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A novel one-pot strategy to prepare β-cyclodextrin functionalized capillary monoliths for enantioseparation of basic drugs

Miaoduo Deng, Meng Li, Yu Zhao, Zhen Jiang, Xingjie Guo*

School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning Province, PR China

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ABSTRACT

With native β-cyclodextrin (β-CD) added into the polymerization mixture directly, a novel, convenient and low-cost one-pot strategy was developed to prepare the β-CD functionalized organic polymer monolithic capillary column. Diazabicyclo[5.4.0]undec-7-ene (DBU) as a basic catalyst for the ring opening reaction between β-CD and glycidyl methacrylate (GMA) was introduced into the polymerization system for the first time. Thereby, two consecutive reactions namely the in situ methacrylation of β-CD and copolymerization reaction can be achieved in one pot. The preparation conditions including the type and composition of porogens , the ratio of functional monomer to crosslinker and amount of 2-acrylamido-2-methyl propane sulfonic acid (AMPS) were optimized. The specific surface area and morphology of the prepared monolith were characterized using scanning electron microscopy (SEM) and nitrogen adsorption analysis, respectively. Raman spectroscopy and nuclear magnetic resonance (NMR) spectroscopy confirmed that β-CD was covalently bonded onto the monolith successfully. Then, the monolithic column was applied to enantioseparation of six basic drugs in capillary electrochromatography (CEC). Under the optimal conditions, tropicamide, homatropine, homatropine methylbromide, brompheniramine, chlorpheniramine and clorprenaline were all totally separated with the resolution (Rs) values of 2.84, 4.70, 4.61, 3.01, 2.57 and 2.33, respectively. Furthermore, the column demonstrated satisfactory stability and repeatability of retention time and enantioselectivity using homatropine as model analyte.

1. Introduction

In the past decades, monolithic materials as the ideal chromatographic stationary phases have attracted considerable attention in the separation fields. In comparison to traditionally packed columns, the main advantages of monolithic column are faster mass transfer, higher permeability and modifiable pore size [1]. Moreover, it eliminated most of the problems associated with in-situ frit formation and particle packing [2,3]. Meanwhile, the monolithic column show satisfactory selectivity and loading capacity superior to that of open tubular columns [4].

For enantiomeric separation, cyclodextrin (CD) and its derivatives as one of the most widely used chiral selectors [5,6] were introduced into the monolithic column by Koide et al. as early as 1998 [7]. Since then, great efforts have been devoted to developing more efficient, simple and stable preparation methods. Usually, CDs can be immobilized on the monoliths by chemical bonding or physical coating. The chemical bonding is the preferred methodology because the resulting monolith have higher stability and longer life time. So far, CDs were covalently bonded onto monoliths mainly via a triazole linkage, an amino linkage or an ether linkage [8].

In case of triazole linkages [9–12] and amino linkages [13–16], most of CDs functionalized monoliths were used for the enantioseparation of acidic and neutral analytes. For example, Guerrouache and coworkers grafted a 6-azido-6-deoxy-β-CD on the surface of the monolith through the click reaction [9]. The enantioselectivity of this monolithic column was evaluated using flavanon as the model analyte. A similar monolithic column prepared by one-step copolymerization strategy showed a partial separation toward 4-bromomandelic acid [11]. By using the amino linkage, a monolith grafted with a 4-dimethylamino-1,8-naphthalimde-β-CD derivative was prepared and baseline separations of racemic naproxen and ibuprofen were obtained on this monolithic column [13]. Additionally, Zhang et al. synthesized a poly(GMA-ethylenediamine-β-CD-*co*-EDMA) monolithic column by one-pot copolymerization strategy and the column was applied to the enantioseparation of chiral acidic compounds [16].

Also some papers reported the ether as linkage for immobilization of CDs onto the monolith [17–22]. Differently, these columns could also serve as good chiral stationary phase for resolving basic analytes. A representative example was reported by Gu and Shamsi [18]. In this

* Corresponding author.
E-mail address: gxjhyz@gmail.com (X. Guo).

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method, a monolith was successfully synthesized by classic one-step copolymerization strategy in the presence of glycidyl methacrylate bonded β-CD as functional monomer and the resulting monolith showed moderate enantioresolution values for the tested analytes. Likewise, the post modification strategy was reported for the preparation of the β-CD functionalized monolith with the ether as linkage [20,22]. Commercial-available β-CD or hydroxypropyl-β-CD were immobilized onto the epoxy activated surface of the monoliths under harsh reaction conditions. The resulting monoliths showed enantioselectivity toward several amino acids.

Even though many methods were developed for anchoring CDs onto the monolith so far, the preparation of the CDs bonded monolith is still not an easy task. The post modification strategy is tedious and difficult to control [23]. Therefore, more studies were dedicated to the development of the direct polymerization strategy including classic one-step strategy and one-pot strategy. In particular, one-pot strategy has considerable prospects due to its merits of excellent column performance and simple preparation procedures [23]. Nevertheless, the applications of reported direct polymerization strategies are still limited, because compounds as functional monomer have to contain special anchoring groups like the vinyl group [15,25], methacrylate group [11,12,18,19] or amino group [16,23,24] in the polymerization system. It is known to us that β-CD derivatives which can be used as functional monomer are very expensive compared to native β-CD and most of them are not available in commercial market. Synthesis and characterization of the β-CD modified functional monomers are time-consuming and require a lot of experiences.

