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ARTICLE TYPE

Facile one-pot preparation of chiral monoliths with a well-defined framework based on thiol–ene click reaction for capillary liquid chromatography

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A novel chiral cyclodextrin (CD) monolith was easily prepared *via* a one-pot process based on the thiol–ene click reaction of allyl-β-CD with pentaerythritol tetra-(3-mercaptopropionate) in a fused-silica capillary. The effects of both the composition of prepolymerization solution and reaction temperature on the morphology, permeability, and selectivity of the β-CD chiral monolith were investigated in detail. The conditions were optimized to fabricate a homogeneous and permeable chiral monolith. In this study, the β-CD monolith was used as the stationary phase of capillary liquid chromatography for the chiral separation of several pharmaceutical enantiomers including flavanone, flubiprofen, naproxen, synephrine, isoprenaline sulfate, ketoprofen, and atropine sulfate monohydrate. Compared to the previously reported two-step method, this one-pot method for the preparation of a β-CD chiral monolith is simple and time-saving. Moreover, good resolutions were obtained for chiral isomers in a shorter analysis time compared to that reported in the literatures. These results indicate that the thiol–ene click chemistry provides a simple and robust method for the preparation of a chiral β-CD monolith.

1. Introduction

Cyclodextrin (CD) is naturally occurring cyclic oligosaccharide consisting of several (6, 7, 8) glucose units. Notably, the secondary 2- and 3-hydroxyl groups of β-CD lie along the mouth of the cavity, and the primary 6-hydroxyl groups are loated at the rear of the molecule. The β-CD is one of the most commonly used chiral selectors for enantioseparation. Because of their ability to form host–guest inclusion complexes with all types of chiral compounds, and the the advantages of monolithic columns including a continuous porous structure, high porosity, and large pores with small-size skeletons, native or derivatized β-CD-based monolithic columns have been successfully prepared and used for enantioseparation. In most instances, silica-based monolithic matrices are mainly prepared by the sol–gel technology, and then the β-CD is attached by covalent bonding,[1-4] physical adsorption,[5] or encapsulation.[6,7] To date, some studies have also been reported on the preparation of chiral β-CD polymer monoliths. For instance, Ahmed et al.[8] prepared a chiral β-CD-functionalized polymer monolith via the copolymerization of β-CD methacrylate and ethylene glycol dimethacrylate in a fused silica capillary for the enantioseparation of racemic pharmaceuticals in reversed-phase nano liquid chromatography. Zhang et al.[9] prepared perphenylcarbamoylated β-CD-silica hybrid monolithic columns for enantioseparations of 13 racemates by capillary liquid chromatography. Zhao et al.[10] developed a vancomycin-capped β-CD-bonded chiral stationary phase for the separation of aromatic positional isomers and the enantiomers of chiral compounds by liquid chromatography. Tian et al.[11] fabricated a novel β-CD-functionalized polymethacrylate-based monolithic column for enantioseparation of ibuprofen and naproxen by capillary electrochromatography.

Chiral separations are crucial for drug discovery research and pharmaceutical industry because the enantiomers of a drug compound can differ significantly in potency, toxicity, and biological activity.[12,13] Therefore, the development of novel chiral stationary phases (CSPs) for enantioseparation is still a hot research topic.[14-16]In recent years, "click chemistry," especially, thiol–ene click reaction, has attracted much attention because of some advantages. For example, this reaction tolerates water, oxygen, and many functional groups, can be carried out under mild reaction conditions, and without using a metal catalyst.[17] The formation of a thiol ether linkage provides a firm and stable covalent bond, which can tolerate tough conditions. Thiol–ene click reaction has been widely used in dendrimer synthesis, new polymer synthesis, biomolecules functionalization and surface modification.[18-20] Because of the abovementioned advantages, CD-based CSPs have been prepared by thiol–ene click reaction. Yao et al.[21] synthesized a novel cationic CD chiral stationary phase by immobilizing vinyl imidazolium-β-CD onto thiol silica based on thiol–ene click reaction for the separation of dansyl amino acids, carboxylic aryl compounds and flavonoids. Huang et al.[22] synthesized a novel perphenylcarbamated β-CD-based CSP by immobilizing mono/di(10-undecenoyl)perphenylaminocarbonyl β-CD on the 3-mercaptopropyl-functionalized silica gel via thiol–ene click reaction. Notably, it is tedious and time-consuming to prepare β-CD-based CSPs by postmodification. To the best of our

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knowledge, chiral monolithic columns have not been prepared by a one-pot thiol–ene click reaction.

