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Short communication

Preparation of novel β-cyclodextrin functionalized monolith and its application in chiral separation

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ABSTRACT

A novel β-cyclodextrin (β-CD) functionalized organic polymer monolith was prepared by covalently bonding ethylenediamine-β-CD (EDA-β-CD) to poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) (poly(GMA-co-EGDMA)) monolith via ring opening reaction of epoxy groups. SEM characterization was performed to confirm the homogeneity of the monolithic polymer. The resulting monolith was then characterized by DSC and XPS elemental analysis to study the thermal stability of the monolith, and to prove the successful immobilization of β-CD on the polymer substrate. The β-CD ligand density of 0.68 mmol g^{-1} was obtained for the modified monolith, indicating the high reactivity and efficiency of the EDA-β-CD modifier. The ethylenediamine-β-CD functionalized monoliths were used for the chiral separation of ibuprofen racemic mixture and showed promising results.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six or more α-(1, 4)-linked D-glucopyranose units. The β-cyclodextrin molecule has secondary 2- and 3-hydroxyl groups lining the mouth of the cavity and primary 6-hydroxyl groups at the rear of the molecule. The cavity permits inclusion of hydrophobic portions of the solute molecules. Interaction of any polar regions of a solute molecule with the surface hydroxyls combined with the hydrophobic interactions in the cavity provides the 3-point interaction required for chiral recognition [1–6]. Native or derivatized β-cyclodextrins bonded to appropriate solid supports as chiral HPLC stationary phases [7–11] are extensively used for the enantiomeric separation of optical isomers. However, the conventional porous materials in chromatography, such as packed materials are prone to hinder the mass transfer of the analytes resulting in high back pressure and long retention time.

Monolithic columns, especially organic polymer based monoliths, are promising and attractive alternatives to packed HPLC columns [12–14]. The major merits of organic polymer based monoliths are their high chemical stability over a broad range of pH and that they are amenable to surface functionalization. Native β-cyclodextrin is quite stable. The derivatization of β-CD is normally needed for the modification of organic polymer based monoliths [15–17]. As a result, the preparations of novel, facile and highly reactive β-CD derivatives are extremely attractive. Additionally, the β-CD derivatives might need suitable spacers for the reaction with the monolithic substrate to afford enough flexibility for the substitution and chiral separation.

In this paper, a comparatively novel and efficient β-CD derivative, ethylenediamine-β-CD (EDA-β-CD) was synthesized for the surface modification of the monolith. The EDA-β-CD was covalently bonded to the poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) organic polymer monolith via ring opening reaction of epoxy groups (Scheme 1) by continuously flushing the heated column with modifier solution until reaching the maximum functionalization. The monolith was applied for the rapid resolution of ibuprofen isomer by HPLC. Effects of organic solvent, buffer concentration and pH value of the mobile phase on the resolution (R_s) and selectivity factor (α) of ibuprofen were systematically studied and discussed.

2. Results and discussion

2.1. Characterization of β-CD modified monoliths

The morphology of β-CD modified monolith was studied by scanning electron microscopy (SEM). As shown in Fig. 1, the porous structure of the monolith was homogenous. The monolithic polymer consisted of aggregates of irregular micro-globules, between which the interspaces formed highly interconnected channels allowing fast flow of the mobile phases. The pressure drop across the column was measured at different flow rates (Fig. 4, supporting information) with water and methanol as mobile phases. The