In this study, we introduced a catalyst into the polymerization system for the first time. The hydroxyl groups of β-CD were activated by the facile catalyst (Diazabicyclo[5.4.0]undec-7-ene), which made it possible to achieve fast methacrylation of native β-CD and the subsequent copolymerization in one pot. From easy-obtained and low-cost raw materials to the final monolithic column, the entire process needed less than 24 h only. The prepared monolith was successfully used for the enantioseparation of six basic drugs in capillary electrochromatography (CEC).

2. Experimental

2.1. Chemicals and reagents

β-cyclodextrin (β-CD), tris(hydroxymethyl)aminomethane (Tris) and ammonium acetate (NH_4OAc) were obtained from TianJin Bodi Chemical Holding (Tianjin, China). β-CD is dried under vacuum at 110 °C for 12 h to remove any moisture. Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), 2,2′-azobis(2-methylpropionitrile) (AIBN), γ-methacryloxy propyltrimethoxysilane (γ-MAPS), 2-acrylamido-2-methyl propane sulfonic acid (AMPS) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Sodium hydroxide, n-propanol (POH), 1,4-butylene glycol (BOH), hydrochloric acid, acetone of analytical grade and acetonitrile (ACN), glacial acetic acid of HPLC grade were purchased from Shandong Yuwang industrial Co., Ltd (Shandong, China). Anhydrous dimethyl sulfoxide (DMSO) were purchased from Aladdin Bio-Chem Technology Co., LTD (Shanghai, China). Water used throughout all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Bedford, MA, USA). Tropicamide, homatropine Hydrobromide, chlorphenamine maleate and clorprenaline hydrochloride were obtained from National Institutes for Food and Drug Control. Brompheniramine maleate and homatropine methylbromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The structures of pharmaceutical racemates are shown in Fig. 1.

2.2. Apparatus

All CEC experiments were performed on a CE apparatus (CL1030, Beijing huayanglimin instrument Co., Ltd, Beijing, China). A syringe pump (SPLab04, Shenchen Precision Pump Co., Ltd, Baoding, China) was used to pump liquid through fused-silica capillaries. An HPLC pump (PU-1580, Jasco corporation, Japan) was used to flush monolithic columns with mobile phase for conditioning. The fused-silica capillaries (375 μm o.d. × 75 μm i.d.) were purchased from Ruifeng Chromatography Ltd. (Yongnian, Hebei, China). A microwave-ultrasound combined reactor (XH-300A, Beijing Xianghu Technology Co., Ltd.) provided continuous and homogeneous ultrasound and microwave irradiations. Morphological characterizations of monoliths were taken with a Hitachi S4800 scanning electron microscope (Hitachi, Ltd., Japan) after a gold coating of the samples. Microscopic pictures were taken with an Olympus microscope (BX60, Olympus, Germany). Raman spectra of monolithic materials (confined within capillary support) were obtained with a HORIBA labRAM HR Evolution raman spectrometer (Horiba Group, Japan) equipped with an Olympus objective LMPLAN FLN 50/0.5 and a double-frequency Nd: YAG laser (532 nm). The specific surface area was examined by nitrogen adsorption experiment on a Micromeritics TriStar II Plus apparatus (Micromeritics Instrument Ltd, USA). Nuclear magnetic resonance (NMR) spectroscopy was obtained with a Bruker Ultrashield Plus 600 MHz spectrometer (Bruker Corporation, Switherland).

2.3. Electrochromatographic conditions

The mobile phases were a mixture of water and acetonitrile containing 30 mM Tris and 5 mM NH_4OAc. The pH values of the mobile phases were adjusted by glacial acetic acid. UV detection wavelength was set at 214 nm for homatropine, clorprenaline and homatropine methylbromide, and 254 nm for clorpheniramine, brompheniramine and tropicamide, respectively. The analyte solutions were injected using a voltage of + 3 kV for 3 s. Mobile phases and the analyte solutions were filtered by 0.22 μm millipore filter before use.

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

As illustrated in Fig. 2, the one-pot copolymerization approach was employed for the preparation of the β-CD functionalized organic polymer monolith. Prior to the polymerization, the inner wall of the 50-cm-long capillary was vinylized with γ-MAPS as reported in the literature [19]. The polymerization solution was prepared using the following procedures: (1) GMA (22 mg), DBU (6 mg), β-CD (60 mg) and DMSO (0.15 g) were accurately weighted into a 3-ml vial (2) The vial was placed into a DMSO bath at 100 °C for 30 min with ultrasound (300 W, 25 kHz) and microwave irradiations (500 W); (3) EDMA (0.024 g), POH (0.15 g), BOH (0.1 g), AMPS (7.5 mg) and AIBN (2.5 mg) were added into the same vial; (4) The resulting mixture was sonicated at around 15 °C for 15 min and then bubbled with nitrogen for 5 min. Subsequently, the pretreated capillary was filled with the prepared polymerization solution to a length of 30 cm, sealed with rubber stoppers and then submerged into a pre-adjusted water bath at 60 °C for 20 h. After the polymerization was completed, the capillary was rinsed with acetonitrile by an external HPLC pump to remove porogens and unreacted chemicals. Finally, the detection window was created at a 10-cm distance from the outlet end.