In this study, a new chiral stationary phase composed of allyl-β-CD and pentaerythritol tetra(3-mercaptopropionate) (PETMP) was prepared by thiol–ene click reaction, and used for the rapid resolution of several pharmaceutical enantiomers by capillary liquid chromatography (cLC). The effects of both composition of prepolymerization mixture and reaction temperature on the morphology, permeability, and selectivity of the chiral monolith were studied in detail. The prepared monolith exhibited good mechanical strength and thermal stability.

2. Experimental

2.1 Reagents and materials

Allyl-β-CD was purchased from Shandong Binzhou Zhiyuan Biotechnology Corporation. Diethylene glycol diethyl ether (DEGDE, 98%) and polyethylene glycol 200 (PEG-200) were obtained from Sigma (St. Louis, MO, USA). γ-Methacryloxypropyltrimethoxysilane (γ-MAPS) was purchased from Alfa Aesar (Ward Hill, MA, USA). Azobisisobutyronitrile (AIBN) was purchased from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized from ethanol before use. PETMP, flavanone, flubiprofen, naproxen, synephrine, isoprenaline sulfate, ketoprofen, atropine sulfate monohydrate were obtained from J&K Chemical Ltd (Beijing, China). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Shanghai Chemical Reagents Corporation (Shanghai, China). Other reagents were of analytical grade. Ultrapure water was used for the preparation of solutions and produced using a Milli-Q water system (Millipore, Bedford, MA, USA). Fused silica capillary of 75-μm i.d. was obtained from Hebei Yongnian Optical Fiber Factory (Hebei, China).

2.2. Instrumentation

A TriSep-2100 pressurized CEC system was used to perform the chromatographic experiments. A PE Spectrum One FT-IR spectrometer (PE, USA) was used to perform Fourier-transform infrared spectroscopy (FT-IR) in the range (4000–400 cm^{-1}) using KBr pellets. The microstructure of monolith was examined using a FEI Quanta 200 FEG SEM (Philips, Netherlands). A Pyris Diamond TG (PE, USA) was used to perform thermogravimetric analysis (TGA).

2.3. Preparation of chiral monolith via thiol–ene click reaction

The vinylized capillary was prepared as follows: A 50% γ-MAPS MeOH (v/v) solution was used to introduce vinyl groups onto the inner surface of the capillary following a previously reported procedure.[23] The chiral monolith was prepared via an one-pot thiol–ene click reaction (Fig. 1). Allyl-β-CD, PETMP, DEGDE, PEG-200, and AIBN with different quantities (as listed in Table 1) were mixed to form a uniform solution under ultrasound irradiation. After sonication for 20 min, the mixture was artificially injected into the vinylized capillary with an effective length of 30 cm using a syringe, and then the ends of the capillary were sealed with rubbers. The capillary was immersed in a water bath (50-60 °C) for 22 h. Finally, the acquired monolithic capillary columns were flushed with MeOH to remove the unreacted chemicals.

