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Preparation of a β-cyclodextrin functionalized monolith *via* a novel and simple one-pot approach and application to enantioseparations

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ABSTRACT

A novel and facile one-pot copolymerization approach was developed for the preparation of a β-cyclodextrin (β-CD) functionalized organic polymer monolith. The proposed one-pot process involved two major reactions occurring in sequence in the same vial: (1) the ring opening reaction between the epoxy groups of glycidyl methacrylate (GMA) and the primary amino groups of ethylenediamine-β-CD (EDA-β-CD); (2) the copolymerization of glycidyl methacrylate-ethylenediamine-β-CD (GMA-EDA-β-CD) and ethylene dimethacrylate (EDMA) using 2,2′-azobisisobutyronitrile (AIBN) as the polymerization initiator. This approach avoids the time-consuming post-polymerization derivatization of the traditional two-step strategy. Compared to the previously reported two-step strategy, the monolith prepared by this one-pot method exhibited higher β-CD ligand density and better column efficiency in HPLC. Satisfactory column permeability and separation selectivity were also obtained on the optimized poly(GMA-EDA-β-CD-*co*-EDMA) monolithic column. Additionally, the column was also applied to the enantioseparation of some racemic acidic compounds with promising results.

1. Introduction

Although well advanced, the development of novel chiral stationary phases (CSPs) in HPLC is still a hot research topic [1–3]. In recent years, chiral monolithic columns have attracted considerable interest owing to their facile preparation methodology and good column characteristics, such as permeability, efficiency, *etc.* [4,5]. Cyclodextrins and their derivatives being some of the most popular chiral selectors in chromatography, CD modified silica based monolithic columns have been successfully developed, which could be attributed to the large amount of available knowledge for preparing traditional CD modified silica gel packings [6–9]. In most cases, the silica based monolithic matrix was first prepared by the sol–gel technology and the CDs were then immobilized by covalent bonding [10–12], by physical adsorption [13] or by encapsulation [14,15]. Wistuba et al. also developed particle-fixation methods to embed ChiraDex particles into a silica based matrix by sintering at high temperatures [16] or gluing *via* a sol–gel process [17]. However the enantioselectivity in these monoliths was found to be low, which could be caused by the shielding of the CDs in the procedure of entrapment.

The CDs could also been incorporated into an organic polymer matrix by grafting after polymerization [18–20], or by copolymerization of CD containing functional monomers [12]. At the beginning of the 2000s, charged low cross-linked polyacrylamide homogeneous gels with physically or covalently incorporated β-CD moieties have been extensively studied [21,22]. Unfortunately, the low permeability of homogeneous gels limited their application in HPLC. Additionally, a high amount of β-CD bonded to the monolith surface could not be expected because of the restriction of mesopores for the high molecular weight chiral selector.

Recently, more efforts have been dedicated to the preparation of CD-modified rigid monoliths [18,19,23,24]. So far, the two-step process involving copolymerization and post-polymerization modification has been the most commonly adopted strategy to prepare CD-modified rigid monoliths. Li et al. developed a series of monolithic CSPs by introducing β-CD derivatives into an epoxy-activated monolith *via* direct and indirect methods under mild conditions [23]. Guerrouache and co-workers developed a method for coupling β-CD to the azido-reactive surface of a poly(*N*-acryloxysuccinimide-*co*-ethylene dimethacrylate) (poly(NAS-*co*-EDMA)) monolith *via* click reaction [24]. Lv et al. prepared a β-CD functionalized organic polymer monolith by covalently bonding ethylenediamine-β-CD (EDA-β-CD) to a poly(glycidyl methacrylate-*co*-EDMA) (poly(GMA-*co*-EDMA)) monolith *via* ring opening reaction of epoxy groups [19]. Unfortunately, the two-step strategy has some shortcomings such as

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laborious, tedious and sometimes uncontrollable fabrication with poor reproducibility.

Instead of the two-step post-modification strategy, the one-step or one-pot strategy with a CD-modified functional monomer is a potential approach to simplify the fabrication and improve the reproducibility. Few one-step strategies have been reported so far [2,12,25]. The limited studies on CD-modified rigid monolithic columns are largely due to the lack of commercially available CD-modified monomers. Wu and Zou et al. developed a simple one-pot approach for preparing hybrid organic–inorganic monolithic capillary columns [2]. This one-pot approach was realized *via* the polycondensation of alkoxysilanes and *in situ* copolymerization of mono (6^A-*N*-allylamino-6^A-deoxy)-per-phenylcarbamoylated-β-CD (Ph-β-CD) and vinyl groups on the pre-condensed siloxanes. However, the inter-laboratory reproducibility seems to be rather poor since this fabrication procedure requires a lot of experience and very careful control of the polymerization conditions. More recently, our group also developed a one-step copolymerization strategy for preparing β-CD functionalized monolithic columns [25]. The novel monomer mono-(1*H*-1,2,3-triazol-4-ylmethyl-2-methylacryl-β-CD) (PMA-β-CD) was first synthesized *via* click reaction between propargyl methacrylate and mono-6-azido-β-CD, and then monolithic columns were prepared through a one-step in situ copolymerization of the PMA-β-CD monomer and EDMA. Nevertheless, unsatisfactory enantioselectivity was obtained, which might be attributed to the short carbon chain of PMA-β-CD or the presence of triazole groups. Shamsi et al. recently reported a one-step approach through the copolymerization of glycidyl methacrylate bonded β-CD (GMA-β-CD) with EDMA [26,27]. GMA-β-CD was first synthesized by covalently bonding primary hydroxyl groups of β-CD with the epoxy groups of GMA. The obtained β-CD modified monolithic column exhibited good enantioselectivity for 32 chiral compounds. However, plenty of efforts still have to be paid on the preparation and purification of the GMA-β-CD monomer since the degree of substitution (DS) of the bonding reaction has an obvious effect on enantioselectivity and separation efficiency.