3. Results and discussion

3.1. Optimization of preparation conditions

The major advantage of our strategy is its simplicity for straightforward preparation of the chiral monolithic column by using the native β-CD. The method described herein process the in situ methacrylation of native β-CD and the subsequent copolymerization in one pot. Fig. 2 illustrates two consecutive reactions: (1) the ring opening reaction between the hydroxyl groups of β-CD and the epoxy groups of GMA

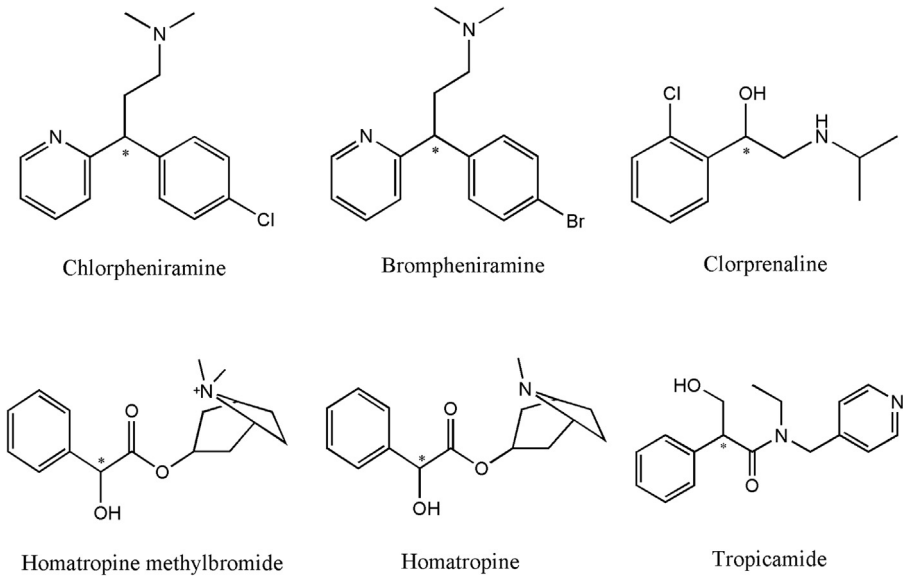


Fig. 1. Chemical structures of six pharmaceutical racemates.

catalyzed by DBU; (2) the copolymerization of GMA-β-CD, AMPS and EDMA. Since preparation conditions have great influences on the structure, permeability and enantioselectivity of the monolith, several parameters were optimized including the type and composition of porogens, ratio of β-CD functionalized monomer to EDMA and amount of AMPS.

It is well known that the type and composition of porogens have a significant effect on the skeleton, morphology and permeability of monoliths. Several frequently used porogens including DMSO, POH, BOH, cyclohexanol, 1-dodecanol were initially attempted to prepare β-CD functionalized monoliths. As a result, a ternary solvent system of DMSO/POH/BOH was regarded as the most suitable one. On the one hand, DMSO can serve as the reaction medium of the ring opening reaction due to good solubility for all reactants. On the other hand, the permeability of the monolith can easily be obtained by changing the proportion of POH in the porogen mixture. The optical microscope images of obtained columns with the varied proportion of POH were displayed in Table 1. Only the proportion of POH at 37.5% (Column B) could result in homogeneous monolithic structure with good permeability. By comparison of their SEM images (Fig. 3), it can be easily observed that the increased proportion of POH would provide more flow through pores for the monolith. Finally, the ternary porogen system containing 37.5% (w/w) DMSO, 37.5% (w/w) POH and 25% (w/w) BOH, was selected for further optimizations.

The ratio of functional monomer to crosslinker is also crucial to the permeability and structure of monoliths. In our experiment, an appropriate ratio of β-CD functionalized monomer to EDMA should be determined keeping the molar ratio of GMA/β-CD at 3:1. Column D, B, and E were prepared corresponding to the ratio of β-CD functionalized monomer to EDMA of 82:18, 77:23, 72:28, respectively. SEM images of resulting columns are shown in Fig. 3. It is clearly seen that increasing the content of EDMA can result in a significant decrease in the size of large pores and increase in the amount of microglobules. Column E with the highest content of EDMA has too narrow pore size to allow mobile phase flow (Table 1). When the content of EDMA was decreased and the ratio of monomer to crosslinker was 77:23, column B provided good permeability and homogeneous monolithic bed. Further decrease the content of EDMA led to less permeable and inhomogeneous monolith (column D). This might be because column D had a low degree of crosslinking and the monolithic structure collapsed under pressure, which would lead to clogging of the pores.

The previous research [26] found that the amount of AMPS in the polymerization mixture has a significant effect on the enantioselectivity of the prepared monolith. As comonomer in the formation of the monolith, AMPS not only supports the positive electroosmotic flow [27] (EOF) but also helps in retaining positively charged chiral analytes and then enhances enantioseparation [26]. In order to obtain the monolith with the best enantioselectivity, a series of monolithic capillaries with increased amounts of AMPS were prepared, keeping all other parameters constant. Six chiral drugs were selected as model compounds to evaluate their enantioselectivity. Under the same separation condition, the enantioseparation results of column B, B1, B2 and B3 responding to

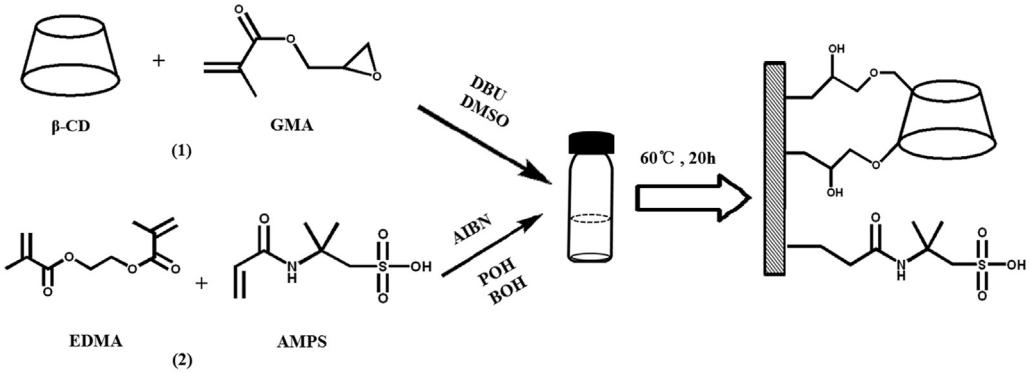
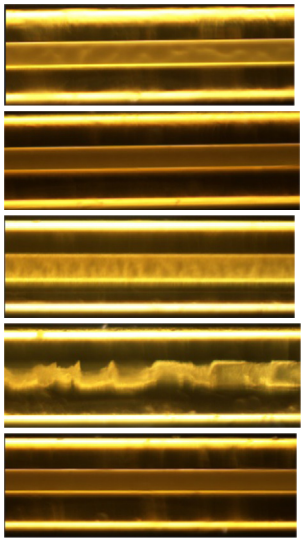


Fig. 2. One-pot preparation scheme of the poly(GMA-β-CD-*co*-EDMA) monolithic capillary columns.