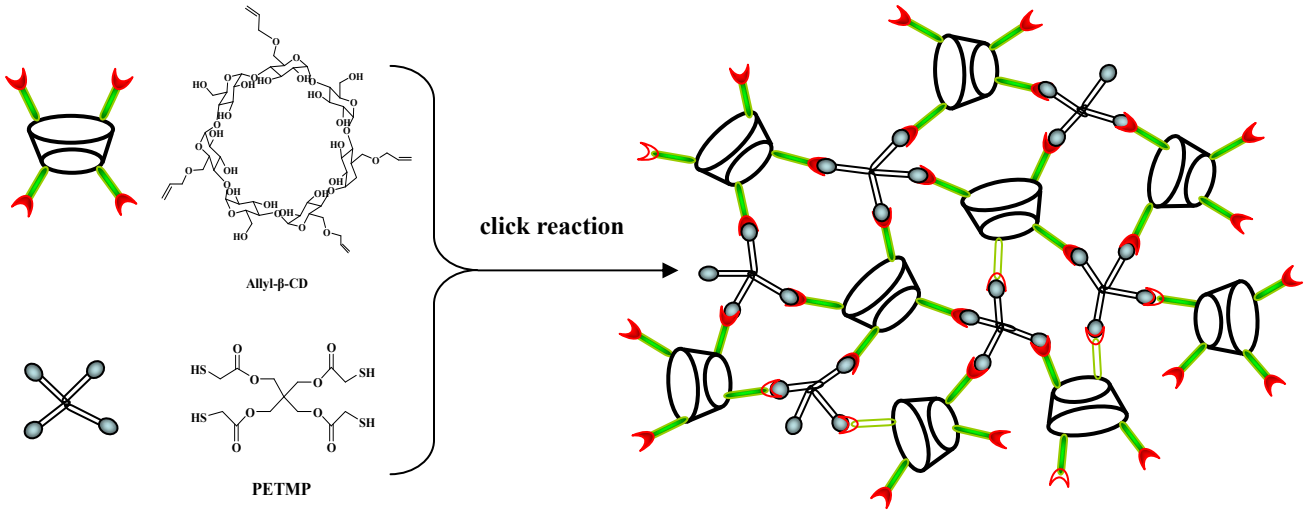

Fig. 1 Preparation of a chiral monolith via thiol–ene click reaction of allyl-β-CD with PETMP.

2.4 Standard solutions and sample preparation

Standard solutions of several pharmaceutical enantiomers in MeOH with concentrations of 1 mg/mL were prepared. Before the injection, the standard solutions were diluted by 100× and filtered through a 0.22-μm membrane. Fig. 2 shows the structures of the racemates.

2.5 cLC conditions

A detection window of 2-mm length was prepared by removing the polyimide coating at the end of the separation monolithic column. The mobile phase used was a mixture of triethylammonium acetate (TEAA) buffer/ACN with different volume ratios. Based on the previous report,[22] the triethylamine acetate buffer was prepared by dissolving the required amount of pure triethylamine in water to obtain 0.1% concentration, and then glacial acetic acid was added to obtain the required pH. mobile phase were filtered through 0.22-μm membrane and sonicated for 20 min before use. The analyses were performed at their optimal wavelength.

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Table. 1 Detail preparation conditions for chiral monolithic column and their permeability

Column	allyl-β-CD/PETMP (mol/mol)	DEGDE (mg)	PEG-200 (mg)	T (°C)	Backpressure (MPa)	Permeability ($\times 10^{-14}$ m^2)
A	1 : 1.0	120	80	55	>25(too hard to pump)	---
B	1 : 2.0	120	80	55	15.6	2.96
C	1 : 3.0	120	80	55	12.8	3.61
D	1 : 4.0	120	80	55	1.4	32.99
E	1 : 3.0	120	80	50	5.4	8.55
F	1 : 3.0	120	80	60	17.6	2.6
G	1 : 3.0	60	140	55	0.8	57.75
H	1 : 3.0	80	120	55	9.8	4.7
I	1 : 3.0	140	60	55	>25(too hard to pump)	---

[a] Other preparation conditions: allyl-β-CD 25 mg; AIBN 6.0 mg.

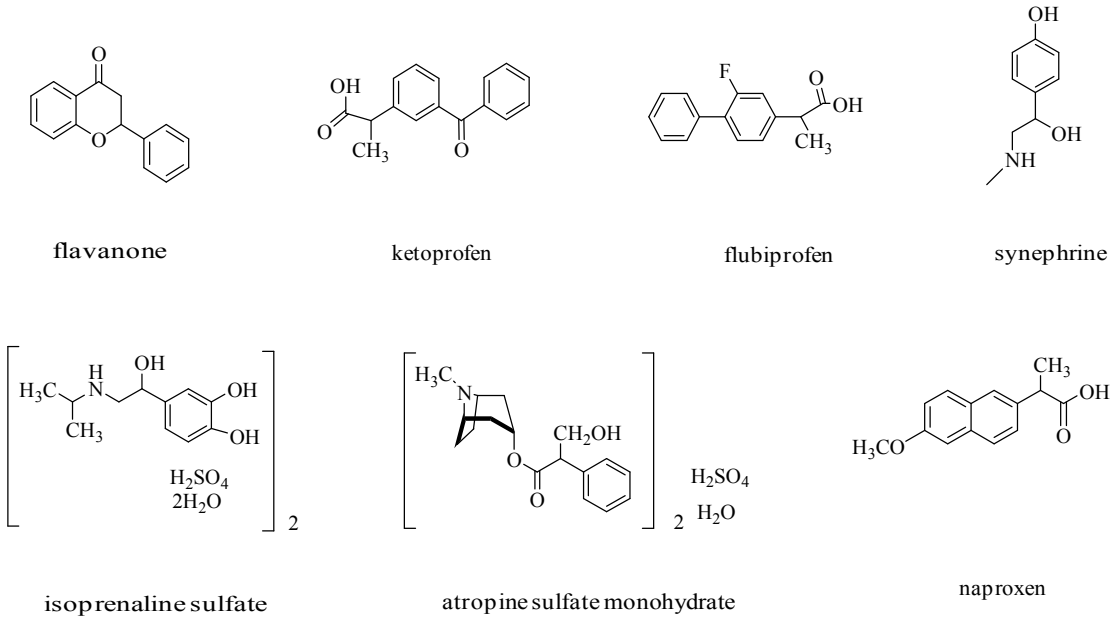

Fig. 2 Structures of racemates.

