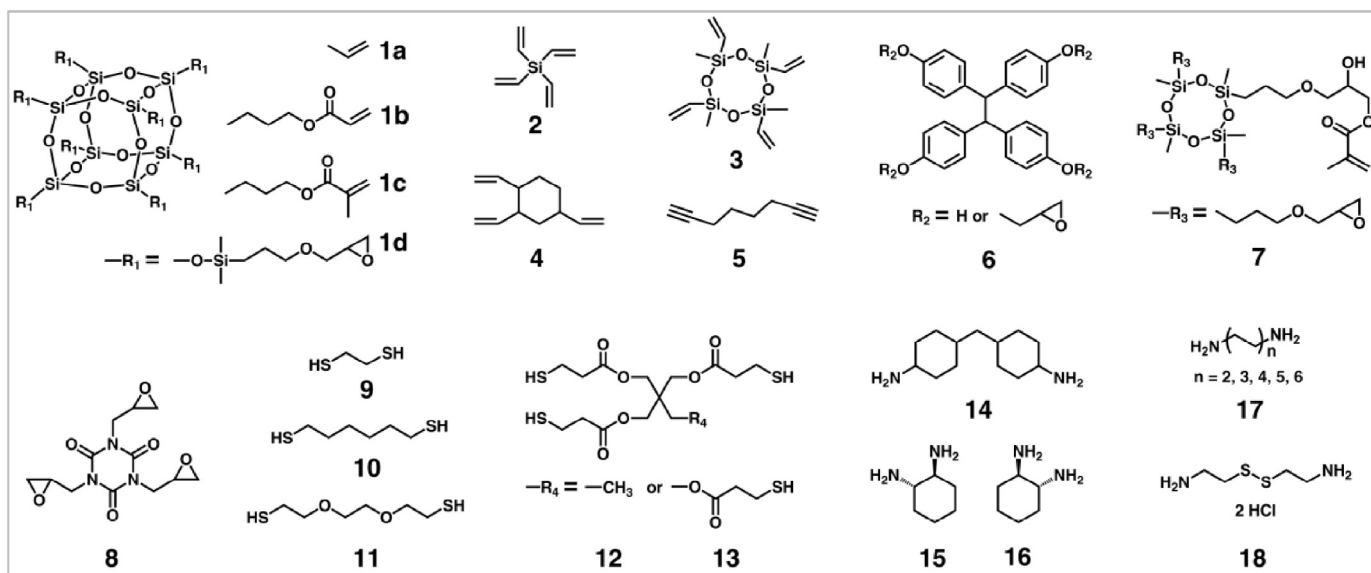


Fig. 3. Various monomers for preparation of monolithic materials by click polymerization. (1a) Octavinyl-octasilsesquioxane; (1b) Acrylopropyl polyhedral oligomeric silsesquioxane; (1c) PSS-methacryl substituted; (1d) PSS-octa[3-glycidyloxypropyl]dimethylsiloxy substituted; (2) Tetravinylsilane; (3) 2,4,6,8-Tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane; (4) 1,2,4-Trivinylcyclohexane; (5) 1,7-Octadiyne; (6) Tetraphenylethane glycidyl ether; (7) Methacrylate epoxy cyclosiloxane; (8) Tris(2,3-epoxypropyl) isocyanurate; (9) 1,2-Ethanedithiol; (10) 1,6-Hexanedithiol; (11) 2,2'-(Ethyleneedioxy)diethanethiol; (12) Trimethylolpropanetri(3-mercaptopropionate); (13) Pentaerythritol tetrakis(3-mercaptopropionate); (14) 4,4'-methylenebis(cyclohexylamine); (15, 16) Trans-1,2-cyclohexanediamine; (17) Alkyl diamines, (18) Cystaminedihydrochloride.

conditions, such as thermal or photo initiation, nucleophilic or alkali catalysis [51–54]. Most of monolithic columns synthesized by click polymerization demonstrated relative homogeneous network structures, which are obviously distinct from those obtained by conventional chain-growth free radical polymerization and facilitate excellent chromatographic performance for separation of small molecules, even reaching $H = 5.9 \mu\text{m}$ ($H = L/N$, H – plate height; L – column length; N – theoretical plate number) [55]. In this review, we would summarize the progress on click polymerization for direct construction of monolithic columns. We then will discuss the phase separation process of click polymerization in order to well understand the formation of homogeneous porous structures, and hope to open further possibilities for precise control of network structure in monolithic columns.

2. Click polymerization for direct construction of monolithic columns

According to the click reaction paradigm, many reactions have been labelled with the click term in published papers, such as azide-alkyne cycloaddition, Diels-Alder addition, oxime/hydrazone formation and thiol-based reactions [40,41]. Click reaction for preparation of monolithic columns should be insensitive to oxygen and keep low reaction activity before the prepolymerization mixture was introduced into capillaries. Up to now, thiol-based click reactions and epoxy-based ring-opening reactions have received much attention to prepare monolithic columns. Herein, we will describe these developments in three categories: thiol-ene/yne polymerization, thiol-(meth)acrylate polymerization and ring-opening polymerization.

2.1. Thiol-ene/yne polymerization

Thiol-ene and thiol-yne click polymerization can be initiated in free radical condition. Previous works have confirmed that both polymerization systems are insensitive to oxygen and show step-growth process [38]. For the first time, Nischang and co-worker have

prepared monolithic materials using vinylPOSS (1a, Fig. 3, the same below) and multifunctional thiols [52]. The radical-mediated thiol-ene click polymerization was carried out under UV light and by heat, respectively. The vinyl groups in vinylPOSS are difficult to undergo homopolymerization but show high reactivity with thiol groups (Fig. 4a, $n = 0$). The porous structure was obtained by removing porogenic solvents (Fig. 5a and 5b). Later, they have investigated the chromatographic characteristics in detail [56]. Before their work was published, we have achieved many results about thiol-ene click polymerization for preparation of monolithic column. Although vinylPOSS is a good cross-linker, we found it was difficult to be dissolved in common solvents. The alkene monomers 2 and 3 may be easily dissolved in binary porogenic solvents, such as diethylene glycol diethyl ether (DEGDE)/ tetrahydrofuran (THF), DEGDE/1-propanol and DEGDE/poly(ethylene glycol) (PEG, $M_w = 200$). The polymerization was initiated under UV light ($\lambda = 365 \text{ nm}$) using 2,2-dimethoxy-2-phenylacetophenone (DMPA) as photoinitiator [57]. A continuous reticular skeleton was clearly observed, and monolithic column exhibited high column efficiencies for alkylbenzenes ($H = 6.3\text{--}8.3 \mu\text{m}$). Additionally, 1,2,4-trivinylcyclohexane (4) and pentaerythritol tetrakis(3-mercaptopropionate) (13) were also used to prepare organic polymer monolith (Fig. 5c) [58]. By comparing the morphology and permeability of monolithic column, we have found many factors had influence on thiol-ene click polymerization process, including the ratio of porogenic solvents, the ratio of alkene to thiol groups, the polymerization time and the content of photoinitiator. Finally, the optimal monolithic column generated the highest column efficiency with $H = 7.5\text{--}13.7 \mu\text{m}$ for alkylbenzenes. However, the dried bulk materials generally suffered from shrinkage and showed negligible surface areas, which was measured by nitrogen adsorption (such as $3.8 \text{ m}^2/\text{g}$). Therefore, the monolithic column was characterized by size exclusion chromatography (SEC) using benzene and polystyrenes with different molecular weight as probes (Fig. 5d). The total porosity of this organic polymer monolithic column was calculated to be 71.5%, while the volume of through-pores was 53.3%.