Table 1
Permeability and skeleton of monolithic columns prepared by different preparation conditions.

Column	Monomer (%, w/w)		Porogen (%, w/w)			AMPS	Permeability	Microscope image
	GMA β-CD	EDMA	DMSO	POH	BOH	(mg)		
A	77	23	37.5	25	37.5	2.5	poor	
B	77	23	37.5	37.5	25	2.5	good	
C	77	23	37.5	50	12.5	2.5	good	
D	82	18	37.5	37.5	25	2.5	poor	
E	72	28	37.5	37.5	25	2.5	poor	

Key to abbreviations: GMA, glycidyl methacrylate; β-CD, β-cyclodextrin; EDMA, ethylene dimethacrylate; DMSO, dimethyl sulfoxide; POH, n-propanol; BOH, 1,4-Butylene glycol; AMPS, 2-acrylamido-2-methyl propane sulfonic acid.

2.5, 5.0, 7.5, 10 mg AMPS in the polymerization mixture are shown in Table 2. As the amount of AMPS was increased from 2.5 to 7.5 mg in the polymerization mixture, the retention time and Rs values for all analytes exhibited an increase trend. Further increase in AMPS comonomer showed longer retention time but decreased Rs values for 4 out of 6 analytes. Hence, 7.5 mg of AMPS as the optimal condition was added in the polymerization mixture.

3.2. Characterization of the β-CD functionalized monolith

3.2.1. The specific surface area of monolithic stationary phase

For nitrogen adsorption analysis, the bulk monolith was prepared in a 10 ml glass vial under the same condition. After the polymerization was completed, the obtained bulk monolith was removed from the container carefully, soxhlet extracted with methanol for 24 h and vacuum dried at 70 °C. A subsequent nitrogen adsorption experiment was performed and the specific surface area of the monolithic material in the dry state was calculated as 43.8655 m^2 g^{-1} according to BET equation.

3.2.2. NMR spectroscopy

The product of ring opening reaction was characterized by ^{1}H NMR spectroscopy. To obtain a solid product for NMR analysis, 3 ml of toluene was added into the reaction solution after the ring opening reaction. The resultant precipitate was collected by centrifugation at 5000 rpm for 10 min, washed with 3 ml of toluene and then 3 ml of acetone at least four times to remove unreacted chemicals and dried under vacuum at 60 °C for 24 h. Finally, the degree of substitution value of β-CD was calculated as 1.43 according to ^{1}H NMR spectroscopy (Fig. S1).

3.2.3. Raman spectroscopy

To gain chemical information on the surface of the monolith, the Raman spectra were recorded directly on the monolith within the confines of the fused-silica capillaries. Fig. 4 shows Raman spectra of the blank monolithic skeleton (a), β-CD modified monolith (b) and β-CD (c). Inspection of Fig. 4, the scissoring vibration of CH for β-CD appeared at ~1260 and ~1334 cm^{-1} in the β-CD modified monolith and β-CD but they were absent in the blank monolith [28]. On the basis of the NMR spectroscopy analysis, it could be confirmed that β-CD was covalently bonded onto the monolith successfully.

3.3. Enantioseparation of basic pharmaceutical racemates

During our study, the prepared monolithic column showed enantioselectivity toward six analytes, including tropicamide, chlorpheniramine, brompheniramine, homatropine, homatropine methylbromide and clorprenaline. In order to obtain the optimum results of enantioseparation, their electrochromatographic conditions were optimized systematically in term of the pH value of mobile phase and ACN content.

In CEC enantioseparation, the pH value of mobile phase is a crucial parameter. It affects not only the EOF, but also the charge of both stationary phase and analytes, and thus the enantioseparation. In our separation system, mobile phases with pH values ranging from 3.0 to 6.0 were studied. It is well understood that, in this pH range, the sulfonic acid groups on the monolith are completely ionized and generate a steady positive EOF[24]. Therefore, the analyte charge state is the dominant factor affecting the retention time. According to their pKa values (Table S1), all of analytes are all protonated and positively charged over the range of pH 3.0–6.0. When the pH value was increased gradually from 3.0 to 6.0, the analytes were less positively charged and thus retained longer on the monolith (Fig. 5a). However, the effects of pH on the resolution are more complicated. The Rs values of all analytes increased gradually with increasing the pH value from 3.0 to 5.0, reaching the maximum at pH of 5.0. When the pH was increased to 6.0 further, the decreased Rs values were obtained regardless of the long retention time (Fig. 5b). The phenomenon indicated the existence of electrostatic attraction between the positively charged analytes and the negatively charged stationary phase. The inhibited electrostatic attraction at pH higher than 5.0 may preclude the interaction between analytes and stationary phases and then lead to the decreased Rs values. Therefore, a pH value of 5.0 was finally selected for further experiments.