2.6 Calculations

Permeability (K) was calculated using the following formula: [24]

$$K = \frac{F \times \eta \times L}{\Delta P \times \pi \times \gamma^2}$$

where F is the volume flow rate of the eluent, η is the viscosity of the mobile phase, L is the length of the monolithic column, and ΔP is the pressure drop across the monolith. In this study, MeOH was used as the mobile phase, and the corresponding value of dynamic viscosity was 0.580×10^{-3}kg (ms) at 25 °C.[25]

3. Results and discussion

3.1 Preparation of chiral monolith

The chiral monolith was prepared from allyl-β-CD and PETMP by a one-pot thiol–ene click reaction. Because the composition of reactants significantly affects the morphology, permeability and selectivity of the chiral monolith, several parameters, the ratio of monomer/crosslinker, ratio of porogens, and reaction temperature were optimized as shown in Table 1. Different molar ratios of monomer/crosslinker were investigated for the prepolymerization solutions (columns A–D, as shown in Table 1). The backpressure of the chiral monolith gradually decreased with the decrease in the ratio of monomer/crosslinker; however, the permeability of the corresponding monoliths increased from 3.61×10^{-14} to 32.99×10^{-14} m^2. As shown in Fig. 3, when the molar ratio of monomer/crosslinker was 1:1 (column A) or 1:2 (column B), the generated monolith was extremely dense, basically imperforate, and had no separation effect. However, the reactants reacted well when the molar ratio of monomer/crosslinker was 1:4 (column D); however, the generated monolith was significantly macroporous, which

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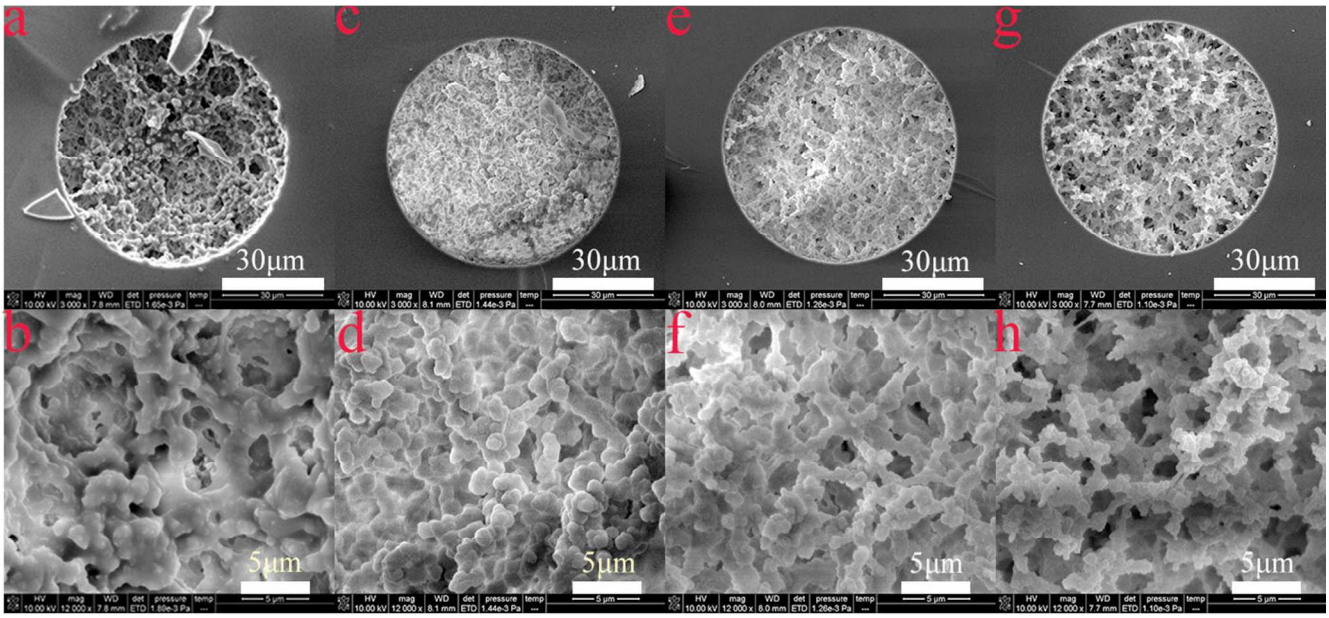

