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SHORT COMMUNICATION

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Preparation of a novel hydroxypropyl-γ-cyclodextrin functionalized monolith for separation of chiral drugs in capillary electrochromatography

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Abstract

In this study, a novel hydroxypropyl-γ-cyclodextrin (HP-γ-CD) functionalized monolithic capillary column was prepared by one-pot sequential strategy and used for chiral separation in capillary electrochromatography for the first time. In one pot, GMA-HP-γ-CD as functional monomer was allowed to be formed via the ring opening reaction between HP-γ-CD and glycidyl methacrylate (GMA) catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and then copolymerized directly with ethylene dimethacrylate (EDMA) and 2-acrylamido-2-methyl propane sulfonic acid (AMPS) in the presence of porogenic solvents via thermally initiated free radical polymerization. The preparation conditions of monoliths were optimized. Enantiomer separations of six chiral drugs including pindolol, clorprenaline, tulobuterol, clenbuterol, propranolol, and tropicamide were achieved on the monolith. Among them, pindolol, clorprenaline, and tropicamide were baseline separated with resolution values of 1.62, 1.73, and 1.55, respectively. The mechanism of enantiomer separation was discussed by comparison of the HP-γ-CD and HP-β-CD functionalized monoliths.

KEYWORDS

chiral drugs, enantiomer separation, hydroxypropyl-γ-cyclodextrin, monolith, one-pot strategy

1 | INTRODUCTION

The separation of enantiomers is an important topic during the past three decades. It is well known that one enantiomer of a racemic drug may exhibit pharmacological activities and/or side effects different from the other one[1]; therefore, it is important to develop an analytical method for enantiomer separation of chiral drugs with different chemical structures. So far, chromatographic approaches as efficient tools for enantiomer separation have been widely utilized.

Capillary electrochromatography (CEC) is an electromigration separation technique which strives to combine the best features of capillary electrophoresis and high-performance liquid chromatography (HPLC). The core of CEC is the development of column technology which comprises packed, monolithic, and open-tubular columns. Among the three types of columns, monolithic columns are slowly attracting more and more attention. In the case of chiral separation, much efforts have been devoted to design and fabricate novel monolithic stationary phase.[2–4]

Over the past few decades, monolithic separation media have emerged as an advantageous alternative to the conventional column packing material in chromatography and electrochromatography. The widespread

acceptance of monolithic separation media has been facilitated by the ease of preparation, favorable mass transfer properties, low back pressure, and high separation efficiency. Moreover, the availability of diverse kinds of functional monomers or reactive precursors from a rich chemistry bank and a wide range of porosity control during the preparation process provide solutions for a variety of separation problems.[5–7] In the early stage, the polymer monoliths are based on soft hydrophilic polyacrylamide gels.[8–10] Svec's group pioneered the fabrication of the polymethacrylate-based monoliths as HPLC separation media.[11–13] In contrast to polyacrylamide gels, the polymethacrylate-based monoliths as rigid polymers are not compressible and do not change their size substantially on swelling. Due to the significant advantage, the rigid polymer monoliths have gained continuously in popularity.[14]

Chiral functionalities on the surface of monolith are the prerequisite for enantiomer separations.[15] Cyclodextrins (CDs) are a family of cyclic oligosaccharides, which possess the truncated cone-shaped structure with relatively hydrophilic exteriors and hydrophobic internal cavity. The unique structure enables their ability to selectively interact with chiral compounds, and then, they can be widely applied in enantiomer separation.[16–18] In the early stages of chiral separation technologies, a number of CDs as chiral mobile phase additives were successfully utilized in CEC. However, CDs in the mobile phase result in relatively low sensitivity with a high consumption of chemicals. Since the discovery of the advantageous property of monoliths, attempts have been made to explore β-CD-based monolithic stationary phase for enantiomer separation.[19–26] With the promising development, new CD derivatives functionalized monoliths need to be prepared in order to achieve altered enantioselectivities and further broaden the application range. Compared with β-CD and its derivatives, hydroxypropyl-γ-CD (HP-γ-CD) provides different enantioselectivities and may serve as a good candidate for enantiomer separation of multiring compounds with rigid structure due to its larger hydrophobic cavity size.[27–29] However, to the best of our knowledge, there is no report on preparing HP-γ-CD functionalized stationary phase.