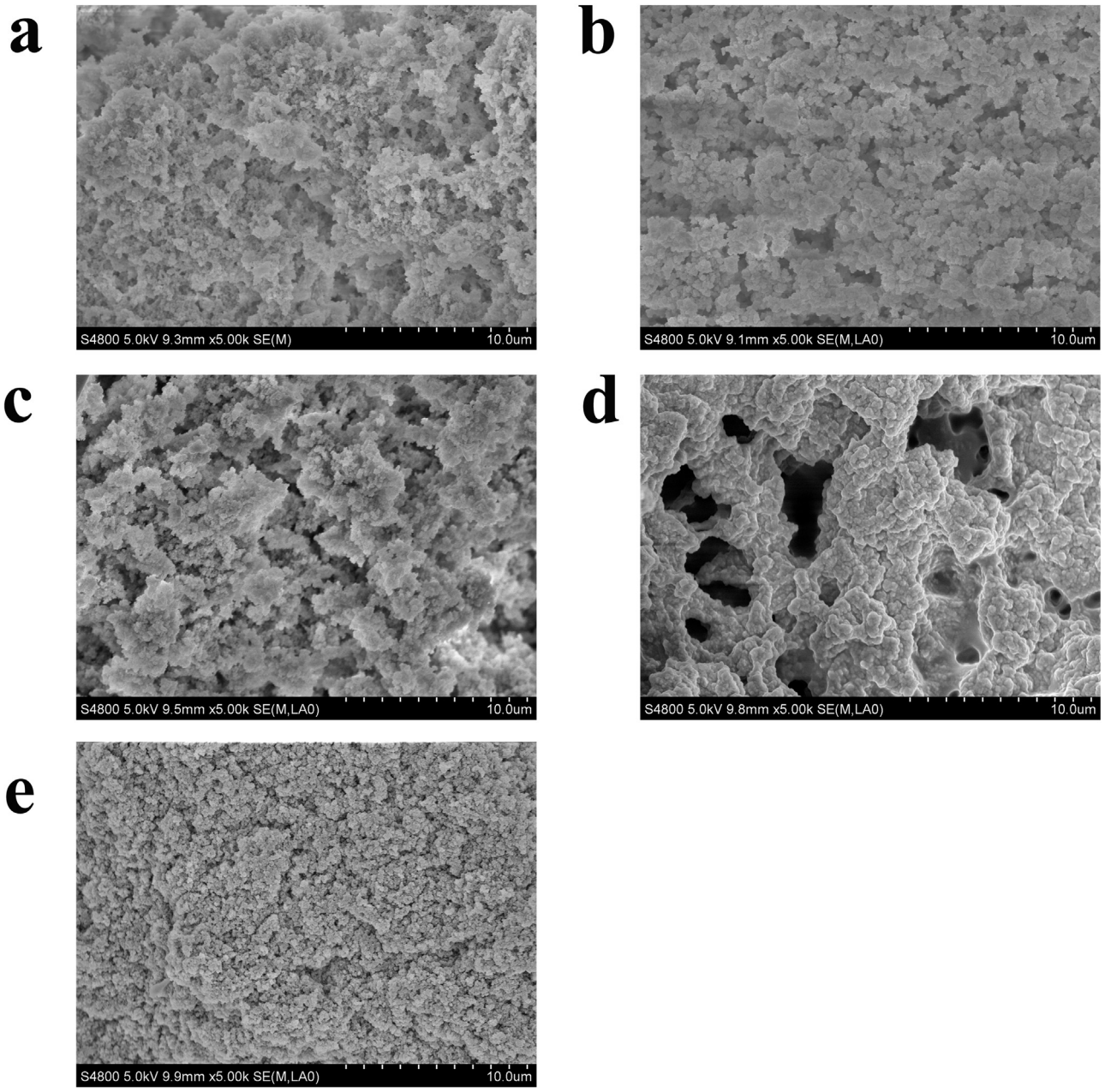


Fig. 3. SEM images of (a) column A, (b) column B (c) column C (d) column D (e) column E.

Table 2
Results of enantioseparation on different monolithic capillary columns.

	Column B		Column B1		Column B2		Column B3	
	t_1(min)	Rs	t_1(min)	Rs	t_1(min)	Rs	t_1(min)	Rs
Chlorpheniramine	13.369	0.80	20.817	1.09	28.513	2.57	36.533	1.08
Brompheniramine	14.786	0.84	27.821	1.12	38.858	3.01	47.188	1.30
Homatropine	12.863	0.95	17.571	2.27	25.615	4.70	34.209	4.93
Homatropine methylbromide	11.684	0.91	15.671	2.16	24.098	4.61	31.893	4.82
Clorprenaline	11.425	0.1	14.271	0.32	20.653	1.43	28.521	0.53
Tropicamide	19.073	0.91	28.004	1.32	32.958	2.84	39.331	2.13

Separation conditions: ACN/H_2O (50:50), 30 mM Tris and 5 mM NH_4OAc, pH 5.0, + 20 kV.
Key to abbreviations: t1 the retention time of the first eluted peak; Rs the resolution value.