Fig. 3 SEM images of chiral monoliths of column A (a, b), column B (c, d), column C (e, f), and column D (g, h).

was bad for the retention of analytes and reduced the separation of the enantiomers (Fig. 4). To our delight, when the molar ratio of monomer/crosslinker was 1:3 (column C), the generated monolith had a well-defined framework, and satisfactory separations were obtained. Finally, a monomer/crosslinker ratio of 1:3 was selected to perform the following experiments. The type and ratio of porogenic solvents in the prepolymerization solution play an important role in the formation of monolith. Therefore, several types of porogenic systems were selected to prepare a chiral monolith, including *n*-propanol/1,4-butanediol and cyclohexanol/decanol. Unfortunately, satisfactory monoliths could not be obtained. According to the results recently reported by group of Zou,[26] we also used DEGDE/1-propanol as the binary porogen. However, a satisfactory monolith could also not be obtained, indicating that DEGDE/1-propanol was not suitable to form the monolith with allyl-β-CD and PETMP as monomers.

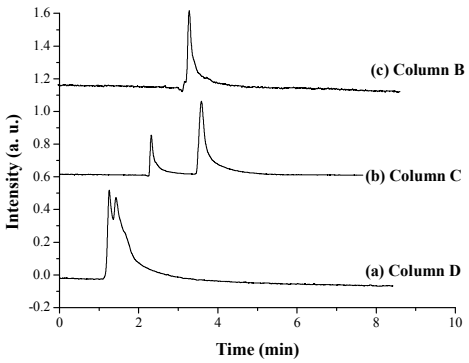

Fig. 4 Effect of the molar ratio of monomer/crosslinker of (a) 1:4 (column D) (b) 1:3 (column C) and (c) 1:2 (column B) on the separation of flavanone using the chiral monolith. Conditions: mobile phase, ACN /0.1%TEAA (70:30, v/v) , pH=4.0; flow rate, 0.05mL/min; detection wavelength, 214 nm; chiral monolith: 55 cm × 75 μm i.d. (30 cm effective length). supplement pressure, 500 psi.

Therefore, 1-propanol was replaced with PEG-200. An opaque white monolith was obtained in the porogenic system of DEGDE/PEG-200. Next, the effect of the ratio (w/w) of DEGDE/PEG-200 on the permeability and morphology of the resulting monolith was investigated. An increase in the content of PEG-200 from 60 mg to 140 mg increased the permeability. This phenomenon is related to the relative speed of phase separation. Although PEG-200 shows excellent compatibility with DEGDE and thiol-containing monomer, it served as a macroporogenic solvent for both the hydrophobic ene-containing monomer and the sulfoether oligomer generated via thiol–ene reaction.[27] The above results are consistent with the fact that a poor solvent leads to an earlier phase separation and forms larger pores in an organic monolith.[28] As shown in Table 1, the ratio of DEGDE/PEG-200 was evaluated from 60:140 to 140:60 (w/w). When the ratio of DEGDE/PEG-200 was >140:60 (w/w), a high backpressure (> 25.0 MPa) was obtained; when the ratio of DEGDE/PEG-200 was < 80:120 (w/w), macropores were obtained, not suitable for separation. However, an appropriate permeability was observed when the ratio of DEGDE/PEG-200 was 120:80 (w/w), good for separating enantiomers.

Thiol–ene click reaction is similar to other radical polymerization reactions, where the reaction temperature significantly affects the morphology of the prepared monoliths. Table 1 shows the effects of reaction temperature on permeability and backpressure. The permeability of the chiral monolith decreased from 8.55×10^{-14} to 2.6×10^{-14} m^2, when the reaction temperature was increased from 50 °C to 60 °C. This is mainly because a higher reaction temperature would change the solubility of the porogenic system, thus delaying the phase separation process.[29] When the click reaction temperature was set at 50 °C and 60 °C, a low column efficiency and resolution were obtained (Fig. 5). However, when the reaction temperature was 55 °C, column C with a reticular monolithic network exhibited a good chromatographic performance. Based on the above investigations, the monolith

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(column C in Table 1) prepared was used for further experiments.

