One-step fabrication of cinchona-based hybrid monolithic chiral stationary phases via photo-initiated thiol-ene polymerization for cLC enantioseparation

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ABSTRACT

Although various click polymerization reactions (thiol-ene, thiol-yne, thiol-Michael, thiol-epoxy and amine-epoxy) have been utilized to prepare either hybrid or organic monolithic columns with homogeneous network structures, there were few reports on fabrication of monolithic CSPs via click polymerization. Herein, a fast and robust approach was explored to fabricate cinchona-based monolithic hybrid CSPs via photo-initiated thiol-ene polymerization within 10 min in one step. A self-synthesized octakis(3-mercaptopropyl) octasilsesquioxane (POSS-SH) was polymerized with phenylisocyanate cinchonidine (PCD) and (+)-N,N'-diallyl-L-tartardiamide (DATDA) or 1,2,4-trivinylcyclohexane (TVCH). The resulting two kinds of as-synthesized monolithic CSPs, poly(POSS-*co*-DATDA-*co*-PCD) and poly(POSS-*co*-TVCH-*co*-PCD), were evaluated for cLC enantioseparation of acidic racemates. It was found that they exhibited different enantioseparation ability due to using different multivinyl crosslinkers. The influence of ACN content in mobile phase on the enantioseparation of acidic racemates was investigated. The separation mechanism was also discussed on the basis of a comparison of enantioseparation on two kinds of hybrid monolithic CSPs.

1. Introduction

It is well known that pharmacological activity of chiral drugs is restricted in most cases due to different biological effects between two optical isomers. The enantioseparation of chiral compounds plays an important role in the fields of chemistry, pharmacology, biology and medicine. Several separation techniques have been developed to pursue efficient enantioseparation with fast analysis, high efficiency as well as large-scale production of pure enantiomers. High performance liquid chromatography (HPLC), capillary liquid chromatography (cLC) and capillary electrochromatography (CEC) equipped with robust chiral stationary phases (CSPs) were intensively developed for direct analysis and detection [1–5]. Up to now, various types of particles-based CSPs were successfully fabricated by immobilization with chiral selectors, including polysaccharides and cyclodextrins (CDs), Pirkle-type, macrocyclic antibiotics, crown ethers and so on [6–16]. Since Lindner and coworkers have developed an anion exchange-type CSPs by immobilization of cinchona alkaloid derivatives in 1996, 9-O-tert-

butylcarbamate derivatives of quinine and quinidine were commercialized as CSPs under the trade names Chiralpak QN-AX and Chiralpak QD-AX, respectively. They exhibited excellent recognition abilities for acidic chiral compounds such as N-protected amino acids [17–19], which were related to a synergistic effect, including ion-pair formation between chiral analytes and selector, hydrophobic, dipole-dipole, hydrogen bonding, π–π and steric interactions [20].

Since monolithic columns had been first introduced by Hjertén and Svec, as a new column technology for fast separation of small molecules and macromolecules in the late 1980s, various monolithic columns including organic polymer, inorganic silica, or organic-silica hybrid matrix have been reported and widely used in the field of separation and analysis. Monolithic CSPs have emerged as an effective tool for chiral separation, allowing the preparation of chromatographic columns with high efficiencies and low column backpressures. There were only a few works on the preparation of cinchona alkaloid-based ion-exchange monolithic CSPs. Lammerhofer and coworkers have fabricated chiral organic monoliths by both thermal- and UV light initiated

polymerization of O-[2-(methacryloyloxy) ethylcarbamoyl]-10,11-di-hydroquinidine, ethylene dimethacrylate and glycidyl methacrylate or 2-hydroxyethyl methacrylate, which exhibited remarkably high efficiencies for the separation of racemic N-2,4-dinitrophenyl valine (DNP-Val) in CEC [21]. Recently, Jiang's group also prepared the same quinidine-based monolith for cLC evaluation of enantioselectively. A mixture of three pairs of 3,5-dichlorobenzoyl-amino acids could be simultaneously enantioseparated in a run [22]. In 2004, Lubda and Lindner fabricated a silica-based monolithic CSP by immobilization of tert-butyl-carbamoylquinine onto a commercially available monolithic silica column [19]. However, the preparation of these silica-based monolithic CSPs was time-consuming with poor reproducibility to control the entire preparation process, and restricted their applications.

As an attractive alternative to organic and silica monoliths, hybrid organic–inorganic monolith has attracted considerable concerns as a potential ideal material with high surface area, excellent mechanical strength and thermal stability [23–26]. Recently, a hybrid β-CD-silica monolith was fabricated by a "one-pot" approach via sol-gel chemistry and in situ copolymerization with a β-cyclodextrin derivative [27]. It would take long time to optimize synthesis conditions. As a result, it was still urgent to develop simple approaches to fabricate novel hybrid monolithic CSPs with improved enantioselectivity and efficiency. A series of organic-silica hybrid monoliths have been developed via thermal-initiated free-radical polymerization and ring-opening polymerization of organic monomers with several vinyl/methacrylate/acrylate/epoxy-containing polyhedral oligomeric silsesquioxanes (POSS) [28–32]. The resulting POSS-containing monoliths exhibited good mechanical and pH stability (pH 1–11) and were successfully applied for separation of various small molecules in cLC. However, there were few reports on the enantioseparation using POSS-containing monolithic CSPs [33].