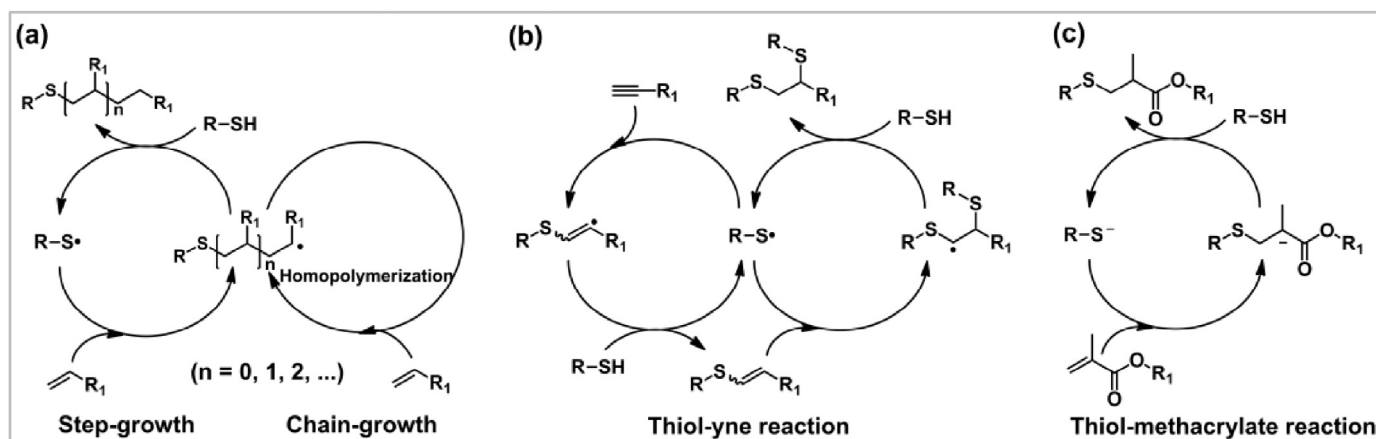


Fig. 4. Mechanisms of (a) free radical thiol-ene reaction; (b) free radical thiol-yne reaction, Reprinted with permission from ref [60]. Copyright 2010 American Chemical Society; (c) thiol-methacrylate reaction.

Bowman and co-workers have systematically investigated thiol-yne click polymerization mechanism that each alkynyl group reacts with two thiol groups (Fig. 4b) and the physical properties of obtained polymers with homogeneous and high- T_g networks [59–61]. On this basis, we introduced the porogenic solvents and kept about 20% (w/w) monomers in final mixture. After optimizing the parameters, we found the combination of DEGDE and THF was suitable for the preparation of porous monolithic column using 1,7-octadiyne (**5**) and 1,6-hexanedithiol (**10**) as monomers [62]. The oligomers with molecular weight ranging from 800 to 1600 Da were detected by MALDI-TOF mass spectrometer. The repeating units with mass difference of 106 and 150 Da were corresponding to 1,7-octadiyne and 1,6-hexanedithiol, respectively (Fig. 5e). The element analysis demonstrated 60.88% carbon loading close to the theoretical value (59.36%). Taking the advantages of photo-polymerization at room temperature, an empty zone at one end of capillary was reserved to make UV detection window for on-column detection, which decreased the extra-column effect in chromatography. Finally, the homogeneous porous structure facilitated lower A-term value and lower plate heights from 10.0 to 12.0 μm at linear velocity of ~ 0.45 mm/s (Fig. 5f, Table 1).

2.2. Thiol-(meth)acrylate polymerization

Under free radical condition, thiol-(meth)acrylate reaction tends to proceed a mixed-mode polymerization (Fig. 4a, $n = 0, 1, 2, \dots$) rather than an ideal click reaction. Previous works have confirmed the thiols could reduce oxygen inhibition and increase the polymerization rate in (meth)acrylic polymerizations [38]. As (meth)acrylates are common and easily available monomers for construction of various polymers via chain-growth free radical polymerization (Fig. 1a), we attempted to employ thiol-acrylate polymerization to prepare monolithic columns. For acryl-POSS (**1b**)/octadecanethiol (ODT) system, the white monolith emerged only after 10 s, suggesting the phase separation took place, while the time delayed to be 30 s for traditional homopolymerization of acryl-POSS [63]. As a result, the monolithic column fabricated without ODT presented the cauliflower-like globular structure (Fig. 6a), which is the typical feature of conventional polymer-based monoliths, while the incorporation of ODT obviously improved the homogeneity of network structure (Fig. 6b). The surface area of ODT-doped monolith in dry state was calculated to be 178.9 m^2/g while that of the pure poly(acryl-POSS) monolith was only 38.2 m^2/g (Fig. 6c). The enhanced surface area may be related to the increment of pores with

the size less than 200 nm (Fig. 6d). Furthermore, this route was also suitable for preparation of sulfonate-containing monolithic columns by replacing ODT with 3-mercapto-1-propanesulfonate.

The ideal thiol-(meth)acrylate click reaction can be carried out under catalysts like bases and nucleophiles, which was also denoted by thiol-Michael click reaction (Fig. 4c) [64]. Keeping equal molar ratio of functional groups, we have studied the thiol-methacrylate click polymerization of POSS-MA (**1c**) with a series of multifunctional thiols (**10**, **12** and **13**) using dimethylphenylphosphine (DMPP) as catalyst [53]. After selecting several kinds of porogenic systems including 1-propanol/1,4-butanediol, toluene/dodecanol and cyclohexaneol/decanol, we finally found the mixture of 1-propanol and PEG 200 was facile to generate ideal porous structure. The permeability of monolithic column could be tuned by changing the ratio of porogens, DMPP content and polymerization temperature. The optimized monolithic column (**1c**+**12**) exhibited the highest column efficiency with $H = 5.1$ μm for butylbenzene. Interestingly, monolithic column post-modified with 1,2-epoxyoctadecane showed stronger retention for alkylbenzenes, suggesting its potential tailorability for special application.

2.3. Ring-opening polymerization

The epoxides can easily react with nucleophiles via ring-opening reaction due to their strong tension of rings. For example, the amine-epoxy reaction can be carried out without any catalysts and has been used to modify the epoxy-containing monolithic columns [65,66]. The ring-opening polymerization based on amine-epoxy (**8** + **14–16**) for preparation of monolithic materials was firstly reported by Hosoya and co-workers [51,67–69]. They have investigated the mechanism and structural properties of the epoxy-based monoliths based on polymerization induced phase separation. The pore size and permeability were controlled by polymerization temperature and PEG with different molecular weight. The morphology of monolithic column was greatly similar to those of silica-based monolithic columns. Surprisingly, the monolithic column showed high permeability and could generate plate height with smaller than 5 μm (Fig. 7). Their excellent works open up a new avenue for tuning the network structure of polymer-based monolithic columns. Later, Irgum and co-workers modified the epoxy-based polymerization method for preparation of hydrophilic monoliths [70,71]. In their strategy, the epoxy monomer, surfactant and an organic diluent were mixed to form dispersed phases, which were then added to aqueous phases composed of amine