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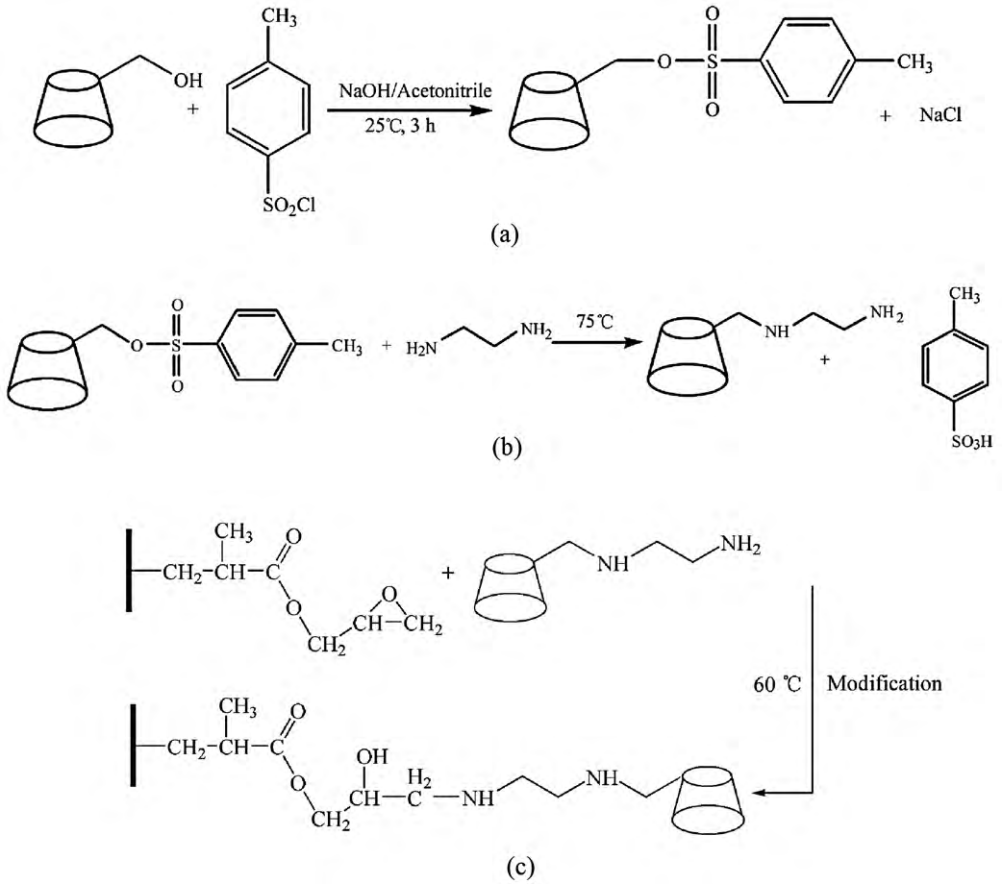

Scheme 1. (a) The reaction process between β-CD and *p*-toluenesulfonyl chloride, (b) the reaction process between Mono-6-OTs-β-CD and EDA, and (c) the synthetic route for the preparation of the β-CD functionalized monolith. Reagents and conditions: 60 °C, 20% EDA-β-CD (w/v) in DMF.

pressure drops of the blank and β-CD modified monoliths were linear with the flow rates, and the regression factor *R* was up to 0.999, indicating a laminar flow regime. The mobile phases can flow through the monoliths at a modest flow resistance. The linearity of the back pressure versus flow velocity confirms the incompressibility of the monoliths, which can withstand a back pressure of up to almost 11 MPa indicating a high mechanical stability.

In the published literatures, the functionalization of the monoliths has been performed by filling the column with modifier solution. The monolith was then sealed at both ends and heated at 60 °C for a period of time by immersion in a water bath [12–15,18,19] or an oven [16]. As a result, the chemical modification of the monolith is limited to the amount of the modifier available in the column, thus producing a modified monolith of low ligand density. In this study, to overcome this limitation, the monolithic column was placed in a column heater and connected to a pump. The functionalization reaction was performed by continuously pumping the modifier solution through the column at a flow rate of 0.2 mL min^{-1} with the column heater at 60 °C until the end of the functionalization processes when the modified monolith reached its maximum ligand capacity. The efficient modification of the monolith was determined by XPS elemental analysis, which showed the appearance of the nitrogen element for the EDA-β-CD modified monolith (Table 1). Compared with the blank monolith, the carbon content decreased and the oxygen content increased significantly for the EDA-β-CD modified monolith, due to the coupling of EDA-β-CD. XPS elemental analysis of the modified monoliths compared to the blank monolith showed changes in the relative atomic composition consistent with the expectation. The β-CD

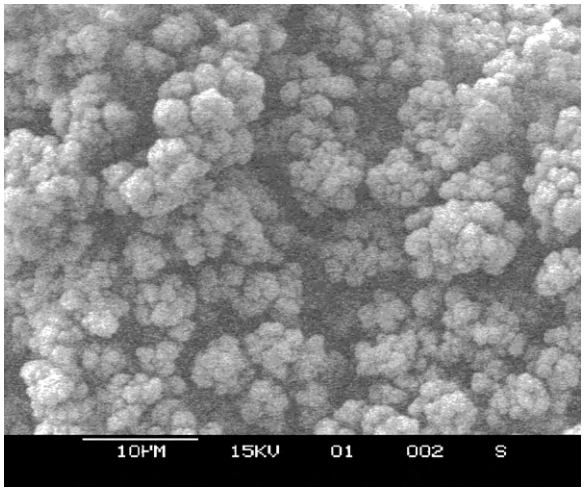

Fig. 1. Scanning electron micrograph of the monolith based on a porogenic solvent of heptane.

Table 1
Elemental compositions (atomic concentration in %, $n = 3$) of the blank monolith and functionalized monoliths (M1–M6) from high resolution XPS spectra.