The electrochromatographic performance of analytes also depended on the composition of the mobile phase. The effect of ACN content (30–60%) on the retention and resolution of the six analytes were studied. As shown in Fig. 6, non-linear relationship between the velocity of EOF and ACN content was observed, which can be explained that the effect of ACN content in the mobile phase on the ratio of its

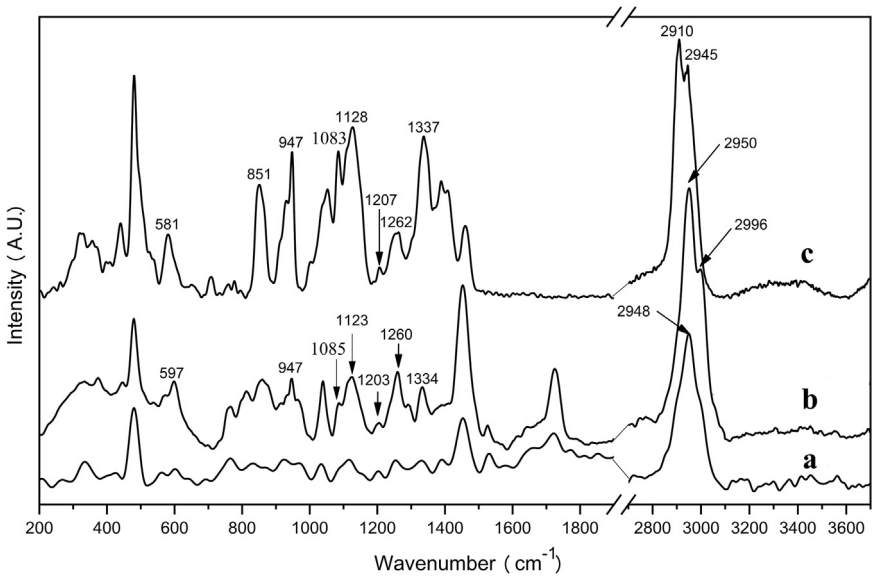


Fig. 4. Raman spectra of monolithic stationary phases within the confines of the fused-silica capillaries: (a) blank monolithic skeleton without β-CD (b) β-CD modified monolith (c) β-CD.

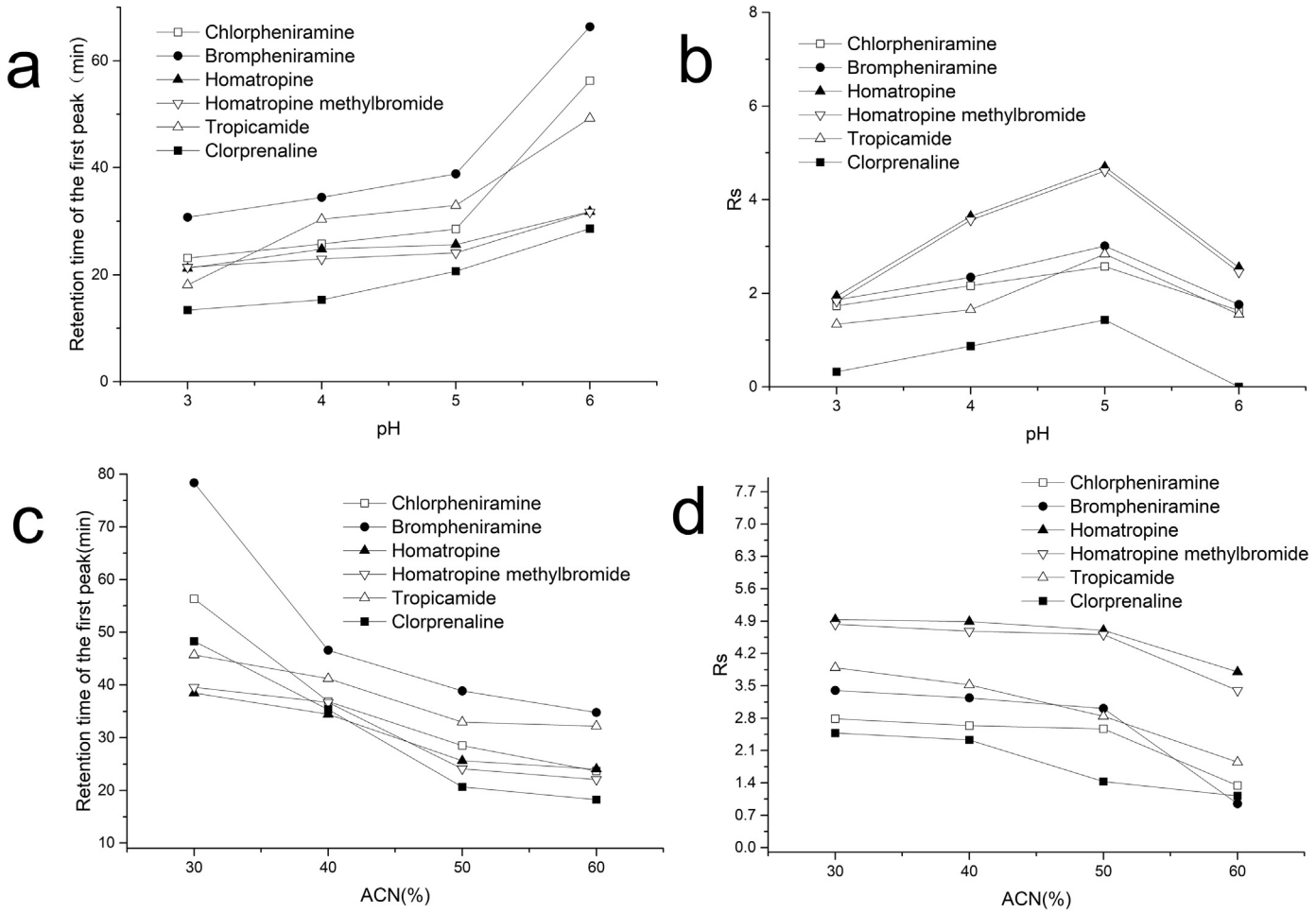


Fig. 5. (a) Effect of pH of the mobile phase on retention time of six pharmaceutical racemates (b) Effect of pH of the mobile phase on Rs values of six pharmaceutical racemates (c) Effect of the ACN content in mobile phase on retention time of six pharmaceutical racemates (d) Effect of the ACN content in mobile phase on Rs values of six pharmaceutical racemates. Separation conditions: (a, b) ACN/H_2O (50:50), 30 mM Tris and 5 mM NH_4OAc, + 20 kV (c, d) 30 mM Tris and 5 mM NH_4OAc, pH 5.0, + 20 kV.