Herein, we would try to synthesize both an octakis(3-mercaptopropyl)octasilsesquioxane (POSS-SH) (POSS-SH) and a chiral selector phenylisocyanate cinchonidine (PCD) as two precursors to directly fabricate novel POSS-containing monolithic CSPs via thiol-ene polymerization. As thiol-ene reaction could be induced photochemically or thermally at ambient temperature in the presence of oxygen without undesirable side reactions such as sulfenyl radical coupling [34], a crosslinker with two or three vinyl groups must be added to form monolithic skeleton in thiol-ene polymerization. As a result, both a chiral (+)-N,N′-diallyl-L-tartardiamide (DATDA) and a nonchiral 1,2,4-trivinylcyclohexane (TVCH) were selected to prepare two kinds of monolithic CSPs, poly(POSS-co-DATDA-co-PCD) and poly(POSS-co-TVCH-co-PCD), which were assigned as CSPs I and II, respectively (Scheme 1).

2. Experimental

2.1. Chemicals and materials

DATDA was obtained from TCI (Shanghai, China). TVCH, 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%) and cinchonidine were purchased from Acros Organics (New Jersey, USA). (3-Mercaptopropyl)trimethoxysilane (MPTMS, 96%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phenyl isocyanate was bought from Chengdu Apotheker chemical reagent (Chengdu, China). Vinyltrimethoxysilane (VTMS, 97.5%), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), polyethylene glycol 200 (PEG200) and dodecanol were from J&K Scientific Ltd. (Beijing, China). Racemic N-(3,5-dinitrobenzoyl)-leucine (DNB-Leu), N-(3,5-dinitrobenzoyl)-α-phenylglycine (DNB-PG), α-methoxyphenylacetic acid and β-phenyllactic acid were purchased from Sigma-Aldrich (Darmstadt, Germany). Their structures are shown in Fig. S1 (Supporting information). HPLC-grade acetonitrile (ACN) and methanol were obtained from Yuwang Group (Shandong, China) and used for preparation of mobile phases. The water used in all experiments was doubly distilled and purified by Milli-

Q system (Millipore Inc., Milford, MA, USA). Hydrochloric acid (HCl), dichloromethane (CH_2Cl_2), pyridine, 1-propanol, ethanol, toluene and other organic solvents were purchased from Kermel Chemical Regent Co., Ltd. (Tianjin, China). Other chemical reagents were of analytical grade. The fused-silica capillary (UV transparent coating) with inner diameter of 75 μm was obtained from Polymicro Technologies (Phoenix, AZ, USA).

The monomer POSS-SH was synthesized by using MPTMS as precursor according to the reported approach [35]. A solution of MPTMS (15 mL) and concentrated HCl (37%, 30 mL) in methanol (360 mL) was stirred at 90 °C for 24 h and produced POSS-SH as a white precipitate. The crude product was washed with cold methanol for three times to remove excess MPTMS. The resulting viscous solution was dissolved in CH_2Cl_2 and then washed three times with water. The CH_2Cl_2 phase was dried with anhydrous Na_2SO_4 and concentrated to obtain POSS-SH in 87% yield.

The chiral monomer of PCD was synthesized as shown in Scheme 1. Cinchonidine (2.94 g, 10 mmol) was first dissolved in 50.0 mL anhydrous pyridine, and then phenyl isocyanate (2.17 mL, 20 mmol) was dropped slowly. Under the protection of nitrogen, the reaction mixture was heated to 80 °C and stirred for 24 h. After removal of pyridine and the unreacted phenyl isocyanate under reduced pressure, the residue was re-dissolved in anhydrous ethanol, and then precipitated in 200 mL of water. After filtration, the residue was recrystallized in ethanol, and 2.0 g of solid product was obtained.

