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Evaluation of newly synthesized derivative of cyclodextrin for the capillary electrophoretic separation

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Abstract

A highly water-soluble new cyclodextrin (CD) derivative 2-*O*-acetonyl-2-*O*-hydroxypropyl-β-CD (2-AHP-β-CD) was synthesized and tested as an effective chiral selector for the capillary zone electrophoretic resolution (Rs) of several basic and acidic analytes. The primary purpose of the research was to explore the capability of the 2-AHP-β-CD as chiral selectors on comparison with the neutral CDs such as β-CD, DM-β-CD and HP-β-CD. Substitution with 2-*O*-acetonyl-2-*O*-hydroxypropyl group at the secondary hydroxyl sites of the CD is aimed at influencing the magnitude and selectivity of analyte–CD interactions. The chiral resolution was strongly influenced by the concentration of the CDs and buffer pH. 2-AHP-β-CD showed the best enantiomer resolution properties among the tested compounds, while the other CDs showed inferior or no performances at all.

Keywords: Capillary electrophoresis; Enantiomer separation; Cyclodextrin; β-Cyclodextrin derivative; Chiral drugs

1. Introduction

Capillary electrophoresis (CE) has been increasingly selected as the technique of choice for enantiomeric separations for its high efficiencies and short analysis time [1–3]. Due to the capillary dimensions required for CE operation, only a very small quantity of the chiral selectors is needed, thereby reducing the operation costs. Another advantage of using CE for enantiomeric separations is that different selectivity can be obtained by simply changing the type of chiral selectors added to the running buffers without changing capillary, therefore allowing an automated approach to method development.

The most commonly used chiral selectors in CE are cyclodextrins (CD) and various derivatives [4–12]. The use of CDs incorporated in gels was reported by Guttman et al. [13], while Fannli [14] published the first paper dealing with the application of CDs in CZE and investigating the chiral separation of several sympathomimetic drugs.

CDs are derived for several reasons: for example, to vary solubility, change complex properties, and introduce certain functional groups [15,16]. Derivation of CDs in CE is mainly targeted to influence complex properties, introduce a charged group, or increase solubility in the CE running buffer. Both charged and neutral CD derivatives have been synthesized and employed for electrophoretic separations of a variety of analytes [17–19].

New derivatives are being synthesized and their properties investigated, because of the importance of these compounds in several fields of application.

In this work, a highly water-soluble 2-*O*-acetonyl-2-*O*-hydroxypropyl-β-CD (2-AHP-β-CD) was synthesized. The enantioresolution properties of newly synthesized 2-AHP-β-CD had been investigated and compared with those of both native and derived CDs commercially available.

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2. Experimental

2.1. Materials

Sodium hydroxide, Tris(hydroxymethylaminomethane), phosphoric acid, morpholinoethanesulfonic acid (MES) were of analytical reagent grade. Commercially available β-CD was recrystallized from water and dried in vacuum (2 mmHg) at 100 °C for 4 h. Pyridine and *N*,*N*-dimethylformamide (DMF) were dried over, and redistilled from CaH_2. Chloroform ($CHCl_3$) was dried over $CaCl_2$. Chlorodimethylethylsilane, trifluoride–etherate ($BF_3{\cdot}Et_2O$) and other reagents were of A.R. grade and used without further purification. Double distilled water was used for the preparation of all solutions. Filters of 0.25 μm pore size were used to filter all the solutions.

Recamic cyclandelate, benzhexol, salbutamol, mexiletine, naproxen, ibuprofen, lobeline, ofloxacin, propafenone, bupivacaine, verapamil, ketamine, properanolol, isoprenaline, metoprolol, pemoline, econazole were purchased from Jinan drug store. Recemic adrenaline, warfarin, ketocanazole, ketoprofen, flurbiprofen were from Sigma (St. Louis, MO, USA). The structure of the analytes are showed in Fig. 1