In this work, a novel HP-γ-CD functionalized monolith was developed and tested in CEC chiral separation for the first time. A simple and efficient one-pot sequential strategy previously reported by our group[24,25] was used for preparation of the monolith. In one pot, HP-γ-CD functional monomer was allowed to be formed via the ring opening reaction of HP-γ-CD with glycidyl methacrylate (GMA) and then directly copolymerized with comonomer and crosslinker to form the final monolith. The newly developed monolithic column showed enantioselectivity toward six chiral compounds including pindolol, clorprenaline, tulobuterol, clenbuterol, propranolol, and tropicamide. The mechanism of enantiomer separation was discussed by comparison of the HP-γ-CD and HP-β-CD functionalized monolith.

2 | EXPERIMENTAL PART

Ammonium acetate (NH_4OAc) was obtained from Tian-Jin Bodi Chemical Holding (Tianjin, China). HP-γ-CD and *n*-hexanol were purchased from Energy Chemical (Shanghai, China). Ethylene dimethacrylate (EDMA), GMA, γ-methacryloxy propyltrimethoxysilane (γ-MAPS), 2,2′-azobis(2-methylpropionitrile) (AIBN), and 2-acrylamido-2-methyl propane sulfonic acid (AMPS) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Cyclohexanol was obtained from Acros (Geel, Belgium). 1-Dodecanol was obtained from China National Pharmaceutical Group Co., Ltd. (Beijing, China). *n*-Butanol (NBA), sodium hydroxide, hydrochloric acid, acetone, toluene, and diethyl ether of analytical grade were purchased from Shandong Yuwang Industrial Co., Ltd (Shandong, China). Acetonitrile (ACN), glacial acetic acid, and triethylamine (TEA) of HPLC grade were purchased from Concord Technology Co., Ltd (Tianjin, China). Anhydrous dimethyl sulfoxide (DMSO) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were obtained from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). All chiral analytes were purchased from National Institutes for Food and Drug Control. The chemical structures of all analytes are shown in Figure 3. Doubly distilled water was used throughout all experiments. All solutions were filtered with a 0.22-μm syringe filter.

All CEC experiments were performed on an HPCE apparatus (CL1030, Beijing Huayanglimin Instrument Co., Ltd, Beijing, China) equipped with a UV detector, a high-voltage power supply, and a HW-2000 chromatography workstation. The polyimide-coated fused silica capillaries (375 μm o.d. × 75 μm i.d.) were obtained from Ruifeng Chromatography Ltd. (Yongnian, Hebei, China). A syringe pump (SPLab04, Shenchen Precision Pump Co., Ltd, Baoding, China) was used to introduce monomer solutions into the previnylized capillaries for polymerization. An HPLC pump (PU-1580, Jasco Corporation, Japan) was used to flush and condition the monolithic capillary. Optical microscopic images of monolithic columns were taken with an Olympus microscope (BX51, Olympus, Germany). Scanning electron microscopy (SEM) images of monoliths were obtained by using a Hitachi S4800 scanning electron microscope

(Hitachi, Ltd., Japan). Fourier transform infrared (FT-IR) spectra of the monolithic columns were recorded using a Bruker EQUINOX55 FT-IR spectrophotometer (Brucker Corporation, Germany). Nuclear magnetic resonance (NMR) spectroscopy was obtained with a Bruker Ultrashield Plus 600-MHz spectrometer (Bruker Corporation, Germany). The specific surface area was determined on a Micromeritics ASAP 2460 apparatus (Micromeritics Instrument Ltd, USA).

The mobile phases were a mixture of ACN and water containing 5 mmol L^{-1} NH_4OAc and 0.4% TEA. The desired pH adjustment was carried out with glacial acetic acid. Electrokinetic injection was used for all samples. The applied voltage was 5 kV, and the injection time was 3 s. The UV detection wavelength was set at 214 nm for clorprenaline, homatropine methylbromide, and tulobuterol; 243 nm for clenbuterol; 254 nm for tropicamide and chlorpheniramine; 259 nm for procaterol; 276 nm for salbutamol and terbutaline; 280 nm for pindolol, propranolol, and tetrahydropalmatine; and 294 nm for ofloxacin.