2.2. Preparation of cinchonidine-based monolithic CSP via photo-initiated thiol-ene polymerization

Prior to use, UV-transparent fused-silica capillary was rinsed by 0.1 mol/L NaOH (4 h), water, 0.1 mol/L HCl (4 h) and water, successively. After flushed with ethanol, the capillary was filled with VTMS/ethanol (50/50, v/v) solution, and then sealed with rubbers at both ends and submerged into water bath at 40 °C for 12 h. Finally, the capillary was rinsed with ethanol to flush out the residual reagents and dried by nitrogen stream at room temperature.
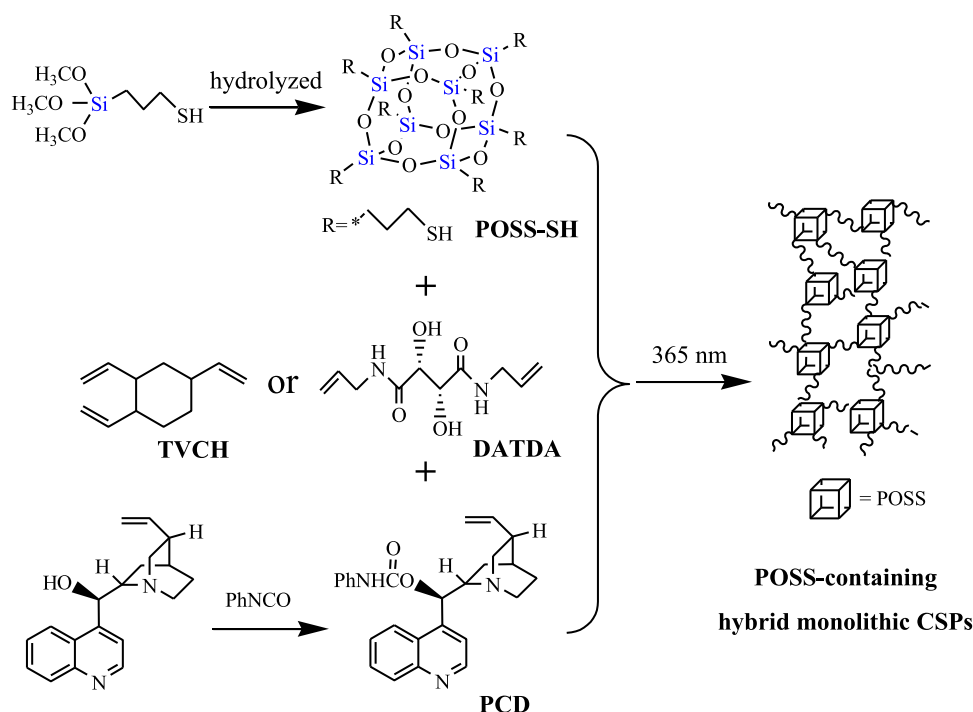
The cinchonidine-based monolithic CSP I was prepared according to our previous approach [36,37]. In brief, appropriate amounts of precursors (POSS-SH, PCD and DATDA) were first dissolved in binary porogenic solvents (DMSO/dodecanol) followed by addition of DMPA in a centrifuge tube. The prepolymerization solution was homogenized by sonication for 10 min and then introduced into the above-mentioned VTMS-pretreated capillary with a syringe. After sealing both ends, the capillary was irradiated in an UV cross-linking instrument (XL-1500A, λ = 365 nm, Spectronics Corporation, New York, USA) for 10 min. The obtained monolithic CSP I was flushed with methanol to remove residuals. The rest of prepolymerization solution in centrifuge tube was also irradiated to form bulk monolithic material under the same conditions. After polymerization, the molded bulk monolith was extracted by Soxhlet extraction for 24 h and then dried in a vacuum oven at 60 °C for 24 h, which could be used for physical characterization.

Similarly, monolithic CSP II was also prepared by using POSS-SH, PCD and TVCH as precursors.

2.3. Instruments and methods

The microscopic image of monolithic material was examined by SEM (JEOL JSM-5600, Tokyo, Japan). FT-IR was obtained by using KBr pellets on Thermo Nicolet 380 spectrometer (Nicolet, Wisconsin, USA). Water contact angle was measured on a JC2000C machine with 5 μL of water drop (Powereach, Shanghai, China). Elemental analysis was performed on EMIA-8100H (HORIBA, Japan).

The permeability was measured according to Darcy's law [38] by the equation: $B_0 = F\eta L/(\pi r^2 \Delta P)$, where F (m^3/s) is the flow rate of mobile phase, η is the dynamic viscosity of mobile phase, L (m) is the effective length of the column and r (m) is the inner diameter of the column, ΔP (Pa) is the pressure drop of column. Mobile phase was

Scheme 1. Schematic preparation of cinchonidine-based hybrid monolithic CSPs via photo-initiated thiol-ene polymerization.

pumped through the monolithic columns at a flow rate of 0.5–1.5 μL/min. The data of ΔP and F were obtained on nanoACQUITY Ultra Performance LC (Waters, USA).

Enantioseparation experiments were performed on an HPLC system equipped with an Agilent 1100 micropump, a 7725i injector with a 20 μL sample loop and a K-2501 UV detector (Knauer, Berlin, Germany), which was set at 214 nm. For obtaining a flow rate of nanoliters per minute (nL/min), a T-union connector was used as a splitter with one end connected to the capillary monolithic column and the other connected to a blank capillary (100 cm × 50 μm i.d.). The outlet of monolithic column was connected to an empty fused-silica capillary (50 μm i.d. and 365 μm o.d.) with a Teflon tube, where a detection window was made by removing a 2 mm length of the polyimide coating in a position of about 5.0 cm from the separation monolithic column outlet. All chromatographic data were collected and analyzed using the software program HW-2000 from Qianpu Software (Shanghai, China). The real flow rate through monolithic column was measured using a blank capillary (10 cm × 75 μm i.d.) connected to the monolithic column. The retention factor (k) and resolution (Rs) were calculated according to standard equations from the literatures [39,40].

3. Results and discussion

3.1. Preparation of hybrid monoliths via photo-initiated thiol-ene

As the composition of prepolymerization solution played vitally important roles in the formation of monolithic columns, several parameters including the type of porogenic system, molar ratio of thiol/ene and the total content of precursors were considered in detail. Various kinds of porogenic systems, such as toluene/dodecanol [41], propanol/1,4-butanediol [42] and 1-butanol/ethylene glycol [32] have been commonly adopted to fabricate POSS-containing monoliths via either free radical polymerization or ring-opening polymerization. However, these porogenic systems could not be selected for preparation of monolithic CSP I as the hydrophobic precursor of cinchonidine derivative and the hydrophilic precursor of DATDA could not be well dissolved. A binary porogenic system of DMSO/1-dodecanol was finally used for fabrication of monolithic CSP I because DMSO could dissolve

both PCD and DATDA. It should be pointed that a little acetic acid in porogenic system would facilitate to dissolve PCD.