β-CD was obtained from Yunan Cyclodextrin Factory (Guangdong province, China). It was crystallized before use. Heptakis-2,6-di-*O*-methyl-β-CD (DM-β-CD) was synthesized according to the literature[20]. The average degree of substitution (DS) refers to the average number of substituting groups on the whole CD molecule. DM-β-CD (DS = 1.8) and 2-*O*-(2-hydroxypropyl)-β-CD (HP-β-CD) (DS = 4.0) were synthesized according to the literature [21]. 2-AHP-β-CD was synthesized in our laboratory. The new CD derivative was actually a mixture of derivatives with different numbers of actonyl and hydroxypropyl groups attached. The CD derivatives often have better aqueous solubility than β-CD. This can be explained by the "impure" nature of the CD derivatives. As they are mixtures of compounds with different degrees and patterns of substitution, the overall solubility can be better than that of the pure derivatives. Double distilled water was used for the preparation of all solutions. Filters of 0.25 μm pore size were used to filter all the solutions.

2.1.1. Background electrolyte (BGE)

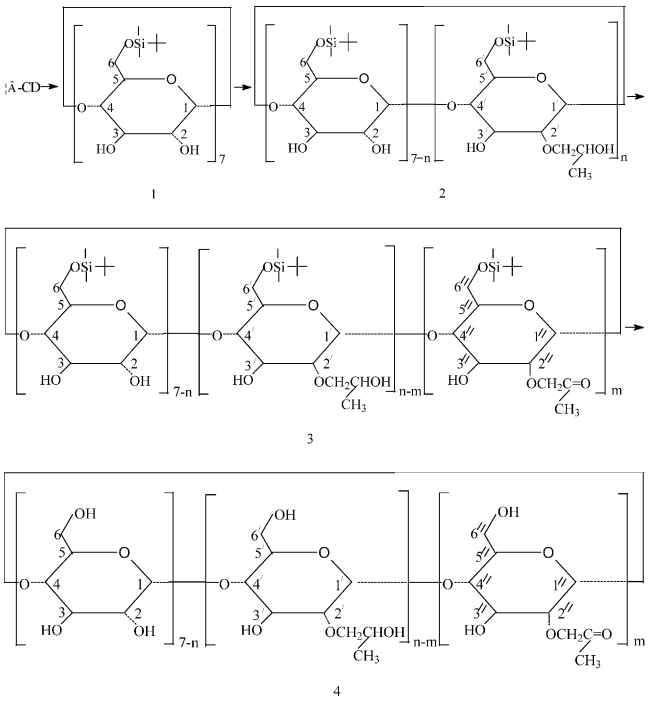
A stock solution 50 mmol/l Tris titrated with phosphoric acid to pH 2.5, 3.0, 3.5, 4.0, 4.5 were prepared and used for electrophoretic experiments in aqueous solution for basic analytes. The CDs were added separately to the stock solution.

A BGE consisting of 0.12 mol/l MES titrated with hydroxide to pH 4.0, 5.0, 6.0, 7.0 were prepared and used for electrophoretic experiments in aqueous solution for acid analytes. The CDs were added separately to the BGE.

2.2. Apparatus

The experiments were carried out with a homemade CE apparatus, equipped with a multiwavelength UV detector. The UV signals were recorded at 214 nm. A fused-silica capillary with a length at 45 cm (effective length 35 cm) and an internal diameter of 75 μm i.d. (Hebei Yongnian Optical Fiber Factory, China) was used as a separation tube. An AM-400 NMR spectrometer (Bucker, Germany) was used. Electrokinetic injection of the sample was done and the applied voltage was 20.0 kV. The temperature of the experiments was 25 °C.

2.3. Synthesis of 2-AHP-β-CD



The *n*–*m* and *m* in 4 were the corresponding average substitution degrees of the hydroxypropyls and acetonyl derivatives. The *n* and *m* values can be controlled by the ratio of reactants and be determined by the ^{1}H NMR spectra of the products ($n = 4.8$, $m = 1$).