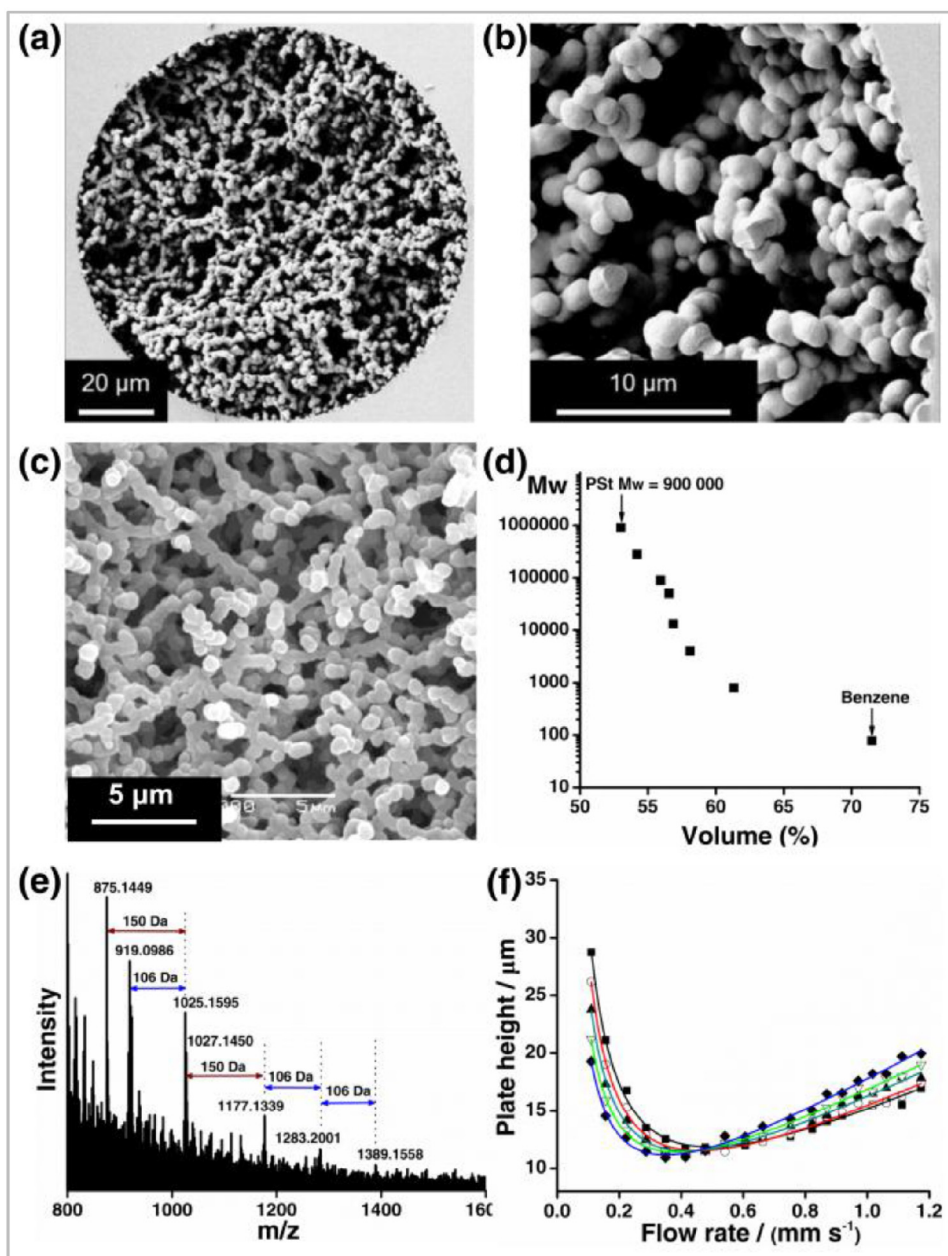


Fig. 5. (a) and (b) SEM images of monolithic column prepared by thermally initiated thiol-ene polymerization (**1a**+**13**, Fig. 3). Reprinted with permission from ref [52]. Copyright 2013 Wiley-VCH; (c) SEM image of monolithic column prepared by photo-initiated thiol-ene polymerization (**4**+**13**) and (d) Porous volume measured by size-exclusion chromatography. Reprinted with permission from ref [58]. Copyright 2015 Elsevier; (e) MALDI-TOF mass spectrum of the oligomers flushed out of monolithic column prepared by photoinitiated thiol-ene polymerization, mass difference of 106 and 150 Da corresponding to monomers **5** and **10**, respectively; (f) Dependence of plate height of alkylbenzenes on linear velocity of mobile phase. Reprinted with permission from ref [62]. Copyright 2014 American Chemical Society.

monomer and calcium chloride. The as-synthesized monoliths were characterized in detail, unfortunately, on which the chromatographic evaluations were seldom reported.

On the basis of ring-opening polymerization protocol, Liu and co-workers have prepared boronate affinity monolithic column by copolymerization with *m*-aminophenylboronic acid (APBA) (Fig. 8a) [72]. The skillful B-N coordination makes the resultant monolithic column very promising for capturing *cis*-diols under neutral conditions and physiological circumstance (Fig. 8b). However, the boronate affinity monolithic column could only specifically bind small *cis*-diol molecules, failing to bind with glycoproteins. They speculated that most of the boronic acid teams were located within the mesopores rather than the macropores. Then they attempted

to replace APBA with 4-mercaptophenylboronic acid (MPBA) to eliminate the possibility of B-N coordination between APBA molecules, as well as to improve the boronic acid density due to the stronger nucleophilicity of thiol group [73]. The novel boronate affinity monolithic column could be a mimic of protein A for specific capture of antibodies, with a binding capacity equivalent to 23.73 mg immunoglobulin G per gram.

Apart from the “one-pot” copolymerization method, the residual epoxy groups, which can be controlled by the ratio of amines to epoxides, were accessible for post-modification. For example, the epoxy-based monolithic column was applied for separation of glycoprotein isoforms in weak anion exchange (WAX) mode after being treated with ammonia aqueous solution [74]. In addition, Hilder and

Table 1
van Deemter coefficients with alkylbenzenes for several monolithic columns

Polymerization	Monomers	Gelation conditions	A-term ^b (μm)	B-term ^b ($\mu\text{m}^2/\text{s}$)	C-term ^b ($\times 10^{-3} \text{s}$)	H_{min} (μm)	Ref.
Thiol-ene	4 + 13	DMPA ^a , UV light	1.9–2.5	2071–3452	3.8–4.1	7.5–13.7	[58]
Thiol-yne	5 + 10	DMPA, UV light	0.1–0.7	1865–3016	12.5–15.5	10.0–12.0	[62]
Thiol-acrylate	1b + ODT ^a	DMPA, UV light	3.8–6.5	1660–2040	13.1–15.9	13.6–16.5	[63]
Thiol-methacrylate	1c + 13	DMPP ^a , 50°C	0.7–5.9	1425–2878	1.5–5.9	7.0–8.5	[53]
Thiol-epoxy	6 + 13	KOH, 50°C	1.0–2.6	1700–1848	4.2–7.1	7.6–8.1	[54]
Thiol-epoxy	1d + 13	KOH, 70°C	0.8–1.7	1817–2462	2.7–4.6	5.9–7.9	[55]
Amine-epoxy	1d + 17 (n = 4)	50°C	1.6–3.6	1514–2266	6.6–8.5	10.0–10.5	[76]
Amine-epoxy	1d + PEI ^{a,c}	50°C	~1.1	1946–2494	10.7–12.9	8.4–9.7	[77]
C ₁₈ silica-based monolithic column (Merck, Germany) ^d			7.4	2094	2.1	~11.6	[82]
C ₁₈ particles packed column (Zorbax ODS) ^e			3.0	3800	4.5	~11.3	[83]

^a ODT: 1-octadecanethiol; PEI: poly(ethyleneimine); DMPA: 2,2-dimethoxy-2-phenylacetophenone; DMPP: dimethylphenylphosphine.

^b Fitted values of A-, B-, and C-terms in van Deemter equation ($H = A + B/u + Cu$) were obtained via plate height (H)-flow rate (u) curves like Fig. 5f. The values of H_{min} were corresponding to those at optimal flow rate.

^c The H - u plots were obtained in HILIC mode using dimethyl formamide, formamide and thiourea as probes.

^d Column: 100 mm \times 4.6 mm i.d. H - u plots for butylbenzene.

^e C₁₈ particles: 6 μm with 8-nm pores, column: 20/28 cm \times 50 μm i.d. H - u plots for m-terphenyl.

co-workers have studied the post-modification of epoxy-based monolithic column with sulphuric acid for hydrophilic interaction liquid chromatography (HILIC) and with 2-aminoethyl hydrogen sulphate for ion-exchange chromatography, as well as with allylamine for further polymers grafting via free radical polymerization [75]. They have evaluated the resulting monolithic columns in the separations of nucleobases/nucleosides, benzoic acids, peptides and proteins. It is worth noting that, in their strategy, 1-decanol and 1-dodecanol were firstly introduced to tune the morphology of epoxy-based monolith. It is a very common and effective way to obtain monoliths with satisfactory permeability and chromatographic performance.