In this study, a novel and facile one-pot copolymerization approach was developed for the preparation of a β-CD modified organic polymer monolith for the first time. The synthesis of the novel monomer glycidyl methacrylate-ethylenediamine-β-CD (GMA-EDA-β-CD) and the subsequent copolymerization were realized in the same vial without any purification step of the chiral monomer, and therefore the whole fabrication process could be finished facilely. Extensive studies on the fabrication conditions were conducted considering permeability, homogeneity, efficiency and porosity of the monolithic columns. The stability, permeability, CD density and reproducibility of the monolithic column were systematically evaluated using elemental analysis, scanning electron microscopy (SEM) and micro-HPLC. The optimized poly(GMA-EDA-β-CD-*co*-EDMA) monolithic columns were applied to the enantioseparation of chiral acidic compounds. Enantioseparation conditions and mechanism have also been investigated in detail.

2. Experimental

2.1. Chemicals and materials

GMA, 3-(trimethoxysilyl)-propyl methacrylate (γ-MAPS), 2,2′-azobisisobutyronitrile (AIBN), dimethyl sulfoxide (DMSO), methanol (MeOH), *N*,*N*-dimethylformamide (DMF), *n*-propanol, ethylene glycol, cyclohexane, acetonitrile (ACN), potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), formic acid (FA), acetic acid (AcA), trifluoroacetic acid (TFA), diethylamine (DEA), triethylamine (TEA), thiourea, toluene, dimethylphthalate, anisole, naphthalene, 4-bromo-mandelic acid, 4-chloro-mandelic acid, 2-bromo-mandelic acid, ibuprofen, fenoprofen, indoprofen were all purchased from Aladdin Chemicals (Shanghai, China). EDA-β-CD and EDMA were obtained from Cyclolab Ltd. (Budapest, Hungary) and Alfa Aesar Chemicals (Tianjin, China), respectively. All reagents were used without further purification. Distilled water was filtered through a 2 μm membrane before experiments. The fused-silica capillaries (375 μm OD × 100 μm ID) were purchased from Ruifeng Chromatography Ltd. (Yongnian, Hebei, China).

2.2. Instrumentation

Elemental analysis was performed on an EA 2400 II CHNS/O elemental analyzer (Perkin Elmer, MA, USA). All scanning electron microscopy (SEM) experiments were carried out using an ultra-high 165 resolution Hitachi S-4800 SEM (Tokyo, Japan) at an acceleration voltage of 3 kV. A water bath was used for thermally initiated copolymerization. All micro-HPLC experiments were carried out on a self-assembled HPLC system that consisted of a DiNa-S nano single pump (Tokyo, Japan), a Valco four-port injection valve with 10 nL internal loop (Houston, TX, USA), and a Shimadzu SPD-15 C UV detector (Kyoto, Japan). Data acquisition and data handling were performed using a Unimicro Trisep™ Workstation 2003 (Shanghai, China). All chromatograms were converted to a txt file and redrawn using Microcal Origin 8.5.

2.3. Preparation of poly(GMA-EDA-β-CD-co-EDMA) monolithic columns

The schematic preparation of poly(GMA-EDA-β-CD-*co*-EDMA) monolithic columns is illustrated in Fig. 1. The desired amounts of GMA and EDA-β-CD were accurately weighted into a 2-mL vial and mixed with the porogens (DMSO and MeOH). After sonication for 10 min and heating at 60 °C for 30 min in order to complete the ring opening reaction between the epoxy groups of GMA and the amino groups of EDA-β-CD, a clear homogeneous solution was formed. The crosslinker (EDMA) and the polymerization initiator (AIBN, 1% w/w with respect to the monomers) were then added into the 2-mL vial. The composition of the polymerization mixture was optimized in order to obtain satisfactory permeability and selectivity, as detailed in Table 1. After sonication and bubbling with nitrogen for 5 min in order to remove dissolved gases, the polymerization mixture was introduced into the capillaries, which have been pretreated with the bi-functional reagent γ-MAPS in order to provide anchoring sites for the polymeric bulk according to a previously reported method [28]. The capillaries were then sealed with GC septa and submerged into a water bath at 60 °C for 12 h. In order to remove any unreacted chemicals and porogens, the obtained monolithic columns were rinsed with MeOH. A 2–3 mm on-column detection window was then created at a distance of 4–5 cm from the end of the column using a thermal wire stripper. In order to measure the density of the CD in the monolith, the bulk polymerization of the mixture was performed in a 1-mL glass vial in parallel to the polymerization within the capillaries. After the polymerization, the polymer was removed from the vial and cut into small pieces, Soxhlet extracted with MeOH for 16 h and then dried under vacuum at 60 °C for 6 h. The finally obtained bulk polymer was taken for elemental analysis. A 3–5 mm length of monolith was then cut, placed on an aluminum stub and then sputter-coated with gold for SEM analysis.

2.4. Chromatographic conditions

Stock solutions of KH_2PO_4 were prepared by dissolving an appropriate amount of salt in deionized water and adjusting to the