1 was prepared from β-CD according to the reported method[22,23]. 7.0 equivalent sodium hydride (1.7 g) was added to the solution of 19.3 g and 1 in 400 ml dry DMF at 0 °C and the mixture was stirred for 2 h. Subsequently, 7.0 equivalent epoxy propane (4.1 g) was added dropwise in 1 h at 0 °C, then was warmed at room temperature for about 12 h to form 2. The solvent was removed in vacuum followed by silica gel column chromatography with eluant of $CHCl_3/CH_3COCH_3/n$-$PrOH/H_2O$ (40:15:5:3 by volume) to obtain pure 2 in 90%. δ_H ($CDCl_3$, ppm): 5.95–5.60 (br, O(2)H, O(3)H, O(3′)H), 5.26–4.84 (m, 7 H, H-1′,1), 4.21–3.22 (m, about 65 H, H-2′, 2, 4, 6, 5, 3, 4′, 6′, 5′, 3′, and $-H_2CCH(OH)-$), 1.02 (d, 17.5 H, $-CH(OH)C\underline{H}_3$, $J = 6.0$ Hz), 0.85 (s, 63 H, $(H_3C)_3CSi$), 0.00 (s, 42 H, $(H_3C)_2Si$); δ_C (ppm): 101.9–100.8 (C-1, 1′), 81.7–80.2 (C-4, 4′), 77.2–71.5 ($-H_2\underline{C}CH(OH)-$, C-2′, 3, 2, 5, 3′, 5′), 66.4 ($-\underline{C}H(OH)CH_3$), 61.2 (C-6, 6′), 26.4–3.2 ($-CH(OH)\underline{C}H_3$,