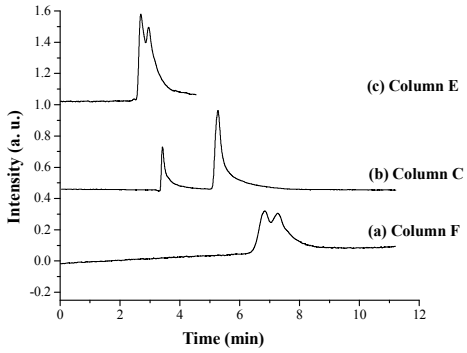

Fig. 5 Effect of thiol–ene click reaction temperature of (a) 60 °C (column F), (b) 55 °C (column C) and (c) 50 °C (column E) on the separation of flavanone using the chiral monolith. The conditions are the same as in Fig. 4.

3.2 Characterization of chiral monolith

Fig. 3 shows the SEM images of columns A–D. The prepared monolithic matrix attached well to the inner wall of the capillary. This is because the alkylene group at the inner wall of the capillary took part in the polymerization during the formation of chiral monolith. Fig. 3 shows that the morphologies of columns A and B are dense. When the content of crosslinker was very high (column D), the prepared monolith had larger pores, reducing separation. However, a continuous reticular skeleton was obtained for column C.

The monolithic material formed in the centrifuge tube was characterized by FT-IR; this provided a strong evidence for the thiol–ene click reaction of allyl-β-CD and PETMP. The peaks at 1643 cm^{-1} can be attributed to C=C signals (Fig. 6a), indicating the presence of vinyl groups of in the allyl-β-CD monomer. The peak at 2560 cm^{-1} was assigned to the thiol group and the peak at 1740 cm^{-1} was assigned to the C=O stretching in the PETMP crosslinker (Fig. 6b). A comparison of Figs. 6a, 6b and 6c and the disappearance of the peak at 2560 cm^{-1} and the decrease in the intensity of the peak at 1643 cm^{-1} shown in Fig. 6c confirmed that the thiol–ene click reaction of allyl-β-CD with PETMP

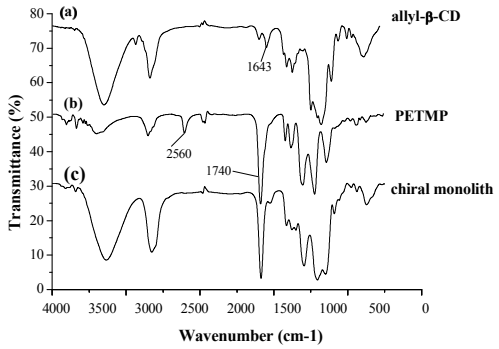

Fig. 6 FT-IR spectra of (a) allyl-β-CD, (b) PETMP and (c) chiral monolith (column C).

occurred.

The thermal stability of the chiral monolith was studied by TGA. As shown in Fig. 7a, because of the pyrolysis of organic moieties, an endothermic mass loss started at 230 °C and continued up to 400 °C, indicating that chiral monolith had good thermostability. Moreover, the mechanical strength of the prepared chiral monolith was investigated using MeOH as the mobile phase to measure the decrease in column pressure. As shown in Fig. 7b, a good linear relationship was observed between the flow rate and backpressure drop even at a high pressure, indicating good mechanical stabilities of the prepared chiral monoliths.

Reproducibility and stability are important factors for the efficiency and practicality of the synthesized chiral monolith. In this study, the reproducibility and stability of the chiral monolith were evaluated in terms of retention times of ketoprofen on column C. The relative standard deviations (RSDs) for the retention time of ketoprofen were less than 1.8% (n = 6) and 2.1% (n = 6) for run-to-run and day-to-day, respectively. The RSD of column-to-column reproducibility with three batches of the column C was less than 6.8% (n = 3). In addition, Figs. 8a, 8b and 8c show the separation of ketoprofen every 15 days. The baseline was very stable, and the enantioseparation results were readily reproducible. The above results indicated that the prepared chiral monolith have good reproducibility and stability.

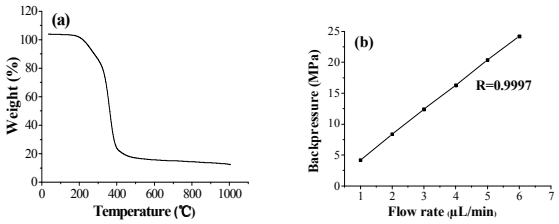

Fig. 7 (a) TGA of chiral monolith (column C) at a heating rate of 10 °C /min in nitrogen. (b) Relationship between the flow rate and backpressure drop of chiral monolith, Conditions: flow rate, 1–6 μL/min for MeOH.