Monolith	C		O		N	
	Mean	Dev	Mean	Dev	Mean	Dev
Blank monolith	0.602	0.013	0.398	0.007	0	0
EDA-β-CD modified monolith	0.545	0.015	0.438	0.006	0.015	0.004

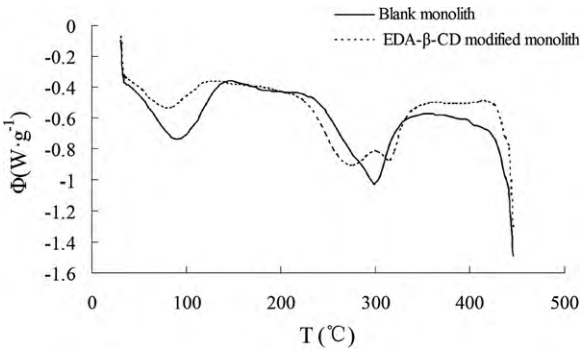

Fig. 2. DSC thermal analysis of a blank organic polymer monolith and an EDA-β-CD modified organic polymer monolith.

ligand density of 0.68 mmol g^{-1} was obtained for the modified monolith according to the nitrogen element content, indicating the high reactivity and efficiency of the EDA-β-CD modifier.

Li et al. [19] have prepared four chiral monolithic columns fabricated with HP-β-CD, Asp-β-CD, NH_2-β-CD and β-CD, respectively, and evaluated their enantioselective performances by separating amino acids and racemic drugs. HP-β-CD and Asp-β-CD modified monoliths showed stronger separation abilities compared to other two types of columns. Compared with HP-β-CD, NH_2-β-CD and β-CD modifiers, EDA-β-CD might have longer spacer to overcome the steric hindrance effect and provide other possibility to chemical bond functionalized chiral selectors [19]. Asp-β-CD and EDA-β-CD have the same hydrophobic cavity as β-CD, but different functional groups, which could provide different interactions to improve the chiral separation. However, the reactive group of Asp-β-CD is secondary amine, which has theoretically lower reactivity with the epoxy group compared with the primary amine of EDA-β-CD. Unfortunately, there was no result regarding the β-CD ligand density in Ref. [19] to support our opinion. Additionally, traditional functionalization of a sealed column (100 mm × 4.6 mm) in a heated water bath is limited to only 1 bed volume of modifier solution, which is approximate 1 mL for our monolith. Our improved method of continuously flushing the heated column with modifier solution until maximum functionalization was reached, significantly increased the ligand density of the monolith.

The thermal stability of the blank and modified monoliths was studied by DSC analysis. As shown in Fig. 2, the DSC curve of the blank monolith displays endothermic peaks at about 90 °C, which was consistent with the evaporation of absorbed water [20]. Another appreciable exothermic peak of the blank monolith at about 303 °C in the DSC curve is assigned to the decomposition of the poly(GMA-co-EGDMA) monolithic polymer. Close examination of the thermal analysis results revealed that the monolith was thermally stable up to 307 °C. For the EDA-β-CD modified monolith, except for the evaporation peak of absorbed water at 82 °C, other two appreciable exothermic peaks at about 277 °C and 316 °C in the DSC curve were assigned to the decompositions of the poly(GMA-co-EGDMA) monolithic polymer and β-CD, respectively. The appearance of the β-CD decomposition peak in the DSC curve of the modified monolith clearly confirmed the successful coupling of the cyclodextrin on the poly(GMA-co-EGDMA) monolithic matrix.

2.2. Chiral separation of ibuprofen racemate

Triethylamine acetate (TEAA) has been proven to be a very good aqueous buffer for the chiral separation of cyclodextrin functionalized stationary phases. Different concentrations of methanol and TEAA in water at different pH values were used as the mobile phases for chiral separation of ibuprofen racemate in HPLC.

2.2.1. Effect of organic solvent

The cavity of β-cyclodextrin is a relatively hydrophobic region and permits inclusion of hydrophobic portions of solute molecules. The addition of organic solvent can alter the hydrophobicity and ionic strength of the aqueous buffer, resulting in changes of the resolution (R_s) and selectivity factor (α). The effects of methanol concentration on R_s and α were investigated by varying the fraction of methanol in the mobile phase from 5% to 40% (v/v), as shown in Table 2 (supporting information). The mobile phase was composed of 0.5% (v/v) TEAA aqueous buffer, and was kept at pH 5. It was found that both resolution (R_s) and selectivity factor (α) increased with the increase of methanol contents from 5% to 30%, in contrast, the dependence is vice versa as methanol contents further increased from 30% to 40%. This result might arise from that more methanol was favorable to the hydrophobic interaction between β-CD and ibuprofen, and consequently increased the chiral separation behavior [15]. However, methanol content above 30% also increased the hydrophobic interaction between poly(GMA-co-EGDMA) monolith and ibuprofen. This non-specific interaction eventually prevailed over the specific one between β-CD and ibuprofen and was not favorable to the chiral separation behavior. As a result, 30% (v/v) methanol was considered as the optimum value for our study.