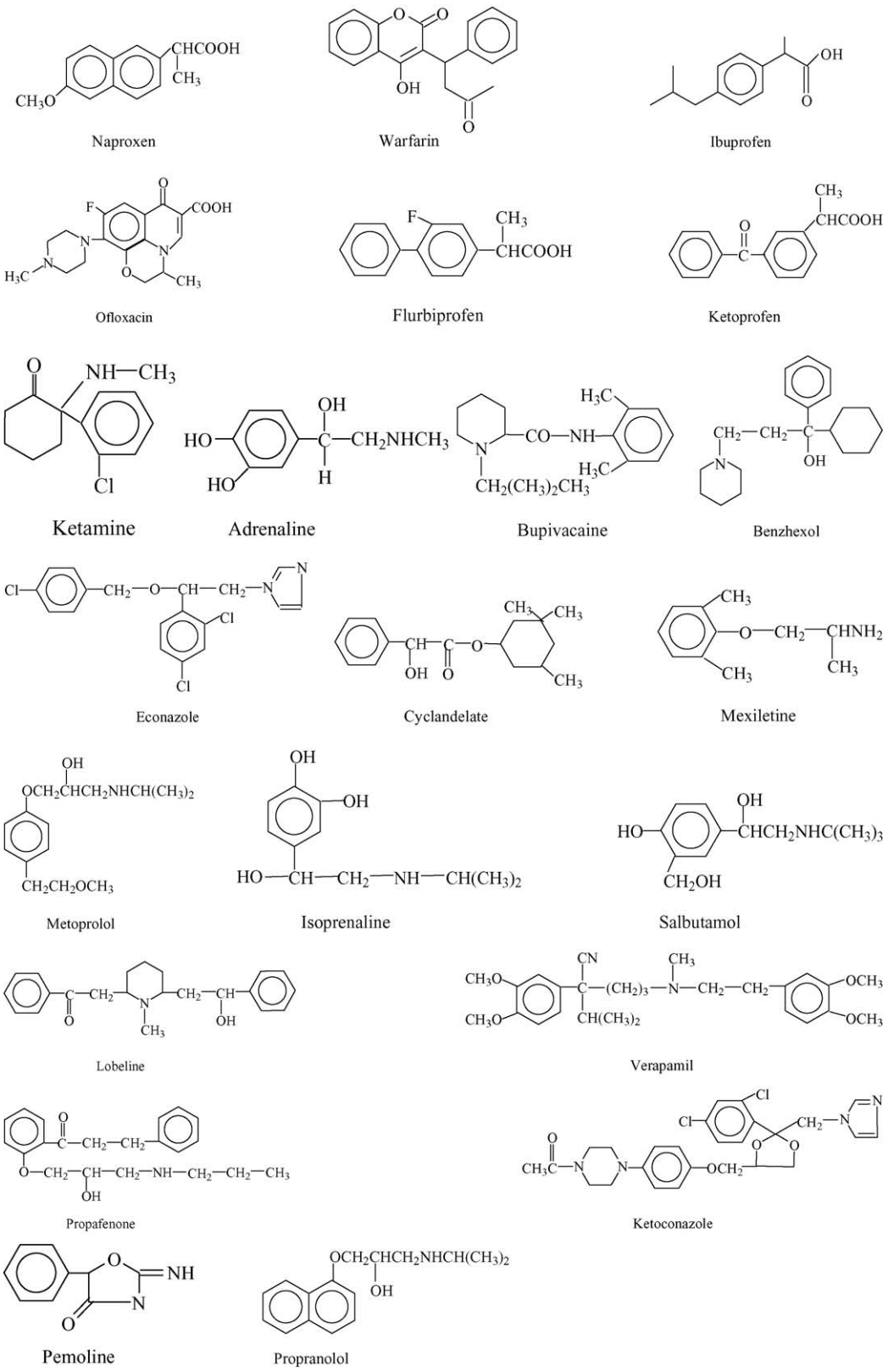


Fig. 1. The structure of the analytes.

$(H_3C)_3C(CH_3)_2Si$–). Anal. found: C, 53.27; H, 8.98 (calc. for $C_{101.4}H_{202.8}O_{40.8}Si_7$: C, 53.48; H, 8.91).

The solution of 2 formed above was directly dispersed in about two parts acetone (based on volume) with a catalytic amount of aluminium isopropoxide (about 1.0 equivalent based on 2), and then refluxed for about 12 h. Most of the solvent was removed in vacuum followed by silica gel column chromatography with eluant of $CHCl_3/CH_3COCH_3$/*n*-PrOH/H_2O (40:15:5:3 by volume) to obtain pure 3 in 80%. δ_H ($CDCl_3$, ppm): 5.96–5.61 (br, O(2)H, O(3)H, O(3″)H, O(3′)H), 5.37–4.88 (m, 7 H, H-1″, 1′,1), 4.67 (s, 2H, –OCH_2CO–), 4.22–3.20 (m, about 60 H, H-2″, 2′, 2, 4, 6, 5, 3, 4″, 4′, 6″, 6′, 5″, 5′, 3″, 3′, and –$H_2CCH(OH)$–), 2.11 (s, 3 H, –$COCH_3$), 1.12–0.95 (d, 14.5 H, –$CH(OH)C\underline{H}_3$, J = 6.1 Hz), 0.85 (s, 63 H, $(H_3C)_3CSi$), 0.00 (s, 42 H, $(H_3C)_2Si$); δ_C (ppm): 219.6 (–CO–), 101.9–100.8 (C-1, 1″, 1′), 81.7–80.2 (C-4, 4″, 4′), 78.7–71.4 ($O\underline{C}H_2CO$–, C-2″, 2′, 3, 2, 5, 3″, 3′, 5″, 5′), 66.4 (–$\underline{C}H(OH)CH_3$), 61.2 (C-6, 6″, 6′), 26.4 to −3.2 (–$CO\underline{C}H_3$, –$CH(OH)\underline{C}H_3$, $(H_3C)_3C(CH_3)_2Si$–). Anal. found: C, 53.34; H, 8.96 (calc. for $C_{101.4}H_{200.8}O_{40.8}Si_7$: C, 53.52; H, 8.83).