The detailed preparation procedures of the HP-γ-CD functionalized monolith are described. At first, polymerization mixture was prepared. HP-γ-CD (60 mg), GMA (16 mg), DBU (6 mg), and anhydrous DMSO (0.15 g) were weighed into a 3-ml vial sealed with a rubber septum-lined screw cap. Then, the mixture was stirred at 100°C for 30 min. After cooling to room temperature, *n*-hexanol (0.25 g), EDMA (24 mg), AMPS (7.5 mg), and AIBN (2.5 mg) were added into mixture. In order to remove dissolved air, the resultant solution was sonicated at around 15°C for 15 min and purged with nitrogen for 5 min; then, the polymerization mixture was introduced into the vinylized capillary with an effective length of 30 cm according the reported procedures.[30] The capillary was sealed at both ends with silicon stoppers and submerged into a preadjusted water bath at 60°C for 20 h. Finally, the obtained monolithic column was washed exhaustively with ACN by an HPLC pump to remove unreacted chemicals and porogens.

3 | RESULTS AND DISCUSSION

As illustrated in Figure 1, the scheme for the one-pot preparation of poly-(GMA-HP-γ-CD-co-EDMA) monolithic column involves two sequential reactions: (1) formation of functional monomer GMA-HP-γ-CD via the DBU-catalyzed ring opening reaction between GMA and HP-γ-CD and (2) free radical polymerization of functional monomer and crosslinker for the formation of poly-(GMA-HP-γ-CD-co-EDMA) monolith (Figure 2). In order to obtain the proper monolithic column for chiral separation, the chemical structure of the product of ring opening reaction was confirmed by NMR spectroscopy. After that, the type and composition of porogenic solvents, ratio of functional monomer to crosslinker, and polymerization temperature were systematically investigated.

The novel functional monomer GMA-HP-γ-CD was formed by the ring opening reaction between HP-γ-CD and GMA catalyzed by DBU at a temperature of 100°C for 30 min; then, the products of ring opening reaction were isolated from the reaction mixture for ^{1}H NMR characterization (detailed procedures are described in the supplementary material). Figure S2 shows the ^{1}H NMR spectra of HP-γ-CD and GMA-HP-γ-CD. As expected, new signals for GMA-HP-γ-CD are observed. The peaks at 5.79 and 6.19 ppm can be assigned to the methylene protons, which demonstrated that hydroxyl groups of HP-γ-CD were successfully substituted with glycidyl methacrylate groups.[31] The average degree of substitution (ADS) of GMA-HP-γ-CD was calculated to be 1.16 by comparison of the corresponding signal areas of HP-γ-CD signals and substituent signals.

For CD-based monoliths, DMSO was found to be a suitable solvent which can offer good solubility for native

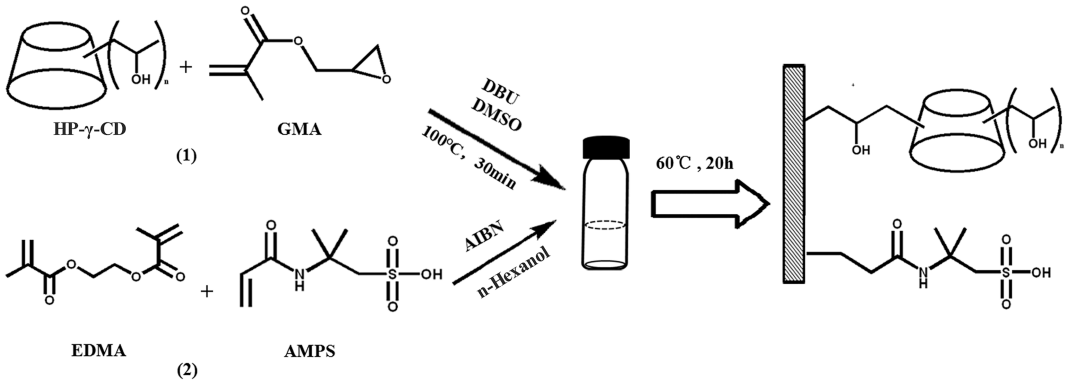

FIGURE 1 One-pot sequential strategy for the preparation of the HP-γ-CD functionalized monolith