In recent years, our group has paid much attention to develop hybrid monolithic columns based on ring-opening polymerization. Polyhedral oligomeric silsesquioxanes (POSS) monomers are

popular building blocks for fabricating organic-inorganic hybrid materials in order to enhance the chemical stability and mechanical strength. We have optimized a versatile polymerization system comprised of POSS-epoxy (**1d**), several diamines (**17**) or poly(ethyleneimine) (PEI, Mw = 1200) and ternary porogenic solvents (1-propanol, 1,4-butanediol and PEG 10,000) [76–78]. For all selected diamines, the monolithic columns demonstrated similar porous structures with narrow pore size distribution, such as ~1 μm measured by mercury intrusion method. The values of plate height were smaller than 10 μm for alkylbenzenes. As mentioned above, the abundant groups like epoxy group, amine group, imine group and hydroxyl group, promote monolithic columns high tailorability for diverse separation modes. For example, γ -gluconolactone-functionalized monolithic column exhibited good chromatographic separation for phenols, basic drug molecules, pyrimidines,

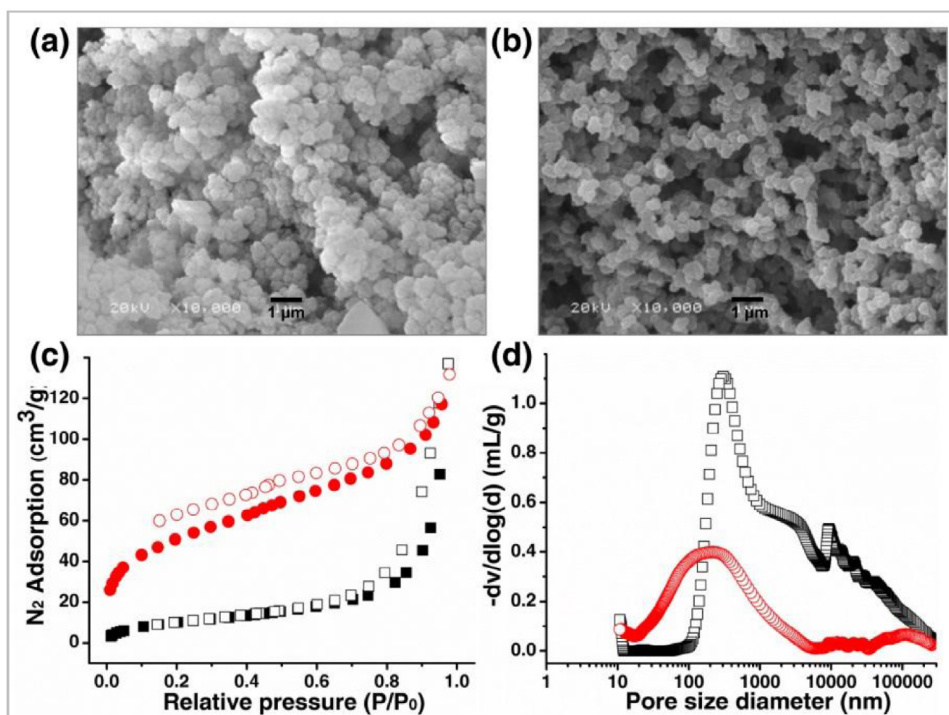


Fig. 6. SEM images of monolithic columns prepared by free radical (a) homopolymerization of pure acrylPOSS and (b) thiol-acrylate polymerization using acrylPOSS and 1-octadecanethiol (ODT); (c) Nitrogen sorption isotherm curves measured at 77 K; (d) Pore size distribution measured by mercury intrusion porosimetry. (square: poly(acrylPOSS) monolith; circle: poly(acrylPOSS-ODT) monolith). Reprinted with permission from ref [63]. Copyright 2015 American Chemical Society.

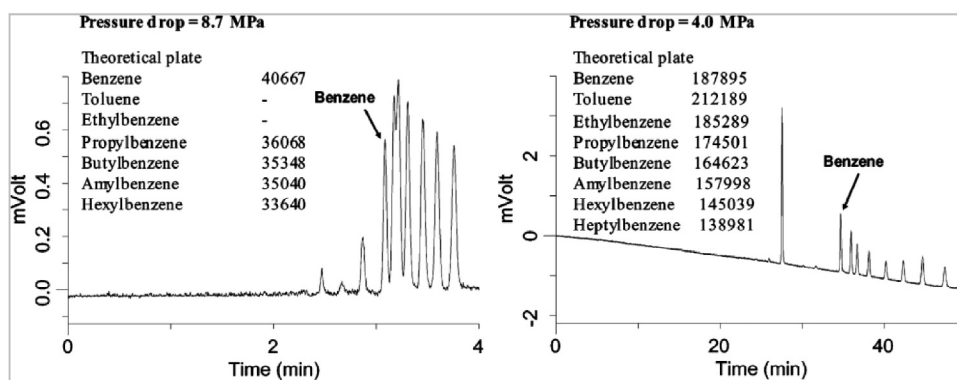


Fig. 7. Separation of alkylbenzenes on polymeric monolithic columns prepared using monomers **8** and **14**. Conditions: Mobile phase, acetonitrile/20 mM sodium phosphate buffer pH 7.0 (60/40, v/v); Column, 18.2 cm \times 100 μ m i.d. (left), 150.5 cm \times 100 μ m i.d. (right); Off column, 9 cm \times 50 μ m i.d.; Detection, 210 nm. Reprinted with permission from ref [51]. Copyright 2006 American Chemical Society.

nucleosides, purines as well as small peptides in HILIC [77]. Taking advantages of high column efficiency, the monolithic column was also adopted for enantioseparation after being physically coated with cellulose tris(3,5-dimethylphenylcarbamate). Based on these works, we have further prepared monolithic column containing disulfide bonds using POSS-epoxy and cystaminedihydrochloride (**18**) [79]. After reduction with dithiothreitol (DTT), the monolithic column was modified with stearyl methacrylate (SMA) via DMPP-catalyzed thiol-methacrylate click reaction, on which the retention factors of alkylbenzenes increased by 2–3 times.

Methacrylate epoxy cyclosiloxane (**7**) contains alkenyl group and epoxy group. We adopted it to prepare monolithic columns by two routes based on ring-opening polymerization and conventional free radical polymerization, respectively (Fig. 9a) [80]. The different morphologies (SEM) images indicate ring-opening polymerization has the potential to improve the uniformity of monolithic columns. Interestingly, two kinds of monolithic columns could be used for post-functionalization to tailor the surface properties owing to the orthogonality of these two polymerization processes.

Analogous to amine-epoxy reaction, thiol-epoxy reaction can react in alkali condition due to the formation of nucleophilic thiolate [41,81]. In our work, potassium hydroxide was selected to catalyze the thiol-epoxy ring-opening polymerization [54]. To obtain homogenous prepolymerization mixture, a quaternary porogenic system consisted of dimethylsulfoxide (DMSO), PEG 200, PEG 10,000 and water was optimized for the polymerization of tetraphenylolthianeglycidyl ether (**6**) with multifunctional thiols (**12**, **13**). It is surprising that the resulting organic monolithic column did not obviously swell in organic solvents such as acetonitrile, methanol and THF, which was then characterized by SEC using THF as mobile phase. The total porosity was calculated to be 86.2% while the volume of through-

pores was about 57.6%, leading to high permeability with $5.4\text{--}19.6 \times 10^{-14} \text{ m}^2$. As the epoxide was replaced with POSS-epoxy, the corresponding hybrid monolithic column also showed homogeneous structure (Fig. 9b) that was similar to those of silica-based monoliths [55]. The resultant both two polymeric and hybrid monolithic columns prepared via thiol-epoxy polymerization exhibited high column efficiencies for small molecules in CLC (Table 1).

2.4. Remarks on click-polymerized monolithic columns

We summarized the van Deemter coefficients (A -, B -, and C -terms) and the minimum plate heights (H_{\min}) for some abovementioned monolithic columns as well as commercial silica-based monolithic column [82] and C_{18} particles packed column [83] (Table 1). The column efficiency was improved by decreasing the values of A - and C -terms, which are associated with the structure homogeneity and permeability of chromatographic columns. Particularly, it is also advantageous to achieve high efficiency and quick separation with lower C -term. Either hybrid or organic monolithic columns prepared by click polymerization demonstrated excellent chromatographic performance, especially for small molecules, which was difficult to achieve by conventional free radical polymerization. It may be related to the step-growth polymerization process that will be discussed later.