Compound 3 (14.5 g) was deprotected by overnight treatment with 14 equivalent $BF_3{\cdot}Et_2O$ in 500 ml $CHCl_3$ at room temperature, followed by ice-water bath. The mixture was concentrated in vacuum, followed by silica gel column chromatography with eluant of $CH_3COOCH_2CH_3$/*n*-PrOH/H_2O (2:3:2 by volume) to give 86% yield of 4. δ_H (D_2O, ppm): 5.36–4.85 (m, 7 H, H-1″, 1′,1), 4.70 (s, 2 H, –OCH_2CO–), 4.25–3.20 (m, about 56 H, H-2″, 2′, 2, 4, 6, 5, 3, 4″, 4′, 6″, 6′, 5″, 5′, 3″, 3′, and –$\underline{H}_2CC\underline{H}(OH)$–), 2.12 (s, 3 H, –$COCH_3$), 1.12–0.95 (d, 14.5 H, –$CH(OH)C\underline{H}_3$, J = 6.0 Hz); δ_C (ppm): 220.6 (–CO–), 101.8–100.8 (C-1, 1″, 1′), 82.6–80.1 (C-4, 4″, 4′), 78.8–71.2 ($O\underline{C}H_2CO$–, C-2″, 2′, 3, 2, 5, 3″, 3′, 5″, 5′), 65.9 (–$\underline{C}H(OH)CH_3$), 61.1 (C-6, 6″, 6′), 24.7 (–$CO\underline{C}H_3$), 20.6 (–$CH(OH)\underline{C}H_3$). Anal. found: C, 47.21; H, 7.22 (calc. for $C_{59.4}H_{102.8}O_{40.8}{\cdot}2H_2O$: C, 47.44; H, 7.11).

2.4. Capillary electrophoresis procedure and resolution calculation

A new capillary was conditioned by flushing successively with 1.0 mol/l NaOH (60 min), 0.1 mol/l NaOH (30 min) and then equilibrated with double distilled water and running buffer each for 30 min before use. Between each injection, the capillary was rinsed with 0.1 mol/l NaOH (2 min), double distilled water (2 min) and with the respective running buffer (2 min).

Resolution (Rs) for a pair of enantiomers was calculated using the following equation:

$$\mathrm{Rs} = \frac{2(t_2 - t_1)}{w_2 + w_1}$$

where t_1 and t_2 are the migration times of the enantiomers measured in minutes; and w_1 and w_2 are the peak widths at the baseline of each enantiomer designated as '1' and '2', and also measured in minutes.

3. Results and discussion

3.1. Chiral separation of acidic compounds

Six chiral drugs such as ibuprofen, ketoprofen, flurbiprofen, naproxen, warfarin and ofloxacin were studied using 2-AHP-β-CD as chiral selector. Flurbiprofen, ibuprofen and ketoprofen were successfully resolved using TM-β-CD [24].

The main parameters affecting chiral separations with CD-based electrolytes are expected to be the nature and concentration of the CD and the buffer pH.

3.1.1. Effect of the pH of the BGE on the resolution (Rs)

The pH change influences the charge of the analytes and the electrostatic interactions with CDs. The effect of pH on the chiral separation of the six acidic compounds was examined at pH 4.0, 5.0, 6.0, 7.0. In order to verify the effect of pH on the resolution of the enantiomers, the same amount of 2-AHP-β-CD (1.48 g/100 ml buffer) was added to the BGE at pH 4, 5, 6 and 7. Fig. 2 showed the effect of pH on the chiral resolution of the six analytes. From Fig. 2, we can see that resolution decrease at low pH value (pH 4) and high pH value (pH 7). From the experiment, we found that the migration time decreased with increasing pH. The reason may be that as pH increased, the carboxydic group of the acidic analytes became more dissociated [24], the stereoselective hydrogen

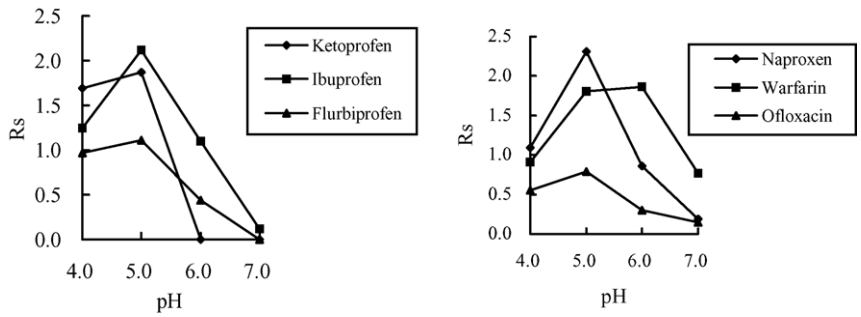


Fig. 2. Effect of pH on the resolution of acidic compounds. Separating conditions: 0.12 mol/l MES; the concentration of 2-AHP-β-CD is 1.48 g/100 ml buffer; applied voltage: 20 kV; injection: 15 kV/5 s; detection wavelength: 214 nm.