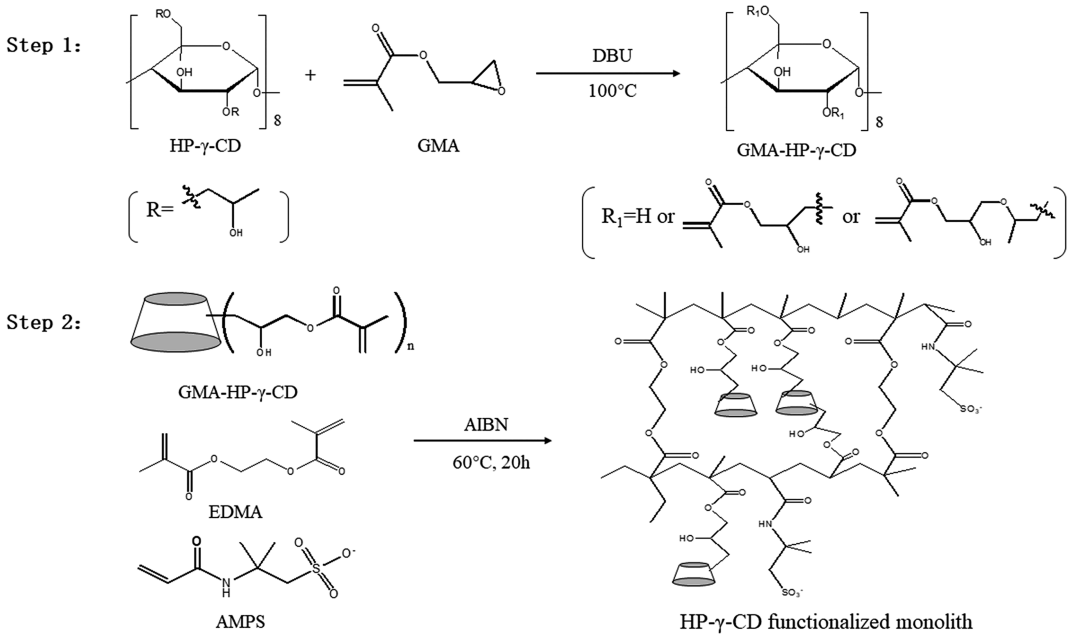

FIGURE 2 Two major reactions in one pot: (1) the ring opening reaction between HP-γ-CD and GMA catalyzed by DBU and (2) the copolymerization reaction of GMA-HP-γ-CD, EDMA, and AMPS

CDs and their derivatives. However, when DMSO was used as a single porogenic solvent, the obtained monolithic matrix was in gel state with low permeability. Therefore, a solvent mixture of DMSO and an alkanol as binary porogens was attempted. After a lot of experiments, DMSO/cyclohexanol, DMSO/1-dodecanol, and DMSO/NBA were found not suitable to produce a monolithic column because they cause the formation of semitransparent gels, whereas an opaque white monolith was formed in the porogenic system of DMSO/*n*-hexanol. Hence, DMSO and *n*-hexanol were finally screened out as optimized binary porogenic solvents to prepare monolith. The multicomponent porogens allow for fine tuning of the monolith morphology by simply changing the ratios of its constituents. Table S1 shows the effect of the component ratio changes of porogenic solvents on morphology and permeability of monoliths. In the case of Column A1, the monolith bed was inhomogeneous with gaps. Most likely 67.5% *n*-hexanol led to so loose monolithic structure and then the monolith collapsed. On the other hand, Column A3 with 57.5% *n*-hexanol was too hard to pump through even though the homogeneous morphology was observed in the microscopic image. Only 62.5% *n*-hexanol in the porogenic system provided the monolith (Column A2) with good balance of permeability and homogeneous morphology.

The content of crosslinker (EDMA) in polymerization mixture was then investigated because it could affect the polymer crosslinking and therefore the permeability, homogeneity, and rigidity. From Table S1, it can be found that when the weight percentage of EDMA was 30% or 36% (Columns B2 and B3), the monolithic columns were impermeable to ACN. This is because the higher crosslinker concentration favors higher crosslinking level, resulting in a lower porosity. An EDMA proportion of 24% (Column A2) provided a homogeneous monolithic matrix with acceptable permeability. However, a further decrease in the EDMA content caused many light and shade stripes in the microscopic image of Column B1. It may be explained by the low crosslinking and mechanical strength resulted from lower EDMA content.