2.2.2. Effect of buffer concentration

In our study, TEAA was used as the co-ion to compete with the anion exchanger for the interaction with ibuprofen. We studied the influence of the TEAA concentrations ranging from 0.1% to 1% (v/v) on the resolution and separation factor of the monolith for ibuprofen, while the aqueous buffer pH value was kept constant at 5 and MeOH content was kept constant as 30% (v/v). As plotted in Table 3 (supporting information), both the resolution (*Rs*) and selectivity factor (α) were slightly influenced by the change of buffer concentration. Ionic strength influences the electrostatic interaction between the chiral selector and the analytes. With the increase of TEAA concentration, the electrostatic competition existing between the positive charged chiral selector and TEAA towards the analytes increased. Hence, to some extent, the chiral separation peak was sharp and symmetrical, resulting in increased resolution and selectivity factor. In addition, increasing the concentration of the buffer could help to overcome the flow effect by enhancing the attractiveness of the CD cavity with respect to the mobile phase. However, too much TEAA would reduce the retention of ibuprofen on the monolith, then leading to decreased resolution and selectivity factor. Consequently, 0.5% (v/v) TEAA was the optimum concentration.

2.2.3. Effect of pH value

The aqueous buffer pH value usually plays an important role in the HPLC enantiomeric separation process. TEAA aqueous buffer was employed to study the effect of pH by adjusting the buffer pH value at 3.3, 4, 5, 6 and 7 before mixing with methanol. The mobile phase comprised methanol and 0.5% (v/v) TEAA aqueous buffer with the volume ratio of 30 to 70. As illustrated in Table 4 (supporting information), at pH 7 virtually no separation was observed, whereas at pH 4 a good separation was achieved. Ibuprofen has a p*K*a value of 4.2 (Merck Index). In solutions of pH less than p*K*a value, the ibuprofen was either partially ionized or remained in uncharged form. Accordingly, in this pH range, the electrostatic interactions between ibuprofen and cyclodextrin were not favorable. In addition, at alkaline pH, the electrostatic interactions between ibuprofen and cyclodextrin as well as monolith were both so strong that the peak tailing might result in the loss of separation.

After optimization, the mixture of MeOH and 0.5% (v/v) TEAA aqueous buffer at pH 4 with the volume ratio of 3 to 7 was chosen

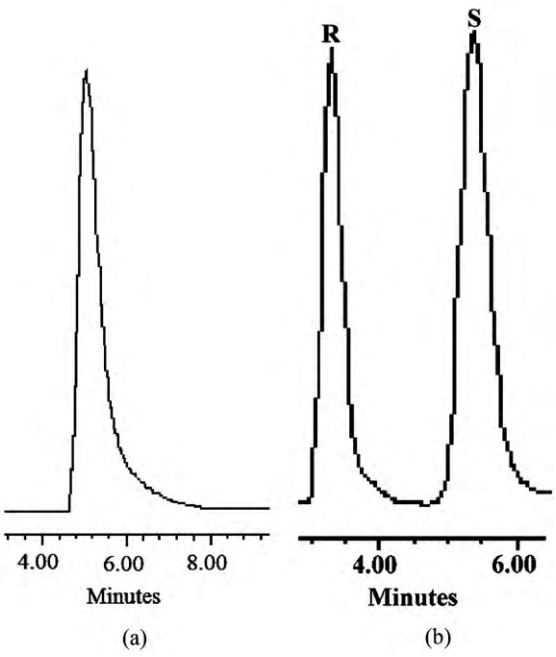

Fig. 3. Enantioseparation chromatogram of ibuprofen racemate on (a) blank and (b) β-CD immobilized poly(GMA-co-EGDMA) monolith. Separation conditions: MeOH/0.5% (v/v) TEA (30:70, v/v), pH 4, flow rate 0.7 mL min^{-1}, injection volume 20 μL, sample 1 mg mL^{-1}, detection wavelength 260 nm.

as the optimum mobile phase for the chiral separation of ibuprofen. The chiral resolution chromatogram was displayed in Fig. 3. Compared with published literatures [21–24] which adopted traditional packed bed chiral columns, the ethylenediamine-β-CD (EDA-β-CD) functionalized organic polymer based monolith has a much faster and symmetrical baseline separation for ibuprofen optical isomer within only 6 min in HPLC.

3. Conclusions

A novel ethylenediamine-β-CD (EDA-β-CD) functionalized poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) monolith was prepared and found to be an effective and efficient method for chiral separation of ibuprofen. The optimum mobile phase was the mixture of MeOH and 0.5% (v/v) TEAA aqueous buffer with the volume ratio of 3 to 7 at pH 4. The coupling methodology developed in this study can be easily adopted for the generation of functional surfaces and modification of separation medium that would attract a broader readership in chromatography and applied chemistry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.07.020.

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