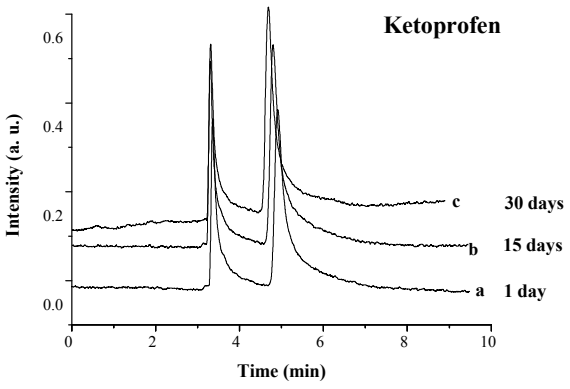

Fig. 8 Comparison of enantioseparation results of ketoprofen on the chiral monolith obtained after (a) 1 day, (b) 15 days and (c) 30 days. Detection wavelength, 254 nm, other conditions are the same as in Fig. 4.

3.3. Chromatographic performance

The enantioselection performance of the optimized chiral

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monolithic column was evaluated using several pharmaceutical enantiomers. Table 2 summarizes the chromatographic results obtained for several pharmaceutical enantiomers under the optimal separation conditions. Compared to the results reported in the literatures, the chiral isomers showed good resolutions in a shorter analysis time. For instance, as shown in Table 2, the resolution value of flavanone was 2.5, higher than that reported by the Fanali's group,[4] and the separation time of flavanone was shorter than theirs (>40 min). The resolution value of naproxen was 3.9, higher than that reported by the Zeng's group,[11] and the separation time was shorter than theirs. The composition of the mobile phase significantly affected the enantioseparation by cLC. Therefore, the enantioseparation conditions including the nature and concentration of organic additives and buffer concentration were systematically optimized. Synephrine was used as the test compound.

Tabe. 2 Enantioseparation on chiral monolith (column C) by cLC

sample	t_1 (min)	t_2 (min)	α (t_2/t_1)	N_1 (Plates/m)	N_2 (Plates/m)	R_s
flavanone	3.324	5.145	1.548	5133.8	3352.9	2.5
naproxen	3.435	6.317	1.839	7767.5	7857.8	3.9
ketoprofen	3.362	4.916	1.489	7843.8	3020.9	2.3
flubiprofen	2.701	10.073	3.729	3044.9	1898.5	4.1
synephrine	3.156	7.047	2.232	1173.7	1313.8	2.6
atropine sulfate monohydrate	6.130	6.811	1.111	5950.5	6749.5	1.6
isoprenaline sulfate	3.170	6.716	2.119	1022.5	1298.0	2.7

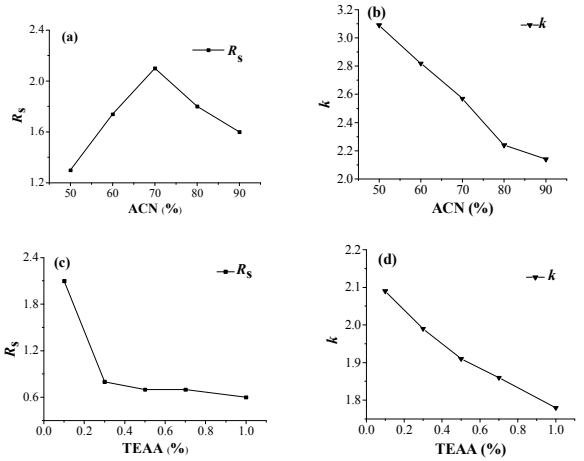

Fig. 9 Effects of ACN content and buffer concentration on the enantioseparation of synephrine. Conditions: mobile phases, (a and b) ACN/0.1%TEAA (at various volume ratios) (pH=5.0); (c and d) ACN/TEAA (70:30, v/v) at various buffer concentrations; chiral monolith, column C; sample, synephrine; flow rate, 0.05 mL/min.

First, the enantioseparations were first attempted using MeOH/TEAA buffer as the mobile phase. This is because although MeOH is commonly regarded as a weaker displacer of analytes from the CD cavity,[30] long retention times and small resolutions are achieved. MeOH generally has a weaker displacing activity than ACN; this might explain why a large retention time was obtained when methanol was used as the organic modifier. Finally, when MeOH was replaced with ACN, all the compounds separated well. Probably because ACN (an aprotic solvent) had a greater tendency to occupy the hydrophobic cavity of CD than MeOH, it was less accessible to the solute.