Compared with thiol-Michael and ring-opening polymerizations, photo initiated thiol-ene and thiol-yne polymerizations completed within less than 30 min at room temperature, providing a rapid approach for preparation of monolithic columns. Considering the controllability of photo-polymerization inside capillary, we believe it is possible to fabricate biphasic or multiphasic columns. Up to now, the thiol-ene/yne click polymerized

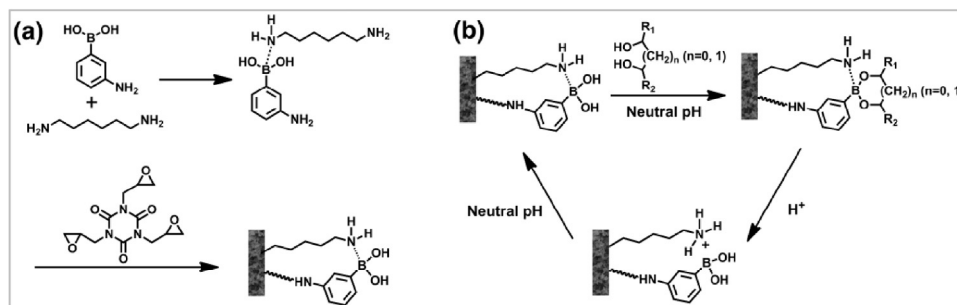


Fig. 8. (a) Ring-opening polymerization for preparation of boronate affinity monolithic column. (b) Inter-molecular synergistic action for the reversible capture/release of cis-diol-containing compounds. Reprinted with permission from ref [72]. Copyright 2009 Wiley-VCH.

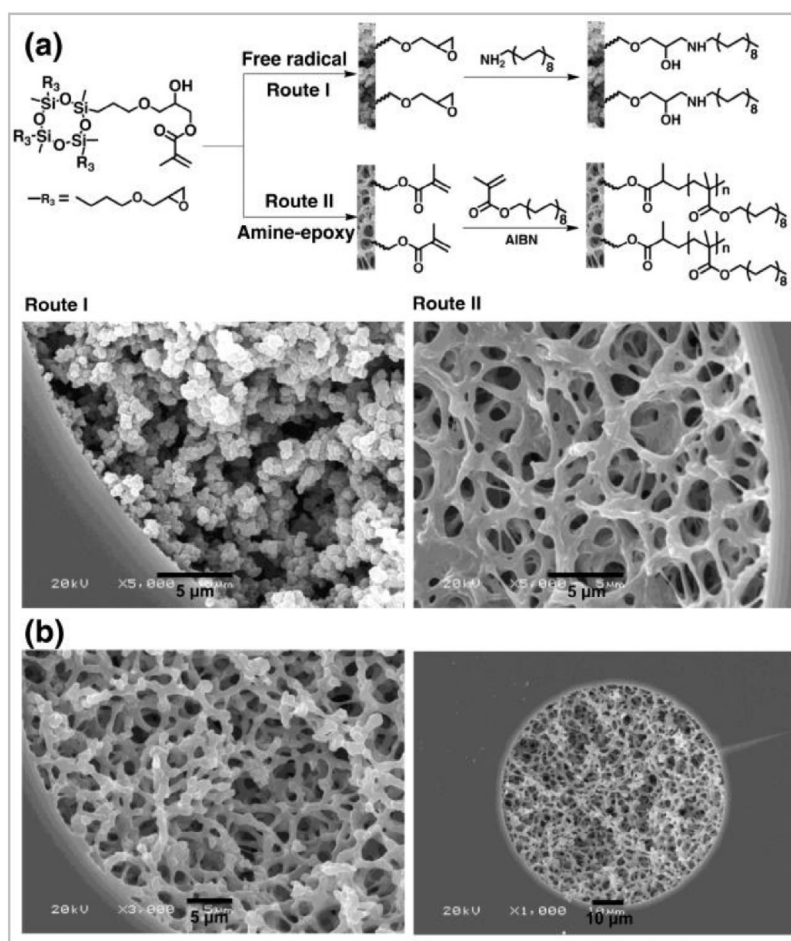


Fig. 9. (a) Two different preparation routes implemented by conventional free radical polymerization and amine-epoxy ring-opening polymerization, respectively, and SEM images of corresponding monolithic columns. Reprinted with permission from ref [80]. Copyright 2014 Elsevier; (b) SEM images of monolithic column prepared by thiol-epoxy polymerization (1d+12). Reprinted with permission from ref [55]. Copyright 2015 Elsevier.

monolithic columns are only used for chromatographic separation in reversed-phase mode, which were limited by the commercially available functional monomers. This limitation may be addressed by synthesizing specific monomers or introducing accessible site like azide group, epoxy group and hydroxyl group for post-modification.

Monolithic columns containing amine group and imine group were rarely prepared by sol-gel chemistry and conventional free radical polymerization. Combining with the reversed-phase characteristic, the existence of amine group and imine group makes the monolithic column promising for CEC separation [78]. Except the abovementioned post-modification via residual epoxy groups, these monolithic columns also can be functionalized by iodomethane for strong anion-exchange chromatography, as well as by long-chain haloalkane for mixed-mode separation.

3. Phase separation process

Monolithic materials fabricated by click polymerization exhibited more homogeneous structure compared with those obtained by conventional polymerization method. Understanding the formation mechanism of such network structure would enable further possibilities for precise control of monolithic materials. Once the homogeneous prepolymerization mixture was initiated, polymerization of monomers would cause the formation of polymers. The growing polymer chains demonstrated different interaction with

porogenic solvents at different stages, finally resulting in the phase separation. Here, the occurrence of phase separation was discussed using the free energy change of ΔG based on the Flory-Huggins mean field theory: [30]

$$\Delta G = RT \left(\frac{\phi_A}{P_A} \ln \phi_A + \frac{\phi_B}{P_B} \ln \phi_B + \chi_{AB} \phi_A \phi_B \right) \quad (\text{Equation 1})$$

Where ϕ_A and ϕ_B are the volume fractions of component A and B; P_A and P_B are the degrees of polymerization; χ_{AB} is the interaction parameter. The first two terms on the right side of equation are related with the mixing entropy, both of which have negative values due to $0 < (\phi_A, \phi_B) < 1$. If component A represents polymer produced by polymerization, the second term is related to the porogenic solvent, of which the value keeps constant. Considering that the value of P_A becomes very large when phase separation occurs, both the first two terms contribute little to the ΔG . Therefore, the third term becomes the predominant factor. When the value of ΔG becomes positive, the phase separation proceeds. As the interaction parameter depends on temperature and composition, the polymerization temperature and porogenic solvents have significant influence on phase separation. This is the reason why these two factors need careful optimization in the preparation process.

The temperature affects not only the solubility of polymers (thermodynamic effect), but also the reaction rates (kinetic effect). At lower temperature, the conversion of monomers is retarded. The

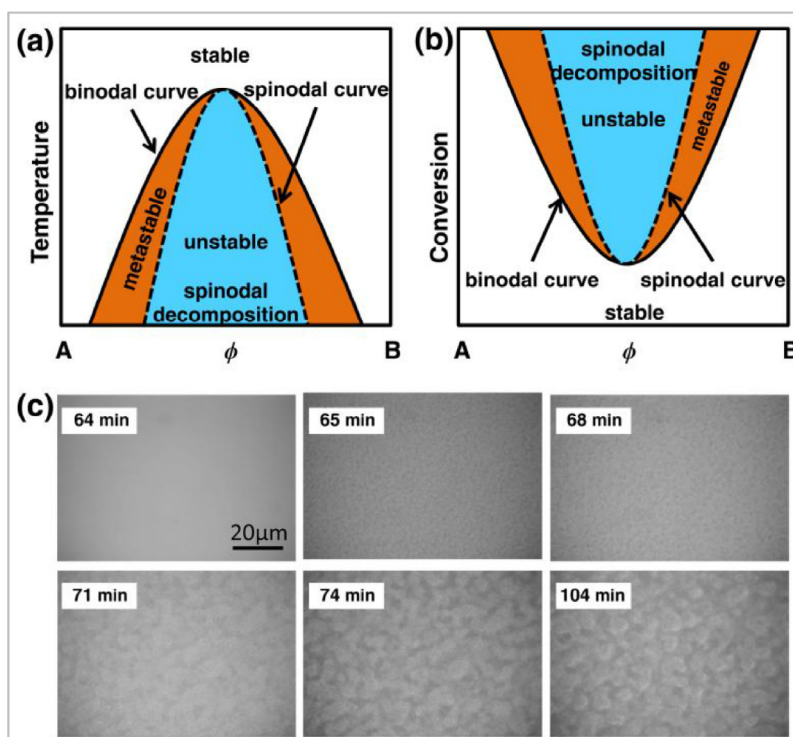


Fig. 10. (a) and (b) Phase diagrams. The binodal and spinodal curves are obtained by interconnecting all the coexisting points (binodal points) and inflection points (spinodal points) of the ΔG - ϕ curves (Equation 1). Reprinted with permission from ref [84]. Copyright 1999 Springer; (c) Optical microscope pictures of phase separation at different reaction time. Reprinted with permission from ref [85]. Copyright 2015 Royal Society of Chemistry.

phase separation may firstly proceed in the metastable region via nucleation and growth mechanism (Fig. 10a), consequently generating particles or connected globule structure [84]. Alternatively higher temperature is favor to high reaction rate. Meanwhile, the solubility of polymers was improved, which delays the onset of phase separation. The two consequences of higher temperature are likely to induce the phase separation via spinodal decomposition. In this case, the domain coarsening is also suppressed in late stage of spinodal decomposition, which contributes small pore size. As an example of amine-epoxy polymerization in the presence of PEG 200, the increased polymerization temperature from 100 to 130°C caused the evolution from connected globule structure to sponge-like morphology [68].