The detailed compositions in the prepolymerization solution for monolithic CSPs were listed in Table 1. It could be found from three columns I-1, I-2 and I-3 that the permeability was increased with an decrease of DMSO content in porogenic system when the molar ratio of thiol/ene/ene groups in POSS-SH/DATDA/PCD was kept constant (8/6/0.7). When the volume ratio of DMSO/1-dodecanol was 58/42 (v/v), the resulting monolith (column I-1) was not permeable. While this ratio was reduced into 52/48 (v/v), the monolith (column I-3) became looser, and its permeability reached $2.5 \times 10^{-14}\, m^2$. These results demonstrated that DMSO served as a good solvent for the generation of micopores, while 1-dodecanol served as a macroporogenic solvent for the formation of satisfactory monoliths.

The molar ratio of thiol/ene also had effect on the formation of monolithic CSPs. Due to high reaction efficiency and conversion of

Table 1
Detail composition of prepolymerization solutions for preparation of monolithic CSPs and their permeability.

CSP[a]	Molar ratio of thiol/ene/ene groups in POSS-SH/DATDA/PCD	Molar ratio of thiol/ene/ene groups in POSS-SH/TVCH /PCD	DMSO/1-Dodecanol[b] (v/v)	THF/PEG200 (v/v)	Permeability ($\times 10^{-14}\, m^2$)
I-1	8/6/0.7	–	58/42	–	–[c]
I-2	8/6/0.7	–	54/46	–	0.4
I-3	8/6/0.7	–	52/48	–	2.5
I-4	8/6/2	–	54/46	–	–[c]
I-5	8/6/0	–	54/46	–	2.7
I-6	5/6/0.7	–	54/46	–	6.8
I-7	9/6/0.7	–	54/46	–	–[c]
II-1	–	3.8/2.9/0.3	–	68/32	2.6
II-2	–	3.8/2.9/0.3	–	74/26	1.1
II-3	–	3.8/2.9/0.3	–	82/18	–[c]

[a] The ratio of monomers/porogens (m/v) was 38.5% for CSP I and 36.8% for CSP II.
[b] A little acetic acid (1.5%, v/v) was added into porogenic solvents.
[c] The resulting monolithic column was too dense to flow through.

thiol-ene polymerization via photo-initiation mode, a nearly equimolar amount of thiol and vinyl groups in the precursors was initially adopted to prepare monolithic CSP. However, the column I-4 could not be obtained when the molar ratio of thiol/ene/ene groups in POSS-SH/DATDA/PCD was kept at 8/6/2, due to the formation of gel-like transparent bulk polymers. If PCD was not added (as for column I-5), a monolithic poly(POSS-*co*-DATDA) was acquired, and its permeability was 2.7×10^{-14} m^2. Additionally, a slight decrease of POSS-SH content would lead to form a monolith with higher permeability of 6.8×10^{-14} m^2 (as for column I-6), while a semitransparent monolith was formed and not even permeable when more POSS-SH was added (as for column I-7). As a result, the column I-3 was finally selected for following characterization and enantioseparation.

Although 1-dodecanol served as a macroporogenic solvent in the formation of monolithic CSP I, transparent monolithic CSP II was formed even using 1-dodecanol as a sole porogen, indicating a binary porogenic system of DMSO/1-dodecanol was not suitable for preparation of monolithic CSP II. We had to seek other porogenic systems. According to our previous result, a binary porogenic system of diethylene glycol diethyl ether/PEG200 has chosen to fabricate an organic monolith via photo-initiated thiol-ene click polymerization reaction of TVCH) and pentaerythriol tetra(3-mercaptopropionate) [43]. Herein, a binary porogenic system of THF/PEG200 was eventually chosen to synthesize monolithic CSP II. As expected, the ratio of THF/PEG200 had effect on the permeability of monolithic CSP II. It could be observed from Table 1 that the permeability was decreased with an increase of THF content, which served as a good solvent. So the column II-1 exhibited a satisfactory permeability of 2.6×10^{-14} m^2 and was further applied for enantioseparation in cLC.

3.2. Characterization of monolithic CSPs

Fig. 1 presents the SEM images of two kinds of monolithic CSPs I and II. It could be observed that the homogeneous matrices were well linked to the inner wall of capillaries (Fig. 1a and b), as well as through-pores with about 1 μm (Fig. 1c and d), which offered a good permeability. It should be noted that both bulk monolithic materials (I and II) formed in centrifuge tubes were subjected to shrinkage in the dry state. So the surface area of monoliths could not be well determined by nitrogen adsorption/desorption isotherm. However, the monolithic matrices inside capillaries could keep stable structure morphology and chromatographic performance even after three weeks usage due to only slight change of retention factor. This phenomenon of shrinkage has been observed in monolithic polymeric columns via photo-initiated thiol-yne polymerization [44].