Temperatures for copolymerization during the preparation of the monolithic columns were also examined. From Table S1, it can be observed that Column C1 was inhomogeneous and poorly permeable. The reason may be that the formed monolithic structures at relatively lower temperature (50°C) are unstable, prone to collapse with pore clogging during the polymerization and/or subsequent workup. When the temperature increased to 60°C, Column A2 showed homogeneous morphology and good permeability. Further increasing the copolymerization temperature to 70°C (Column C2), it was difficult to pump through the column, which may be because the increased temperature leads to a reduction in the average pore diameter.

On the basis of these observations, a polymerization mixture consisting of HP-γ-CD (60 mg), GMA (16 mg), DBU (6 mg), anhydrous DMSO (0.15 g), *n*-hexanol (0.25 g), EDMA (24 mg), AMPS (7.5 mg), and AIBN (2.5 mg) was chosen and polymerized at 60°C to

obtain the homogeneous and permeable HP-γ-CD functionalized monolithic column.

The cross-section morphology of the prepared HP-γ-CD functionalized monolithic column (Column A2) was observed by SEM (SEM sample preparations are described in the Supporting Information), and the obtained SEM images are shown in Figure S1 with two different magnifications. As shown in Figure S1a, a uniform HP-γ-CD functional monolith with large through pores is observed and tightly anchored to the capillary wall. In Figure S1b, it can be seen that many small pores are contained in the monolithic bed which most significantly contributes to the surface area. Furthermore, the specific surface area of the resultant material was 43.3 $m^2\ g^{-1}$ determined by nitrogen adsorption–desorption experiments (sample preparations are described in the Supporting Information).

The chemical groups of the monolith were confirmed by FT-IR spectroscopy (FT-IR sample preparations are described in the Supporting Information). Figure S3 shows the FT-IR spectra of HP-γ-CD, HP-γ-CD functionalized monolith, and blank monolith. The FT-IR spectrum of the HP-γ-CD functionalized monolith (Figure S3b) combines features of the HP-γ-CD (Figure S3c) and the blank monolith (Figure S3a) spectra. The spectrum of HP-γ-CD shows bands at 943 and 1083 cm^{-1}. These bands also exist in the spectrum of HP-γ-CD functionalized monolith but are absent in the spectrum of blank monolith. The FT-IR spectra provide the evidence of the presence of HP-γ-CD on the monolithic material.

In CEC experiment, we attempted to separate 13 compounds by use of the HP-γ-CD functionalized monolith. Their chemical structures are shown in Figure 3. Of the 13 compounds, pindolol, propranolol, clorprenaline, tulobuterol, clenbuterol, and tropicamide could be separated. After the optimization of separation conditions, baseline separation was achieved for pindolol, clorprenaline, and tropicamide with R_s values of 1.62, 1.73, and 1.55, and partial separation of propranolol, clenbuterol, and tulobuterol was obtained with R_s values of 0.53, 0.84, and 0.53. Figure 4 shows the electrochromatograms of the six analytes and the corresponding R_s values, selectivity factors (α), and column efficiencies (n) at the optimum conditions.

Our group has previously reported the fabrication of the HP-β-CD functionalized monolith by the same strategy.[25] Table S2 summarizes enantioseparation results of chiral drugs on the HP-γ-CD and HP-β-CD functionalized monoliths. Compared with HP-β-CD, HP-γ-CD composed