bonds between this group and the substituting group on the CDs rim may be influenced, so the interaction time of CD with the analytes may also be influenced. The resolution of warfarin was almost the same at pH 5 (Rs = 1.81) and pH 6 (Rs = 1.86), all the other analytes were best separated at pH 5.0. So the pH 5.0 was chose to be the optimum pH for the separation of the six acidic analytes.

The value $pK_a + 0.5$ is proposed to be the suitable pH of the background electrolyte for the separation of chiral compounds containing a carboxylic group [25], which is supported by our experimental results. According to literature [25], the pK_a values for ibuprofen, warfarin, naproxen and ketoprofen are 4.40, 4.80, 4.20 and 5.02 respectively. So the suitable pH for ibuprofen, warfarin, naproxen and ketoprofen should be around 5.0.

3.1.2. Influence of the type of CD and its concentration on resolution

The concentration of chiral selector is an important parameter in chiral separation by CE. At extremely low concentration, the amount of chiral selector is not sufficient to form complexes with enantiomers and therefore, the enantiomers cannot be separated. On the other hand, when the concentration of chiral selector is extremely high, both enantiomers will be nearly completely complex. Because the two enantiomer–chiral selector complexes have very similar mobility, the enantiomers cannot be separated either in this case.

According to Wren and Rowe [26], the apparent mobility differences required for the chiral resolution of enantiomers is given as follows:

$$\Delta u = \frac{C(\mu_f - \mu_c)(K_1 - K_2)}{1 + C(K_1 - K_2) + K_1 K_2 C^2}$$

where C is the concentration of the chiral selector, μ_f the electrophoretic mobility of the free analyte, μ_c the electrophoretic mobility of the analyte–selector complex, and K_1, K_2 are the binding constants. The optimum concentration can be calculated as

$$C_{opt} = \frac{1}{\sqrt{K_1 K_2}}$$

From this equation, we can see that the stronger the binding of the solute–CD complex, the lower the CD concentration is needed to achieve optimal resolution.

In order to verify the influence of the CD type on the resolution of the six acidic compounds, various CD was added to the BGE. When β-CD and HP-β-CD were added to a BGE containing variable CD concentrations at pH 5.0. None of the six compounds was separated. When DM-β-CD was added to the BGE containing (10, 20, 30, 40 and 50 mmol/l) DM-β-CD, only flurbiprofen (Rs = 0.38) and ibuprofen (Rs = 0.86) were partly separated at 40 mmol/l DM-β-CD. When 2-AHP-β-CD was used at 0.49, 0.98, 1.48, 1.97 g/100 ml buffer, besides ofloxacin (Rs = 0.79), all the other five compounds were separated. Table 1 give the results obtained for the six analytes studied. From Table 1 we could see that increasing the concentration of 2-AHP-β-CD caused a general increase in the migration times for all the six compounds. This may be that when the concentration of 2-AHP-β-CD increased, the viscosity of the solution increased, and the complexing of CD with the analytes also increased. Besides ofloxacin, all the other compounds were separated. Separations of ibuprofen, flurbiprofen and ketoprofen at different concentration of 2-AHP-β-CD were showed in Fig. 3.

Compared with β-CD, HP-β-CD and DM-β-CD, 2-AHP-β-CD is a good chiral selector for the separation of the six acidic compounds.

3.2. Chiral separation of basic compounds

3.2.1. Selection of buffer pH

Buffer pH is an important parameter in the CE chiral separation. It is known that basic enantiomers exist as positively charged ions in acidic condition. At this condition, basic drugs and their complexes will migrate towards the cathode. At low pH, the surface charge of the capillary and adsorption of cationic analytes to the bare fused-silica surface can be reduced. At the same time, the EOF will be much less than that at high pH, thus providing analytes with a longer time for interaction with CDs as they migrated through the capillary. We can see that low pH seems to be more favorable for separation of basic drugs. Moreover, the positively charged $Tris^+$ interacted with the silica surface to reduce the adsorption of the basic drug, and enhance recognition of CDs.