The bulk monolithic materials (I and II) formed in centrifuge tube were characterized by FT-IR (Fig. 2). The weak peak at 2556 cm^{-1} was assigned to stretching vibrations of thiol group, while the strongly peak at 1085 cm^{-1} was assigned to the Si-O-Si symmetric stretching vibration in POSS-SH. Characteristic peaks at 990 and 919 cm^{-1} were assigned to C=C signals, and the peak at 1645 cm^{-1} was assigned to C=O in DATDA. The peaks at 1640, 990 and 910 cm^{-1} were assigned to C=C signals in TVCH. Disappearance of peak at 2556 cm^{-1}(-SH), decrease of peak at 990 and 919 cm^{-1} (C=C) and existence of peak at 1645 (C=O) cm^{-1} in CSPs I and II confirmed the thiol-ene click polymerization reaction of DATDA/TVCH and POSS-SH quickly occurred within several minutes. As the molar ratio of thiol/ene in prepolymerization solution was approximate 8/7, a few thiol groups should exist in monolithic CSP I,
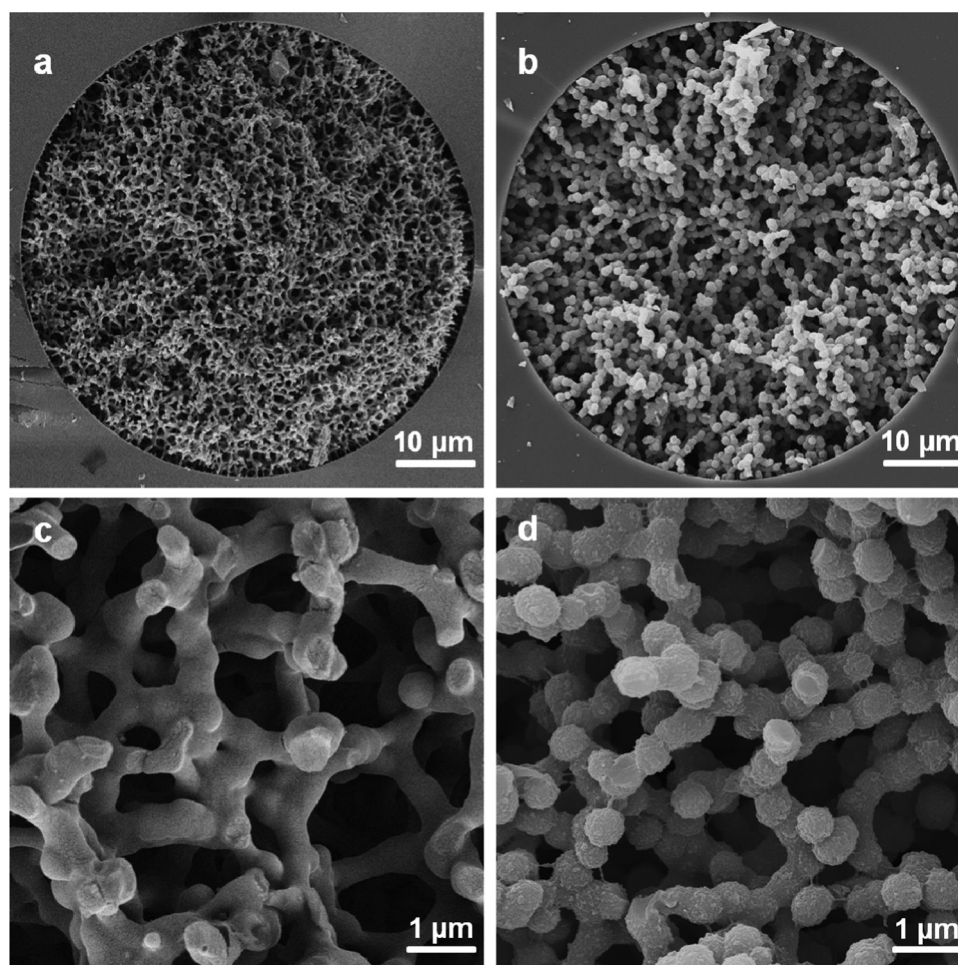

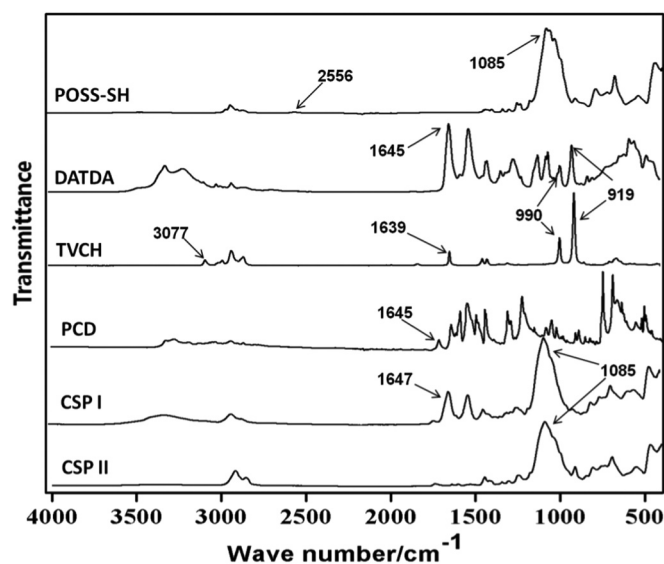**Fig. 1.** SEM images of (a, c) CSP I and (b, d) CSP II.