The effects of ACN concentration on k and R_s were investigated by varying the percentage composition of ACN in the mobile phase from 50% to 90% (v/v). As shown in Fig. 9a, the R_s value for the enantiomers only changed slightly over the studied ACN concentration range. A slightly higher R_s was obtained when the mobile phase contained 70% ACN; therefore, 70% ACN was considered optimal for further experiments. Fig. 9b clearly shows that a higher ACN content in the mobile phase resulted in the lower retention of the analyte. This indicates a hydrophobic retention mechanism on the monolithic stationary phase under the tested separation conditions.

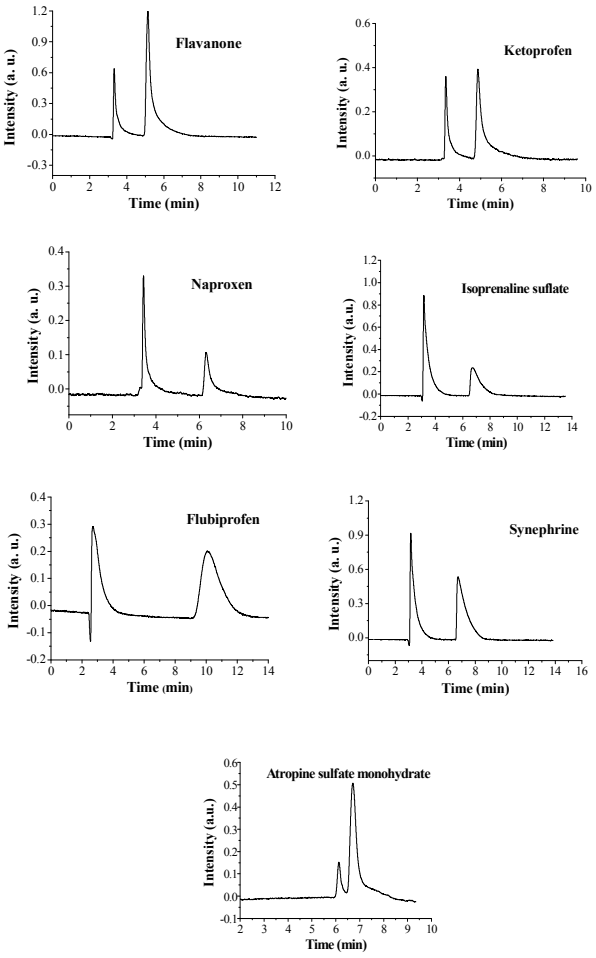

Fig. 10 Enantioseparation of seven racemic compounds on the chiral monolith (column C). Conditions: mobile phase: ACN/0.1%TEAA (70:30, v/v); flow rate, 0.05 mL/min; supplement pressure, 500 psi; flavanone, atropine sulphate monohydrate, detection wavelength, 214 nm, pH=4.0; flubiprofen, naproxen, ketoprofen, detection wavelength, 254 nm, pH=4.0; synephrine, isoprenaline sulfate, detection wavelength, 230 nm, pH=5.0.

TEAA was proven to be a very good buffer for the chiral separation of CD-functionalized stationary phases. In this study, TEAA was also selected as the aqueous buffer. The effect of buffer concentration on enantioseparation was also investigated by adjusting the value from 0.1% to 1.0%. As shown in Figs. 9c and 9d, both the k and R_s values of synephrine slightly decreased with increasing buffer concentration from 0.1% to 1.0%. This phenomenon can be attributed to two reasons: On one hand, when

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the TEA (triethylamine) concentration increased, the electrostatic interactions between chiral selector and analytes altered, causing the variation in resolution; on the other hand, because of electrostatic competition between chiral selector and TEA towards the analytes, the decrease in resolution can be explained by the increased competition effect with the chiral selector.[11] A higher R_s was obtained with 0.1% TEAA; therefore, 0.1% TEAA was considered optimal for further experiments.

The chromatographic performance of the chiral monolith was investigated by separating several pharmaceutical enantiomers by cLC. Fig. 10 shows the separation of enantiomers by cLC using ACN/0.1%TEAA (70:30, v/v) as the mobile phase. All the tested racemates could be enantioseparated well within 13 min. High separation efficiencies were observed for these racemates.

4. Conclusions

In this study, a novel chiral monolith was easily prepared using allyl-β-CD and PETMP by a thiol–ene click reaction. The synthetic method was very simple and convenient. The resulting chiral monolith exhibited good permeability, thermal stability, and mechanical stability. The chiral monolith also demonstrated its excellent enantioseparation of selected racemic compounds by cLC. This study provides possibilities for preparing other chiral monolithic columns that involve click reaction using multienes and multithiols as the precursors.

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Notes and references

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