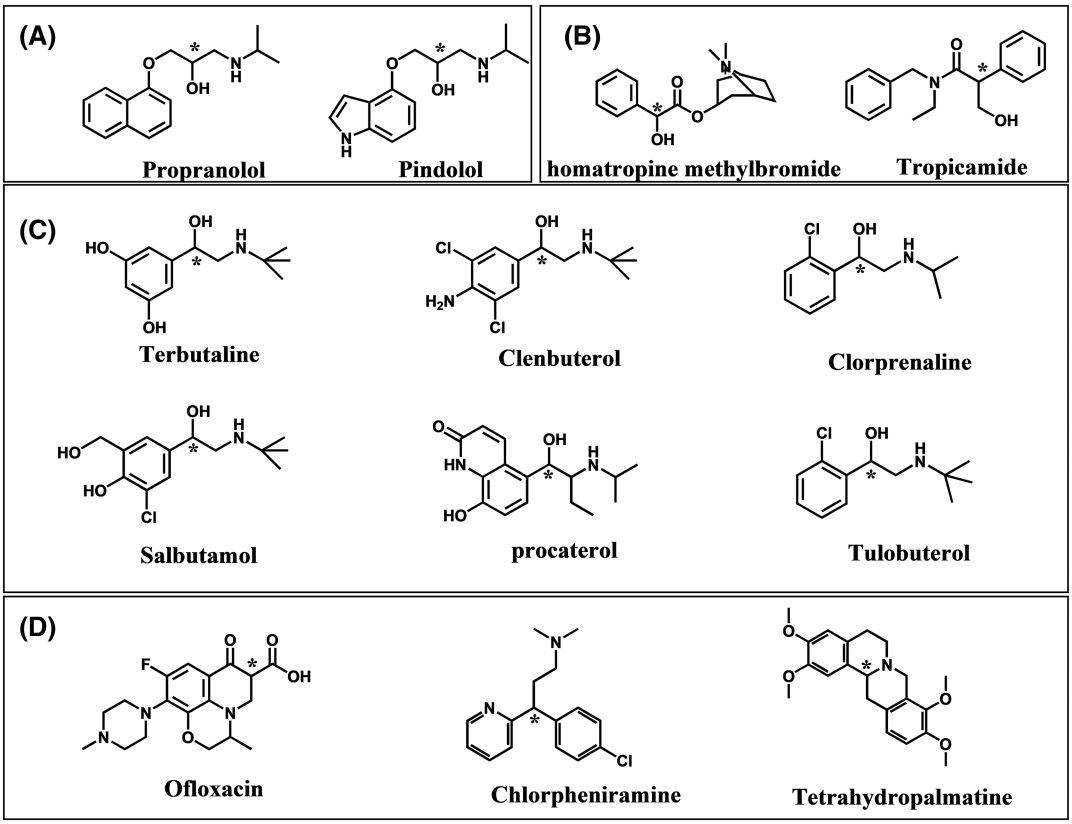

FIGURE 3 Chemical structures of (A) β-adrenergic receptor blockers, (B) anticholinergic agents, (C) β-adrenergic receptor agonist, and (D) others