Fig. 2. ATR-IR spectra of POSS-SH, DATDA, TVCH, PCD, CSPs I and II.

however, thiol signal at 2556 cm^{-1} was not observed. For CSP II, the peaks at 1640 cm^{-1} (assigned to C=C) was hardly observed, which indicated almost all vinyl groups took part in the reaction with sulfhydryl groups. It is well known that thiol-ene reaction is insensitive to oxygen due to the formation of peroxy radical. As a result, one of the major obstacles in traditional free radical photopolymerization was essentially eliminated in thiol-ene polymerization because the polymerization occurs in air almost as rapidly as in an inert atmosphere [45].

Water contact angle was measured for investigating the hydrophobic property of material. As shown in Fig. S2 (Supporting information), the contact angle value of CSP I (82°) was lower than that of CSP II (112°). It might be related to the distribution of hydrophilic hydroxyl and amino groups in the skeletons of monolithic CSP I, which did not exist in the skeletons of monolithic CSP II. Thermal stability was also analyzed by monitoring weight loss with increasing temperature using a TGA. A representative plot of monolithic materials (I and II) was shown in Fig. S3 (Supporting information). Due to the hygroscopic nature of two monoliths, they were normalized for absorbed water from the atmosphere by subtracting detected mass loss from room temperature to 100 °C. Both monoliths exhibited high overall thermal stability until 275 °C, but the weight loss of monolith I was more remarkably than that of monolith II from 275 to 380 °C. In general, cyclohexanes in monolith II should have higher degradation temperature than tartardiamide counterparts in monolith I, and were more thermally stable. As a result, the type of vinyl monomers had impact on thermal degradation onset temperature of resulting monoliths.

The mechanical stability of two hybrid monolithic CSPs was examined, and the backpressures were all linearly (R^2 = 0.999) increased when the flow rate was increased from 50 to 500 nL/min (data not shown). These results demonstrated that two hybrid monoliths exhibited satisfactory mechanical stability. Particularly, run-to-run repeatability was also measured in terms of both t_o and the retention factor of benzene when the mobile phase was 50% ACN/water solution. It was calculated that the relative standard deviations (RSD) for benzene were less than 2.6% (n = 5), further indicating stable matrices in two monolithic columns. The column-to-column and batch-to-batch preparation reproducibility of two monoliths were also evaluated through the RSD for retention factor of benzene. It was calculated that the column-to-column reproducibility and batch-to-batch preparation of monoliths were less than 4.8% (n = 3) and 6.5% (n = 3) RSD, respectively, for two hybrid monolithic columns. These results demonstrated that the reproducibility of hybrid monoliths would be acceptable.

3.3. Enantioseparation on two kinds of hybrid monolithic CSPs in cLC

Applications of cinchona alkaloid-type CSP were widely explored under the mobile phase of polar-organic mode, in which non-aqueous conditions were adopted by employing methanol as mobile phase with acetic acid as an acidic modifier, which represented the counter-ion in the ion-exchange process being necessary to facilitate elution within short time [46,47]. The enantioseparation evaluation of as-synthesized two kinds of monolithic CSPs was performed after carefully optimizing chromatographic conditions. DNB-Leu has been enantioseparated on cinchona alkaloid-type CSPs with exceptionally high selectivity and resolution [22,40,46]. So DNB-Leu was selected as a model racemate for cLC. In order to acquire fast enantioseparation on the monolithic CSP, herein, a 20-cm long monolithic capillary column was evaluated by using various hydro-organic mobile phases. The effect of organic solvent category (methanol and ACN) in the mobile phase on retention time and enantioselectivity was investigated. Although DNB-Leu could be enantioseparated with the mobile phases of either methanol/water (70/30, v/v) or ACN/ water (70/30, v/v) containing HAc and TEA, the retention time was much longer and could not be eluted from column within 60 min. Therefore, a solution of ACN/H$_2$O containing a little of HAc and TEA was chosen to enantioseparate acidic racemates according to several excellent reports. Fig. 3 presented several typical chromatograms of enantioseparations on CSP I by using mobile phase of ACN/ H$_2$O (93/7, v/v) containing HAc (160 mM) and TEA (10 mM). It could be seen that racemic DNB-Leu (Fig. 3a) and DNB-PG (Fig. 3b) could be baseline separated within 15 min, but other racemates, α-methoxyphenylacetic acid (Fig. 3c) and β-phenyllactic acid (Fig. 3d) were not well separated. As shown in Fig. 3a, the resolution (Rs) of DNB-Leu was 5.3. Although the resolution was lower than those obtained on the silica particles-based cinchona-CSPs, fast chiral separation could be performed on monolithic CSP in cLC. These results demonstrated photoinitiated thiol-ene polymerization for fabrication of hybrid monolithic CSPs represents a powerful approach for preparation of monolithic capillary columns for enantioseparations.