To better understanding how the network structures are affected by different polymerization mechanism, the phase diagram as a function of conversion was also given in Fig. 10b [84]. The conventional free radical polymerization generated polymers with high molecular weight even in the early stage, however, which did not show high conversion of monomers. The strong immiscibility of such polymers drove themselves to form microgels, indicating a fast phase separation process prior to spinodal decomposition. The microgels tended to aggregate with each other rather than to react with monomers, which finally led to the formation of heterogeneous morphology in monolithic materials. In contrast, the step-growth polymerization demonstrated high conversion in short time, generating polymers with low or middle molecular weight. The higher conversion would induce the relative homogeneous mixture with weak aggregation into spinodal decomposition region according to the phase diagram (Fig. 10b). In the earlier stage of spinodal decomposition, the phase separation and structural freezing by gelation would happen rapidly, so the synergy led to the formation of bicontinuous structure (Fig. 10c) [85].

The porogenic solvents should have good miscibility with starting monomers before polymerization, and are easily removed after

polymerization. Generally, changing the ratio of solvents is an effective approach to control the permeability of monolithic column. Based on the interaction with polymers, the porogenic solvents are classified into good solvent (microporogenic solvent) and poor solvent (macroporogenic solvent). In polymerization process, the increment of good solvent improves the solubility of polymers delaying the onset of phase separation, consequently generating smaller pore size due to the suppression of coarsening process, while the poor solvent showed opposite effects.

Last but not the least, the phase separation process not only helps us to understand the formation mechanism of porous structure, but also provides significant directions for the design of monolithic columns. However, the fateful and reliable evaluation criteria of monolithic columns are chromatographic performances, such as permeability, separation efficiency, peak capacity, etc. Though the polymerization process in capillary is robust, the optimization of parameters, such as temperature, the type and ratio of porogenic solvents, is critical and time-consuming. That may be the reason why bulk materials with good SEM images cannot guarantee high chromatographic performance when fabricated inside capillary. A suggestion is that the facile, simple and controllable polymerization reaction is conducive to the preparation and repeatability of monolithic columns.

4. Conclusion

In this review, we have summarized recent progress on the construction of monolithic columns via click polymerization. Based on the polymerization-induced phase separation, various polymerization systems were discussed in the aspect of polymerization conditions and component of precursor solutions. Most of monolithic columns exhibited homogeneous structure with high crosslink density, which were greatly improved compared with those prepared by conventional free radical polymerization. The exciting

advances demonstrated click polymerization was a reliable approach to fabricate monolithic columns. However, considering that the excellent chromatographic separations were evaluated using standard samples in reported works, we should recognize there are still numerous challenges to make monolithic columns competitive with the advanced particulate-packed columns. Another problem is that the bulk click-polymerized monoliths shrink in dry state, which limits their application on large size and make characterization very difficult to deduce the information on surface area and micropore or mesopore sizes. We expect that the drawbacks can be addressed by adopting rigid monomers in the future.

Furthermore, taking the advantages of flexible polymerization conditions, click polymerization has potential for in situ preparation of monoliths inside micro-pipette tips or on chips, which are also very useful materials in separation science field. In addition to the common (meth)acrylates, much more functional monomers including the azides, thiols, alkenes, alkynes, halides, isocyanates, epoxides could be possibly employed to prepare monolithic materials by click polymerization (Fig. 1b). It is worth noting that some click reactions between each other are orthogonal, such as thiol-Michael and thiol-yne reactions, which have potential for preparing novel functional monolithic materials by sequential click polymerization [86]. As a result, it is optimistically anticipated that the click polymerization will be further developed as a useful tool in the commercialization of monolithic stationary phases with the aim of affording diverse chromatographic selectivity through the collective research efforts of scientists and technologists.

Acknowledgements

The authors acknowledge the funding support by the China State Key Basic Research Program Grant (2013CB911203, 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. Zou as well as the National Natural Sciences Foundation of China (No. 21175133, 21575141) to J. Ou.