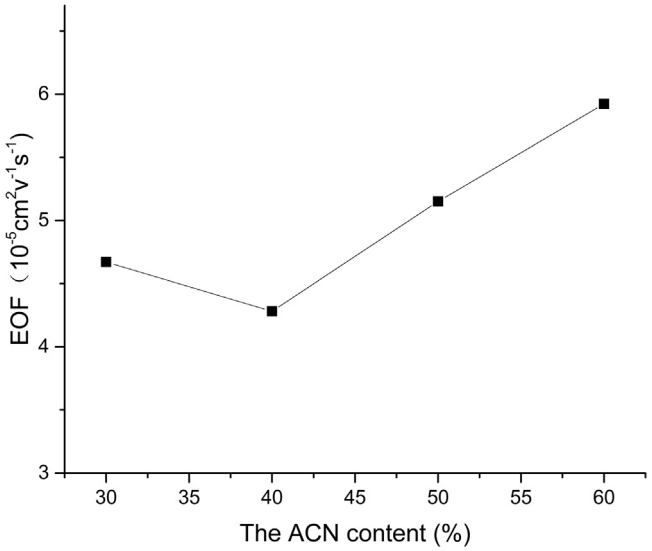


Fig. 6. Effect of the ACN content on EOF.

dielectric constant to viscosity [27,29]. However, all analytes exhibited a trend of increased retention with the decrease in ACN content (Fig. 5c). This suggests that the most predominant mechanism of retention may be based on hydrophobic interactions between analytes and the prepared stationary phase under reversed phase conditions. Furthermore, a general trend of better Rs values for all analytes were obtained as decreasing the ACN content (Fig. 5d). This may be because the lower content of ACN is favor of the formation of inclusion complexes between analytes and the hydrophobic cavity of β-CD. Considering the trade-off between retention time and resolution, 50% ACN as the optimal condition was selected for tropicamide, chlorpheniramine, brompheniramine, homatropine and homatropine methylbromide. Similarly, 40% ACN was selected for clorprenaline.

Under the optimum conditions, tropicamide, homatropine, homatropine methylbromide, brompheniramine, chlorpheniramine and clorprenaline were baseline separated with Rs values of 2.84, 4.70, 4.61, 3.01, 2.57 and 2.33, respectively. Fig. 7 displays the typical electrochromatograms of the six analytes.

3.4. Linearity, repeatability and stability

Under the optimal experimental conditions, linearity of the developed analytical method was investigated. For each pair of enantiomers, a series of solutions at six concentration levels ranging from 10 to 800 μg ml^{-1} were prepared. Each solution was injected in duplicate. The corresponding calibration curve was obtained by plotting the peak area versus the concentration of each enantiomer, respectively. The results are listed in Table 3. All enantiomers showed good linearity with satisfactory correlation coefficients (R^2) greater than 0.9990.

The stability and repeatability of the poly(GMA-β-CD-*co*-EDMA) monolithic columns were further evaluated in terms of relative standard deviations (RSD%) of the retention time, resolution and efficiency