As the content of organic solvent in mobile phases affected the retention and resolution of racemates, DNB-Leu was also selected as a testing analyte for investigating the effect of ACN content in mobile phase on retention factor, resolution, and efficiency for hybrid monolithic CSPs I. The result was shown in Fig. 3e. The retention factor (k) of DNB-Leu decreased with an increase of ACN content in mobile phase from 90% to 96% (v/v), indicating a hydrophobic interaction between cinchonidine derivative and enantiomers. The hydrophobic interaction is a kind of a "long-distance" interaction that pushes the chiral analytes close to the CSPs in RP mode, and cooperates with two "short-distance" interactions of π–π and hydrogen-bonding interactions for chiral recognition [48,49]. Additionally, the resolution of DNB-Leu also decreased from 6.7 to 3.4 with the increase of ACN content from 90% to 96%. The decrease of resolution of other three chiral compounds could be observed in Fig. 3f. These results demonstrated that ACN content had an important effect on both retention and resolution of racemate on hybrid monolithic CSP I.

Another monolithic CSP II was also evaluated in cLC, and the results were given in Fig. 4. It could be observed from Fig. 4a that the resolution of racemic DNB-Leu was only 1.2, which was remarkably lower than that on monolithic CSP I. However, the baseline-enantioseparations of DNB-PG and α-methoxyphenylacetic acid were not acquired even after optimization of chromatographic conditions (Fig. 4b and c). As shown in Fig. 4d, the retention factors of racemate DNB-Leu were hardly changed with an increase of ACN content in mobile phases from 90% to 100%, but its resolution was slightly increased from 0.58 to 0.91. For comparison of two kinds of CSPs, the monolithic CSP I exhibited higher resolution and better selectivity of enantioseparation for acidic racemates than monolithic CSP II. These results indicated that the vinyl monomers also affected enantioseparation ability in cLC, and chiral biene (DATDA) could significant improve both resolution and
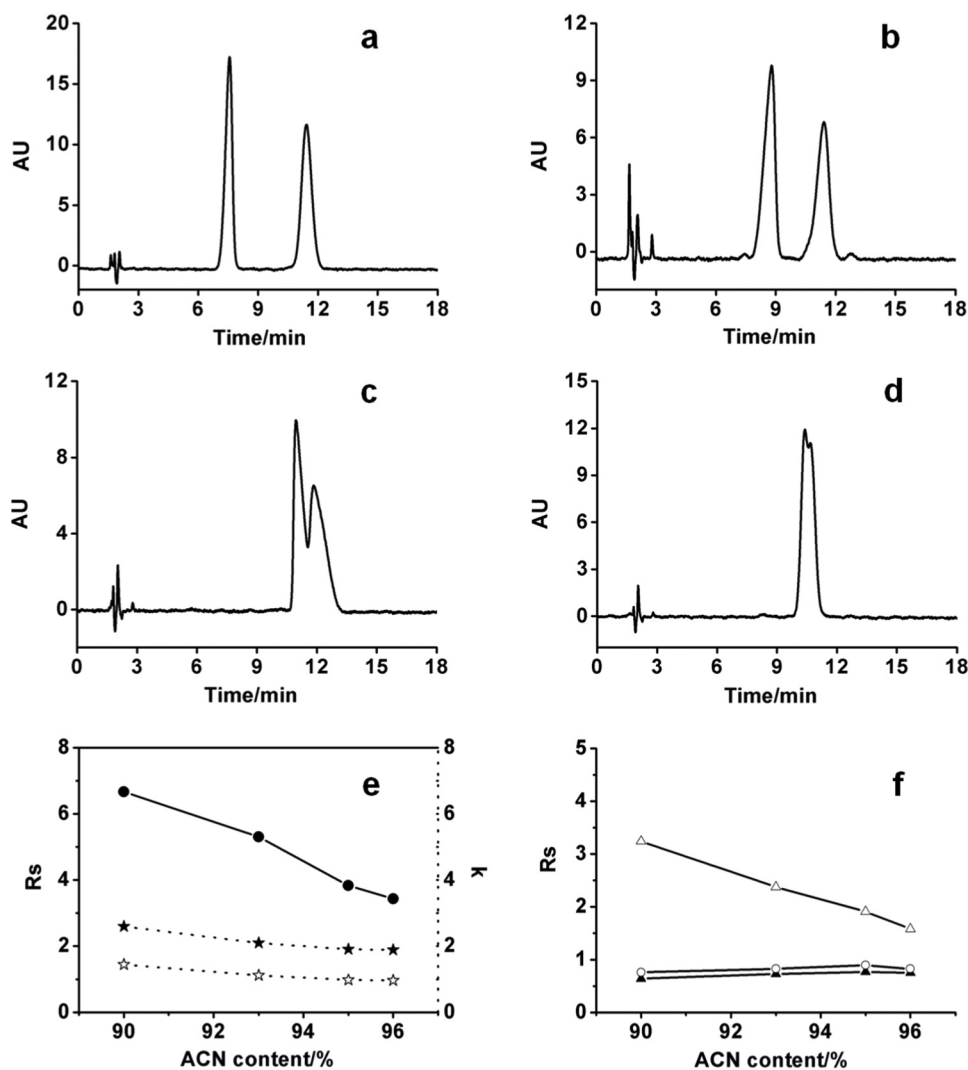
Fig. 3. (a-d) Separations of chiral compounds on monolithic CSP I by cLC. Analytes: (a) DNB-Leu, (b) DNB-PG, (c) α-methoxyphenylacetic acid and (d) β-phenyllactic acid. (e) Effect of ACN content in mobile phases on resolution (*Rs*) and retention factor (*k*) of DNB-Leu. (f) Effect of ACN content in mobile phases on resolution (*Rs*) of other analytes. (△) DNB-PG, (○) DL-α-methoxyphenylacetic acid and (▲) β-phenyllactic acid. Experimental conditions: column dimension, 20.8 cm × 75 μm.; mobile phase, (a-d) ACN (HAc 160 mM, TEA 10 mM)/H$_2$O(93/7, v/v); flow rate, 400 μL min^{-1} (before split); detection wavelength, 214 nm.
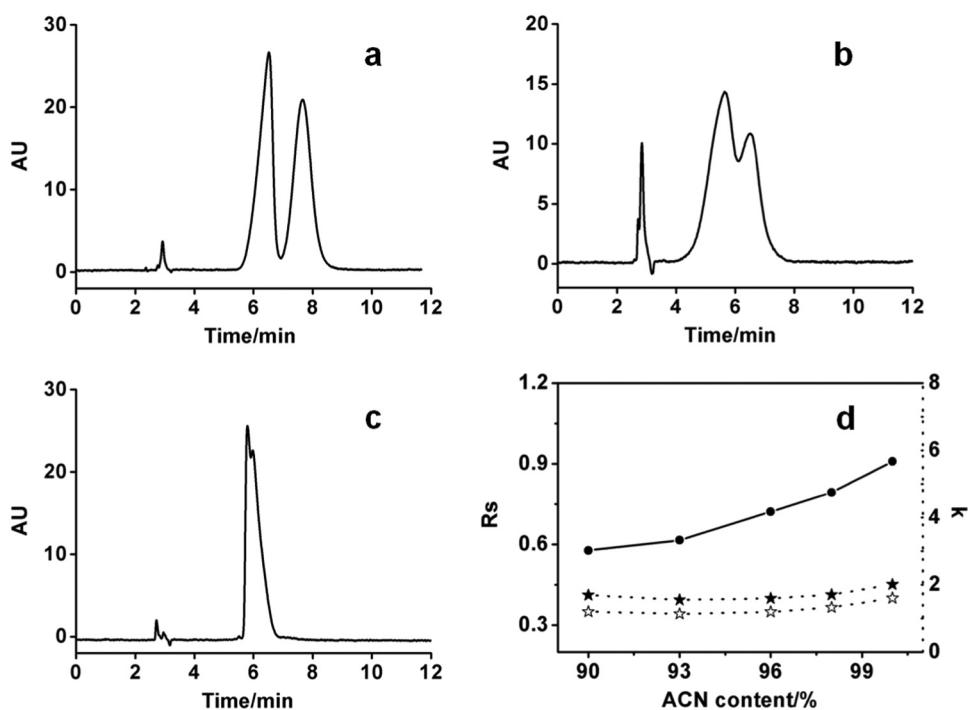
Fig. 4. (a-c) Separations of chiral compounds on monolithic CSP II by cLC and (d) effect of ACN content in mobile phases on resolution (*Rs*) and retention factor (*k*) of DNB-Leu. Analytes: (a) DNB-Leu, (b) DNB-PG and (c) α-methoxyphenylacetic acid. Experimental conditions: column dimension, 21.0 cm × 75 μm i.d.; mobile phase, (a-c) ACN (HAc 160 mM, TEA 10 mM)/H$_2$O (100/0, v/v); flow rate, 150 μL min^{-1} (before split); detection wavelength, 214 nm.