References

- [1] F. Svec, E.C. Peters, D. Sýkora, J.M.J. Fréchet, Design of the monolithic polymers used in capillary electrochromatography columns, *J. Chromatogr. A* 887 (2000) 3–29.
- [2] Q. Tang, M.L. Lee, Column technology for capillary electrochromatography, *TrAC-Trend Anal. Chem.* 19 (2000) 648–663.
- [3] N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, et al., Monolithic silica columns for high-efficiency chromatographic separations, *J. Chromatogr. A* 965 (2002) 35–49.
- [4] H. Zou, X. Huang, M. Ye, Q. Luo, Monolithic stationary phases for liquid chromatography and capillary electrochromatography, *J. Chromatogr. A* 954 (2002) 5–32.
- [5] K. Cabrera, Applications of silica-based monolithic HPLC columns, *J. Sep. Sci.* 27 (2004) 843–852.
- [6] F. Svec, Organic polymer monoliths as stationary phases for capillary HPLC, *J. Sep. Sci.* 27 (2004) 1419–1430.
- [7] K. Nakanishi, K. Kanamori, Organic-inorganic hybrid poly(silsesquioxane) monoliths with controlled macro- and mesopores, *J. Mater. Chem.* 15 (2005) 3776–3786.
- [8] F. Svec, Recent developments in the field of monolithic stationary phases for capillary electrochromatography, *J. Sep. Sci.* 28 (2005) 729–745.
- [9] G. Guiochon, Monolithic columns in high-performance liquid chromatography, *J. Chromatogr. A* 1168 (2007) 101–168.
- [10] K. Nakanishi, N. Tanaka, Sol-gel with phase separation. Hierarchically porous materials optimized for high-performance liquid chromatography separations, *Acc. Chem. Res.* 40 (2007) 863–873.
- [11] O. Núñez, K. Nakanishi, N. Tanaka, Preparation of monolithic silica columns for high-performance liquid chromatography, *J. Chromatogr. A* 1191 (2008) 231–252.
- [12] K.K. Unger, R. Skudas, M.M. Schulte, Particle packed columns and monolithic columns in high-performance liquid chromatography-comparison and critical appraisal, *J. Chromatogr. A* 1184 (2008) 393–415.
- [13] H. Aoki, N. Tanaka, T. Kubo, K. Hosoya, Polymer-based monolithic columns in capillary format tailored by using controlled in situ polymerization, *J. Sep. Sci.* 32 (2009) 341–358.
- [14] Y. Li, M.L. Lee, Biocompatible polymeric monoliths for protein and peptide separations, *J. Sep. Sci.* 32 (2009) 3369–3378.
- [15] F. Svec, Porous polymer monoliths: amazingly wide variety of techniques enabling their preparation, *J. Chromatogr. A* 1217 (2010) 902–924.
- [16] Z. Zhang, R. Wu, M. Wu, H. Zou, Recent progress of chiral monolithic stationary phases in CEC and capillary LC, *Electrophoresis* 31 (2010) 1457–1466.
- [17] P. Aggarwal, H.D. Tolley, M.L. Lee, Monolithic bed structure for capillary liquid chromatography, *J. Chromatogr. A* 1219 (2011) 1–14.
- [18] R.D. Arrua, T.J. Causon, E.F. Hilder, Recent developments and future possibilities for polymer monoliths in separation science, *Analyst* 137 (2012) 5179–5189.
- [19] R.D. Arrua, M. Talebi, T.J. Causon, E.F. Hilder, Review of recent advances in the preparation of organic polymer monoliths for liquid chromatography of large molecules, *Anal. Chim. Acta* 738 (2012) 1–12.
- [20] H. Li, Z. Liu, Recent advances in monolithic column-based boronate-affinity chromatography, *TrAC-Trend Anal. Chem.* 37 (2012) 148–161.
- [21] Y. Liang, L. Zhang, Y. Zhang, Recent advances in monolithic columns for protein and peptide separation by capillary liquid chromatography, *Anal. Bioanal. Chem.* 405 (2013) 2095–2106.
- [22] K. Liu, P. Aggarwal, J.S. Lawson, H.D. Tolley, M.L. Lee, Organic monoliths for high-performance reversed-phase liquid chromatography, *J. Sep. Sci.* 36 (2013) 2767–2781.
- [23] I. Nischang, Porous polymer monoliths: morphology, porous properties, polymer nanoscale gel structure and their impact on chromatographic performance, *J. Chromatogr. A* 1287 (2013) 39–58.
- [24] J. Ou, H. Lin, Z. Zhang, G. Huang, J. Dong, H. Zou, Recent advances in preparation and application of hybrid organic-silica monolithic capillary columns, *Electrophoresis* 34 (2013) 126–140.
- [25] F. Svec, Y. Lv, Advances and recent trends in the field of monolithic columns for chromatography, *Anal. Chem.* 87 (2014) 250–273.
- [26] J. Ou, Z. Liu, H. Wang, H. Lin, J. Dong, H. Zou, Recent development of hybrid organic-silica monolithic columns in CEC and capillary LC, *Electrophoresis* 36 (2015) 62–75.
- [27] N. Tanaka, D.V. McCalley, Core-shell, ultrasmall particles, monoliths, and other support materials in high-performance liquid chromatography, *Anal. Chem.* 88 (2015) 279–298.
- [28] I. Nischang, T.J. Causon, Porous polymer monoliths: from their fundamental structure to analytical engineering applications, *TrAC-Trend Anal. Chem.* 75 (2016) 108–117.
- [29] K. Kanamori, K. Nakanishi, T. Hanada, Rigid macroporous poly(divinylbenzene) monoliths with a well-defined bicontinuous morphology prepared by living radical polymerization, *Adv. Mater.* 18 (2006) 2407–2411.
- [30] K. Kanamori, J. Hasegawa, K. Nakanishi, T. Hanada, Facile synthesis of macroporous cross-linked methacrylate gels by atom transfer radical polymerization, *Macromolecules* 41 (2008) 7186–7193.
- [31] J. Hasegawa, K. Kanamori, K. Nakanishi, T. Hanada, S. Yamago, Pore formation in poly(divinylbenzene) networks derived from organotellurium-mediated living radical polymerization, *Macromolecules* 42 (2009) 1270–1277.
- [32] Y. Shen, L. Qi, L. Mao, Macroporous polymer monoliths with a well-defined three dimensional skeletal morphology derived from a novel phase separator for HPLC, *Polymer (Guildf.)* 53 (2012) 4128–4134.
- [33] K. Liu, P. Aggarwal, H.D. Tolley, J.S. Lawson, M.L. Lee, Fabrication of highly cross-linked reversed-phase monolithic columns via living radical polymerization, *J. Chromatogr. A* 1367 (2014) 90–98.
- [34] G. Hasegawa, K. Kanamori, N. Ishizuka, K. Nakanishi, New monolithic capillary columns with well-defined macropores based on poly(styrene-co-divinylbenzene), *ACS Appl. Mater. Interfaces* 4 (2012) 2343–2347.
- [35] J. Urban, F. Svec, J.M.J. Fréchet, Efficient separation of small molecules using a large surface area hypercrosslinked monolithic polymer capillary column, *Anal. Chem.* 82 (2010) 1621–1623.
- [36] H.C. Kolb, M.G. Finn, K.B. Sharpless, Click-chemistry: diverse chemische funktionalität mit einer handvoll guter reaktionen, *Angew. Chem. Int. Ed. Engl.* 113 (2001) 2056–2075.
- [37] P. Espeel, F.E. Du Prez, “Click”-inspired chemistry in macromolecular science: matching recent progress and user expectations, *Macromolecules* 48 (2015) 2–14.
- [38] C.E. Hoyle, C.N. Bowman, Thiol-ene click chemistry, *Angew. Chem. Int. Ed. Engl.* 49 (2010) 1540–1573.
- [39] J.-F. Lutz, 1,3-Dipolar cycloadditions of azides and alkynes: a universal ligation tool in polymer and materials science, *Angew. Chem. Int. Ed. Engl.* 46 (2007) 1018–1025.
- [40] W. Xi, T.F. Scott, C.J. Kloxin, C.N. Bowman, Click chemistry in materials science, *Adv. Funct. Mater.* 24 (2014) 2572–2590.
- [41] C.E. Hoyle, A.B. Lowe, C.N. Bowman, Thiol-click chemistry: a multifaceted toolbox for small molecule and polymer synthesis, *Chem. Soc. Rev.* 39 (2010) 1355–1387.
- [42] P.L. Golas, K. Matyjaszewski, Marrying click chemistry with polymerization: expanding the scope of polymeric materials, *Chem. Soc. Rev.* 39 (2010) 1338–1354.
- [43] A. Qin, J.W.Y. Lam, B.Z. Tang, Click polymerization, *Chem. Soc. Rev.* 39 (2010) 2522–2544.
- [44] B.C. Yao, J.Z. Sun, A.J. Qin, B.Z. Tang, Thiol-yne click polymerization, *Chin. Sci. Bull.* 58 (2013) 2711–2718.