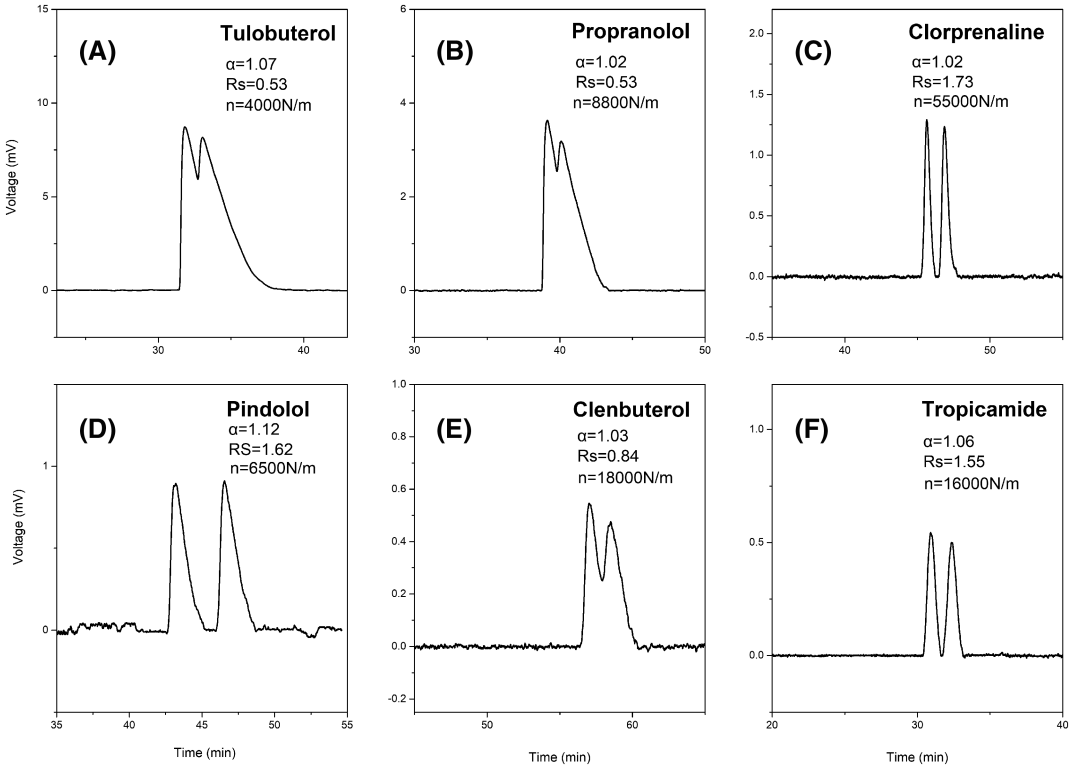

FIGURE 4 Electrochromatograms of six analytes under the optimum conditions on the poly-(GMA-HP-γ-CD-co-EDMA) monolith. Separation conditions: (B) ACN-H_2O (40:60), 5 mmol L^{-1} NH_4OAc, and 0.4% TEA, pH 5.0, +20 kV. (A,C,D,E) ACN-H_2O (30:70), 5 mmol L^{-1} NH_4OAc, and 0.4% TEA, pH 5.0, +20 kV. (F) ACN-H_2O (20:80), 5 mmol L^{-1} NH_4OAc, and 0.4% TEA, pH 5.0, +20 kV

of eight glucose residues symmetrically arranged in a ring, which means HP-γ-CD has a larger hydrophobic cavity and can accommodate large-sized molecules. Therefore, it is our interest to use bicyclic and polycyclic compounds as model compounds to explore the HP-γ-CD monolith to determine if the HP-γ-CD monolith behaves better than the HP-β-CD monolith for chiral separation of large-sized compounds. In details, the model compounds include pindolol, propranolol, homatropine methylbromide, tropicamide, procaterol, ofloxacin, chlorpheniramine, and tetrahydropalmatine. Unfortunately, the resolution of most of these compounds on the HP-γ-CD monolith was lower than that on the HP-β-CD monolith. The reason why the selectivity is reduced may be the large cavity of HP-γ-CD has relatively high degree of flexibility and it is difficult to form enantioselective inclusion complex. It is worth mentioning that the separation performance of pindolol and propranolol was better than that on the HP-β-CD monolith. A comparison of chemical structures of these analytes reveals that only pindolol and propranolol contain large planar π-conjugation systems. Molecules of rigid planar structure are assumed to possess a limited freedom of rotation within hydrophobic cavities of HP-γ-CD, permitting differences in affinity for the CD cavity between enantiomers.

Besides size, other factors such as van der Waals forces, dipole–dipole interaction, hydrogen bonding, and hydrophobic interactions also govern the formation and the strength of an inclusion complex. Therefore, some substituted benzene ring compounds including terbutaline, clenbuterol, clorprenaline, salbutamol, and tulobuterol were still tested on the HP-γ-CD monolith. The results showed that the HP-γ-CD monolith had enantiomer selectivity toward clorprenaline, clenbuterol, and tulobuterol, but only the resolution of clenbuterol on the HP-γ-CD monolith was higher than that on the HP-β-CD monolith. According to Figure 3, the 3,4,5 position of the benzene ring was substituted by chloride, amino group, and chloride, respectively. The presence of the three substituted groups may enlarge the planar conjugated systems of the molecule. To some extent, the substituted benzene ring of clenbuterol seems to resemble naphthalene ring or indole ring. This may be the reason why clenbuterol obtain a better separation result on the HP-γ-CD monolith. More model compounds should be tested in order to demonstrate separation mechanisms of CD functionalized monoliths better.

4 | CONCLUSION

In this study, a novel poly-(GMA-HP-γ-CD-co-EDMA) monolithic capillary column was prepared and found to be efficient for chiral separation. Under the optimal preparation conditions, the resulting monolithic column possesses uniform structure and proper permeability. Furthermore, six pairs of enantiomers including pindolol, clorprenaline, tropicamide, propranolol, clenbuterol, and tulobuterol were separated, and three of them could be baseline separated. Importantly, a comparison of HP-γ-CD and HP-β-CD functionalized monoliths suggests that the proposed HP-γ-CD functionalized monoliths may have better enantioselectivity toward some analytes containing large planar conjugated systems than other CD-based monoliths. In addition, the suggested one-pot sequential procedure could be expanded to prepare other CD-based monolithic stationary phases.

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DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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