Table 1
Effect of the concentration of 2-AHP-β-CD on the enantiomeric resolution of six acidic compounds

The six acidic compounds	2-AHP-β-CD (g/100 ml buffer)							
	0.49		0.98		1.48		1.97	
	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs
Ibuprofen	15.92/16.12	0.64	16.72/17.07	0.91	17.70/18.68	2.12	18.73/19.57	1.00
Ketoprofen	9.57/9.72	0.58	10.38/10.72	1.01	11.35/12.09	1.87	12.40/13.11	1.09
Flurbiprofen	13.65/13.90	0.83	14.43/14.72	0.92	15.44/15.80	1.11	16.32/16.64	1.02
Naproxen	13.58/13.88	1.17	14.39/14.95	1.96	15.52/16.50	2.31	17.01/18.03	2.31
Warfarin	17.74/18.07	1.05	20.01/20.53	2.01	22.83/23.63	1.81	25.07/25.83	1.22
Ofloxacin	15.23/15.35	0.26	16.20/16.37	0.49	17.25/17.50	0.79	18.37/18.55	0.55

Electrophoretic conditions: 0.12 mol/l MES, pH 5.0; applied voltage: 20 kV; injection: 15 kV/5 s; detection wavelength: 214 nm.

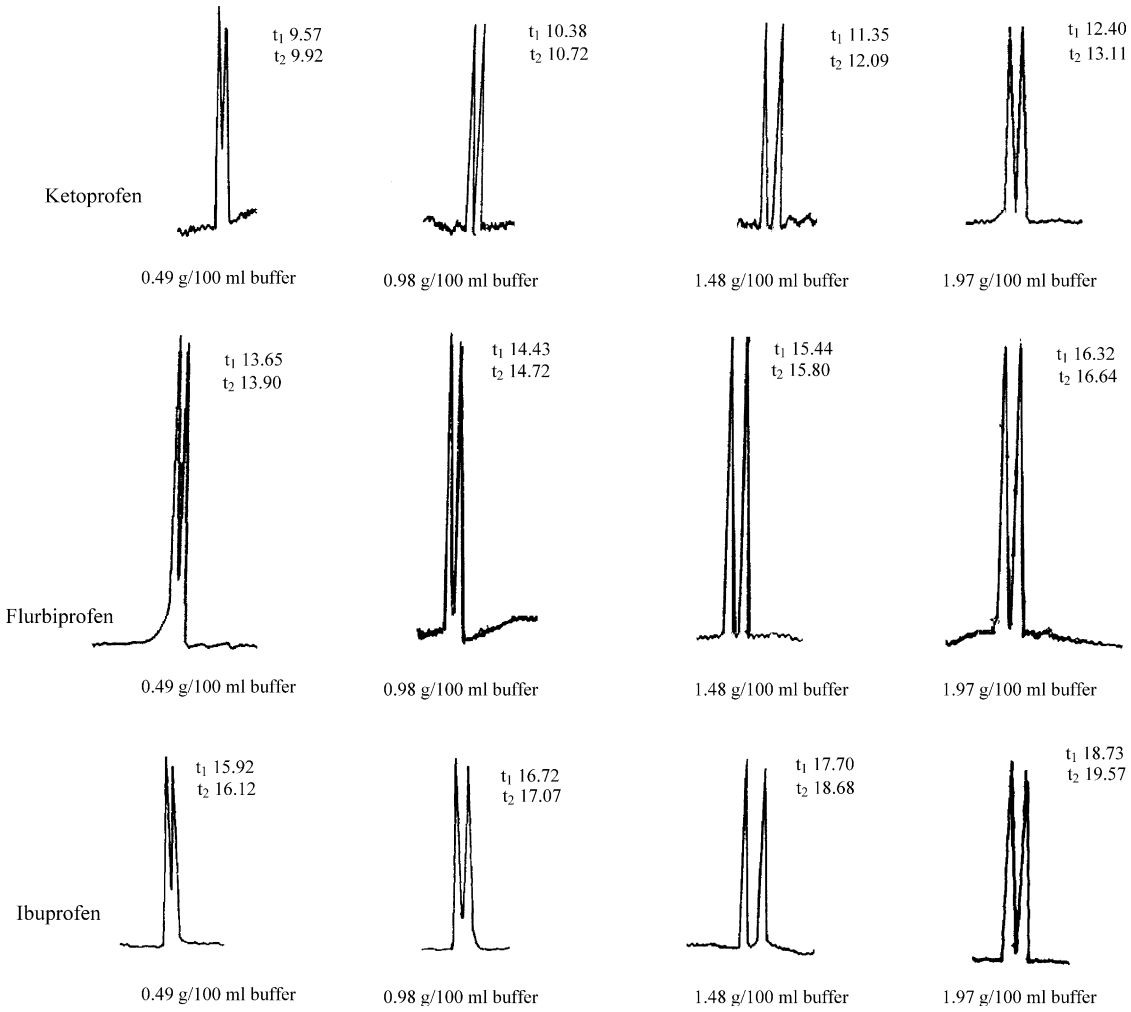


Fig. 3. Enantionmeric separation of ibuprofen, flurbiprofen and ketoprofen at different concentration of 2-AHP-β-CD. Electrophoretic conditions: 0.12 mol/l MES, pH 5.0; applied voltage: 20 kV; injection: 15 kV/5 s; detection wavelength: 214 nm.

Moreover, at pH 2.5, which is near the pK_{a1} value of H_3PO_4 (2.1), the buffer has a high buffer capacity to resist the pH changes caused by electrolysis effect in the CZE process. Fig. 4 shows the effect of pH on resolution of propranolol. It can be seen that the optimized resolution was obtained at pH 2.5.

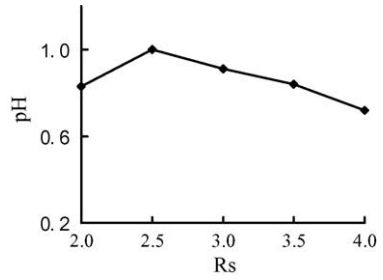


Fig. 4. Effect of pH on the resolution of propranolol. Separating conditions: 50 mmol/l Tris–H_3PO_4; the concentration of 2-AHP-β-CD is 1.85 g/100 ml buffer; applied voltage: 20 kV; injection: 15 kV/5 s; detection wavelength: 214 nm.