- [45] A. Qin, J.W.Y. Lam, B.Z. Tang, Click polymerization: progresses, challenges, and opportunities, *Macromolecules* 43 (2010) 8693–8702.
- [46] M. Malkoch, R. Vestberg, N. Gupta, L. Mespouille, P. Dubois, A.F. Mason, et al., Synthesis of well-defined hydrogel networks using click chemistry, *Chem. Commun. (Camb.)* (2006) 2774–2776.
- [47] T. Gong, B.J. Adzima, N.H. Baker, C.N. Bowman, Photopolymerization reactions using the photoinitiated copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, *Adv. Mater.* 25 (2013) 2024–2028.
- [48] M.A. Daniele, A.A. Adams, J. Naciri, S.H. North, F.S. Ligler, Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds, *Biomaterials* 35 (2014) 1845–1856.
- [49] N. Gupta, B.F. Lin, L.M. Campos, M.D. Dimitriou, S.T. Hikita, N.D. Treat, et al., A versatile approach to high-throughput microarrays using thiol-ene chemistry, *Nat. Chem.* 2 (2010) 138–145.
- [50] R.A. Prasath, M.T. Gokmen, P. Espeel, F.E. Du Prez, Thiol-ene and thiol-yne chemistry in microfluidics: a straightforward method towards macroporous and nonporous functional polymer beads, *Polym. Chem.* 1 (2010) 685–692.
- [51] K. Hosoya, N. Hira, K. Yamamoto, M. Nishimura, N. Tanaka, High-performance polymer-based monolithic capillary column, *Anal. Chem.* 78 (2006) 5729–5735.
- [52] F. Alves, I. Nischang, Tailor-made hybrid organic–inorganic porous materials based on polyhedral oligomeric silsesquioxanes (POSS) by the step-growth mechanism of thiol-ene “click” chemistry, *Chem. Eur. J.* 19 (2013) 17310–17313.
- [53] H. Lin, J. Ou, Z. Liu, H. Wang, J. Dong, H. Zou, Facile construction of macroporous hybrid monoliths via thiol-methacrylate Michael addition click reaction for capillary liquid chromatography, *J. Chromatogr. A* 1379 (2015) 34–42.
- [54] H. Lin, J. Ou, Z. Liu, H. Wang, J. Dong, H. Zou, Thiol-epoxy click polymerization for preparation of polymeric monoliths with well-defined 3D framework for capillary liquid chromatography, *Anal. Chem.* 87 (2015) 3476–3483.
- [55] H. Lin, L. Chen, J. Ou, Z. Liu, H. Wang, J. Dong, et al., Preparation of well-controlled three-dimensional skeletal hybrid monoliths via thiol-epoxy click polymerization for highly efficient separation of small molecules in capillary liquid chromatography, *J. Chromatogr. A* 1416 (2015) 74–82.
- [56] F. Alves, I. Nischang, Radical-mediated step-growth: preparation of hybrid polymer monolithic columns with fine control of nanostructural and chromatographic characteristics, *J. Chromatogr. A* 1412 (2015) 112–125.
- [57] Z. Liu, J. Ou, H. Lin, Z. Liu, H. Wang, J. Dong, et al., Photoinduced thiol-ene polymerization reaction for fast preparation of macroporous hybrid monoliths and their application in capillary liquid chromatography, *Chem. Commun. (Camb.)* 50 (2014) 9288–9290.
- [58] L. Chen, J. Ou, Z. Liu, H. Lin, H. Wang, J. Dong, et al., Fast preparation of a highly efficient organic monolith via photo-initiated thiol-ene click polymerization for capillary liquid chromatography, *J. Chromatogr. A* 1394 (2015) 103–110.
- [59] B.D. Fairbanks, T.F. Scott, C.J. Kloxin, K.S. Anseth, C.N. Bowman, Thiol-yne photopolymerizations: novel mechanism, kinetics, and step-growth formation of highly cross-linked networks, *Macromolecules* 42 (2008) 211–217.
- [60] J.W. Chan, J. Shin, C.E. Hoyle, C.N. Bowman, A.B. Lowe, Synthesis, thiol-yne “click” photopolymerization, and physical properties of networks derived from novel multifunctional alkynes, *Macromolecules* 43 (2010) 4937–4942.
- [61] J.W. Chan, H. Zhou, C.E. Hoyle, A.B. Lowe, Photopolymerization of thiol-alkynes: polysulfide networks, *Chem. Mater.* 21 (2009) 1579–1585.
- [62] Z. Liu, J. Ou, H. Lin, H. Wang, Z. Liu, J. Dong, et al., Preparation of monolithic polymer columns with homogeneous structure via photoinitiated thiol-yne click polymerization and their application in separation of small molecules, *Anal. Chem.* 86 (2014) 12334–12340.
- [63] H. Zhang, J. Ou, Z. Liu, H. Wang, Y. Wei, H. Zou, Preparation of hybrid monolithic columns via “one-pot” photoinitiated thiol-acrylate polymerization for retention-independent performance in capillary liquid chromatography, *Anal. Chem.* 87 (2015) 8789–8797.
- [64] D.P. Nair, M. Podgórski, S. Chatani, T. Gong, W. Xi, C.R. Fenoli, et al., The thiol-michael addition click reaction: a powerful and widely used tool in materials chemistry, *Chem. Mater.* 26 (2013) 724–744.
- [65] Y. Lv, Z. Lin, F. Svec, “Thiol-ene” click chemistry: a facile and versatile route for the functionalization of porous polymer monoliths, *Analyst* 137 (2012) 4114–4118.
- [66] Q. Li, X. Tu, J. Ye, Z. Bie, X. Bi, Z. Liu, Nanoconfining affinity materials for pH-mediated protein capture-release, *Chem. Sci.* 5 (2014) 4065–4069.
- [67] N. Tsujioka, N. Hira, S. Aoki, N. Tanaka, K. Hosoya, A new preparation method for well-controlled 3D skeletal epoxy resin-based polymer monoliths, *Macromolecules* 38 (2005) 9901–9903.
- [68] N. Tsujioka, N. Ishizuka, N. Tanaka, T. Kubo, K. Hosoya, Well-controlled 3D skeletal epoxy-based monoliths obtained by polymerization induced phase separation, *J. Polym. Sci. Part A Polym. Chem.* 46 (2008) 3272–3281.
- [69] K. Hosoya, T. Mori, M. Sakamoto, T. Kubo, K. Kaya, Properties of a non-aromatic epoxy polymer-based monolithic capillary column for μ -HPLC, *Chromatographia* 70 (2009) 699–704.
- [70] A.M. Nguyen, K. Irgum, Epoxy-based monoliths. A novel hydrophilic separation material for liquid chromatography of biomolecules, *Chem. Mater.* 18 (2006) 6308–6315.
- [71] A.M. Nguyen, N.P. Dinh, Q.M. Cam, T. Sparrman, K. Irgum, Preparation and characterization of sizable macroporous epoxy resin-based monolithic supports for flow-through systems, *J. Sep. Sci.* 32 (2009) 2608–2618.
- [72] L. Ren, Z. Liu, Y. Liu, P. Dou, H.-Y. Chen, Ring-opening polymerization with synergistic co-monomers: access to a boronate-functionalized polymeric monolith for the specific capture of cis-diol-containing biomolecules under neutral conditions, *Angew. Chem. Int. Ed. Engl.* 48 (2009) 6704–6707.
- [73] Y. Liu, Y. Lu, Z. Liu, Restricted access boronate affinity porous monolith as a protein A mimetic for the specific capture of immunoglobulin G, *Chem. Sci.* 3 (2012) 1467–1471.
- [74] J. Liu, L. Ren, Y. Liu, H. Li, Z. Liu, Weak anion exchange chromatographic profiling of glycoprotein isoforms on a polymer monolithic capillary, *J. Chromatogr. A* 1228 (2012) 276–282.
- [75] M. Talebi, R.D. Arrua, A. Gaspar, N. Lacher, Q. Wang, P. Haddad, et al., Epoxy-based monoliths for capillary liquid chromatography of small and large molecules, *Anal. Bioanal. Chem.* 405 (2013) 2233–2244.
- [76] H. Lin, J. Ou, Z. Zhang, J. Dong, H. Zou, Ring-opening polymerization reaction of polyhedral oligomeric silsesquioxanes (POSSs) for preparation of well-controlled 3D skeletal hybrid monoliths, *Chem. Commun. (Camb.)* 49 (2013) 231–233.
- [77] H. Lin, J. Ou, S. Tang, Z. Zhang, J. Dong, Z. Liu, et al., Facile preparation of a stable and functionalizable hybrid monolith via ring-opening polymerization for capillary liquid chromatography, *J. Chromatogr. A* 1301 (2013) 131–138.
- [78] H. Lin, Z. Zhang, J. Dong, Z. Liu, J. Ou, H. Zou, Preparation of polyhedral oligomeric silsesquioxane based hybrid monoliths by ring-opening polymerization for capillary LC and CEC, *J. Sep. Sci.* 36 (2013) 2819–2825.
- [79] Z. Liu, J. Ou, H. Lin, H. Wang, J. Dong, H. Zou, Preparation of polyhedral oligomeric silsesquioxane-based hybrid monolith by ring-opening polymerization and post-functionalization via thiol-ene click reaction, *J. Chromatogr. A* 1342 (2014) 70–77.
- [80] H. Wang, J. Ou, H. Lin, Z. Liu, G. Huang, J. Dong, et al., Chromatographic assessment of two hybrid monoliths prepared via epoxy-amine ring-opening polymerization and methacrylate-based free radical polymerization using methacrylate epoxy cyclosiloxane as functional monomer, *J. Chromatogr. A* 1367 (2014) 131–140.
- [81] S. De, A. Khan, Efficient synthesis of multifunctional polymers via thiol-epoxy “click” chemistry, *Chem. Commun. (Camb.)* 48 (2012) 3130–3132.
- [82] M. Kele, G. Guiochon, Repeatability and reproducibility of retention data and band profiles on six batches of monolithic columns, *J. Chromatogr. A* 960 (2002) 19–49.
- [83] E. Wen, R. Asaie, C. Horváth, Dynamics of capillary electrochromatography: II. Comparison of column efficiency parameters in microscale high-performance liquid chromatography and capillary electrochromatography, *J. Chromatogr. A* 855 (1999) 349–366.
- [84] J. Kiefer, J.L. Hedrick, J.G. Hilborn, Macroporous thermosets by chemically induced phase separation, *Adv. Polym. Sci.* 147 (1999) 161–247.
- [85] Y. Mi, W. Zhou, Q. Li, D. Zhang, R. Zhang, G. Ma, et al., Detailed exploration of structure formation of an epoxy-based monolith with three-dimensional bicontinuous structure, *RSC Adv.* 5 (2015) 55419–55427.
- [86] H. Peng, C. Wang, W. Xi, B.A. Kowalski, T. Gong, X. Xie, et al., Facile image patterning via sequential thiol–Michael/thiol-yne click reactions, *Chem. Mater.* 26 (2014) 6819–6826.