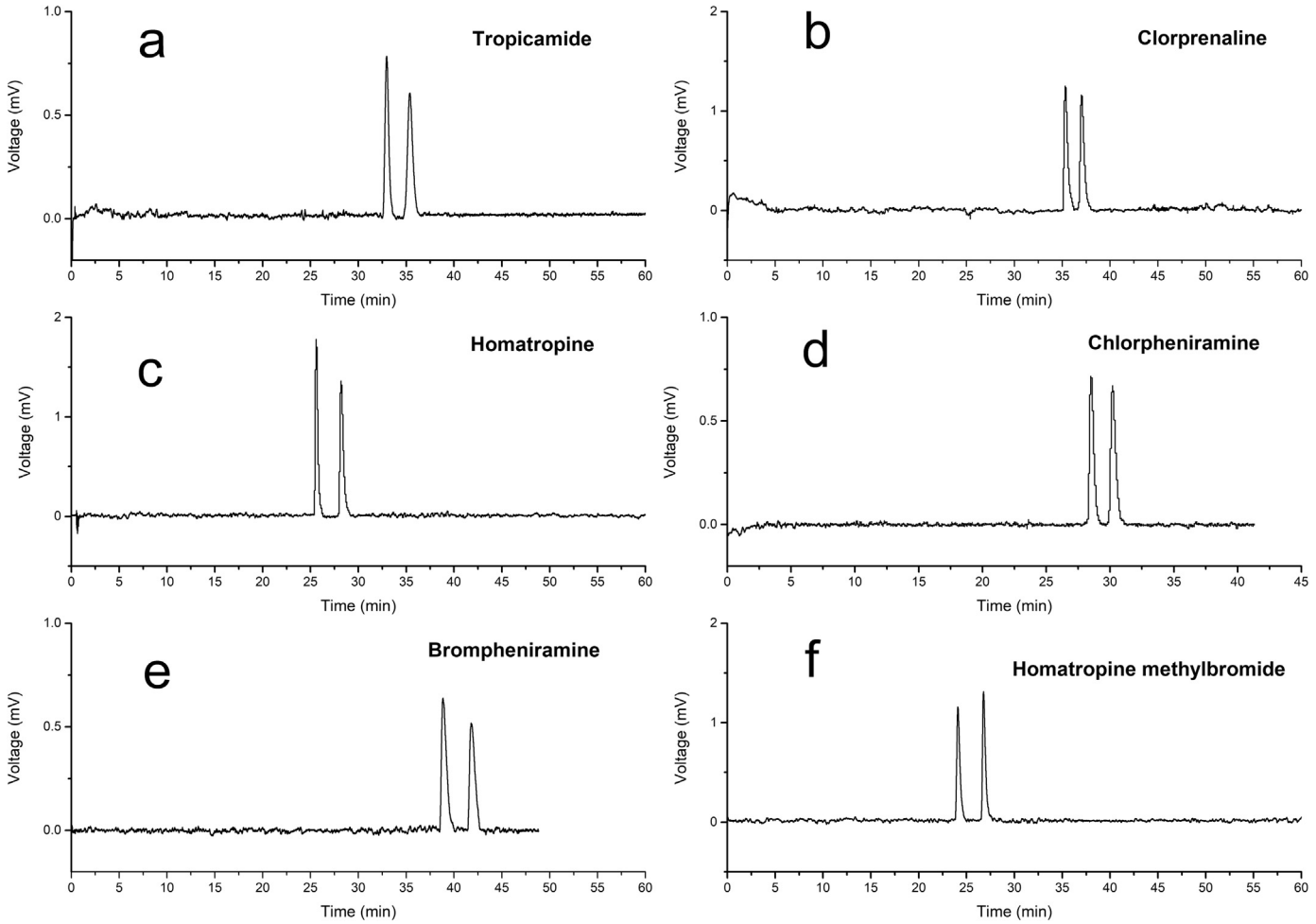


Fig. 7. Electrochromatograms of the six analytes under the optimum conditions. Separation conditions: (a,c,d,e,f) ACN/H_2O (50:50), 30 mM Tris and 5 mM NH_4OAc, pH 5.0, + 20 kV (b) ACN/H_2O (40:60), 30 mM Tris and 5 mM NH_4OAc, pH 5.0, + 20 kV.

Table 3
Linearity of six pharmaceutical racemates.

	Peak 1 linear equation	R^2	liner range (μg·ml^{-1})	Peak 2 linear equation	R^2	linear range (μg·ml^{-1})
chlorprenaline	y = 263.50 × +341.12	0.9996	10–800	y = 262.26 × +322.34	0.9995	10–800
brompheniramine	y = 242.16 × +218.24	0.9994	10–800	y = 242.78 × +232.58	0.9995	10–800
tropicamide	y = 328.24 × −200.12	0.9995	10–800	y = 329.62 × −189.34	0.9991	10–800
homatropine	y = 459.86 × +336.13	0.9994	10–800	y = 461.42 + 373.57	0.9994	10–800
homatropine methylbromide	y = 363.48 × −143.95	0.9991	10–800	y = 364.22 × −122.63	0.9995	10–800
clorprenaline	y = 705.72 × +230.18	0.9990	10–800	y = 708.58 × +263.87	0.9996	10–800

Key to abbreviations: R^2 correlation coefficients.

Table 4
Reproducibilities of retention time, resolution value and efficiency for homatropine on the poly(GMA-β-CD-*co*-EDMA) monolithic capillary columns.

	Retention time, min (% RSD)		Rs	Efficiency, plates/m (% RSD)	
	Peak 1	Peak 2	(% RSD)	Peak 1	Peak2
Run-to-run (n = 6)	26.160 (2.3)	28.110 (3.0)	4.71 (3.1)	47,634 (3.6)	42,876 (3.2)
day-to-day (n = 3)	26.114 (3.5)	28.601 (3.5)	4.63 (3.9)	46,981 (3.4)	43,543 (3.8)
column-to-column (n = 3)	25.598 (4.1)	28.235 (4.3)	4.61 (4.4)	47,134 (7.2)	43,758 (7.8)

Separation conditions: ACN/H_2O (50:50), 30 mM Tris and 5 mM NH_4OAc, pH 5.0, + 20 kV.
Key to abbreviations: Rs the resolution value; RSD relative standard deviation.

with racemic homatropine as tested analyte. The number of replicates of run-to-run and day-to-day experiments was six and eighteenth, respectively. As shown in Table 4, the RSD of run-to-run for retention time of each enantiomer was 2.3% and 3.0%, respectively. The RSD of run-to-run for resolution was 3.1%. As for the day-to-day repeatability, the result was good with RSD of 3.5% and 3.9% for retention time and resolution, respectively. The reproducibility of the three freshly prepared chiral columns from three different polymerization mixture was also investigated with homatropine as model analyte. The RSD of the retention time and resolution were all lower than 4.4%, indicating good column-to-column reproducibility of three chiral column. Moreover, there was no significant change in the separation efficiency for at least 100 injections. These results indicated that the stability and repeatability of the monolithic columns were satisfactory.

4. Conclusion

In this paper, a novel one-pot strategy was successfully developed for the preparation of the β-CD functionalized monolithic capillary column by directly adding native β-CD into the polymerization mixture followed by in situ polymerization. Compared to other strategies, this method is more rapid, simple and low-cost. After the optimization of preparation conditions, the β-CD modified monolithic column afforded good enantioselectivity for the studied six racemic drugs in CEC. Meanwhile, each enantiomer exhibited good linear between its concentration and peak area in the range of 10–800 μg ml^{-1}. Stability and repeatability for enantioseparation were proved to be satisfactory. The electrochromatographic retention of analytes on the proposed column was found to be based on a mix mode of hydrophobic interaction and electrostatic interaction.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.07.041.

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