retention of acidic racemates on monolithic CSP I. According to "three-point" attractive interactions model, the interaction can take place such as electrostatic interaction, π-π interaction between the PCD and the benzene ring of analytes and dipol-dipol interaction [48,49]. In our case, DATDA possibly played two roles in enantioseparation. First, hydrogen bonds could be formed between these acidic racemates and DATDA, as stronger retention was performed on monolithic CSP I under higher ACN content of mobile phase (> 90%) via hydrophilic interaction chromatography (HILIC). Second, chiral DATDA might be served as one of the preferential interaction responsible for chiral recognition, which would improve the resolution of racemates and enantioselectivity ability.

4. Conclusions

A robust and fast approach has been successfully developed to fabricate hybrid monolithic cinchona-based CSPs via one-step photo-initiated thiol-ene polymerization. Although several kinds of organic-silica hybrid monoliths have been developed via thiol-based click polymerization, such as thiol-ene/yne polymerization, thiol-(meth)acrylate polymerization and ring-opening polymerization with commercially available POSS-containing reagents and other precursors, but they were not applied for preparation of monolithic CSPs. This photo-initiated thiol-ene polymerization would be faster than conventional sol-gel chemistry, free radical polymerization and ring-opening polymerization for preparation of hybrid monolithic CSPs in cLC. It is also insensitivity to oxygen, not requiring nitrogen protection in the process of synthesis and provides a time-saving approach to optimize preparation conditions. It would be expected to apply this approach and other polymerizations labelled with "click" term in published papers, such as azide-alkyne cycloaddition, Diels-Alder addition and oxime/hydrazone reactions for fabrication of other hybrid monolithic CSPs in the future.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2019.02.046.

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