3.2.2. Effect of CD type and concentration on the resolution of basic compound

The β-CD was used as chiral selector for the separation of the 16 basic compounds. As can be seen from Table 2 that only ketamine (Rs = 1.13) is separated and ketoconazole (Rs = 0.26) is partly separated by β-CD. The migration times were faster than with any other CD derivatives.

The DM-β-CD has been extensively used in CE chiral separations, often demonstrating enhanced resolutions over native β-CD. Among the 16 analytes studied, five analytes were separated and two analytes were partly separated. The probable explanation is that methylation of the hydroxy group of β-CD enlarged the rim of CD, and makes the hydrophobic cavity flexible, so the conformation of DM-β-CD is better than β-CD (Table 3).

The HP-β-CD is another widely used CD derivative. Table 4 showed the compounds resolved or partly resolved among the 16 compounds. From Table 4, we can see that five compounds were separated and one analyte was partly separated. The presence of the hydroxypropyl chain makes the

Table 2
Effect of β-CD concentration on Rs

Chiral compounds	Concentration of β-CD (mmol/l)					
	5		10		15	
	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs
Ketoconazole	8.54/8.54	0	8.79/8.83	0.26	9.09/9.15	0.26
Ketamine	5.53/5.61	0.79	6.10/6.26	1.05	6.51/1.68	1.13

Electrophoretic conditions: 50 mmol/l Tris–H_3PO_4, pH 2.5; applied voltage: 20 kV; injection: 15 kV/5 s; detection wavelength: 214 nm.

Table 3
Effect of DM-β-CD concentration on Rs

Chiral compounds	Concentration of DM-β-CD (mmol/l)							
	10		20		30		40	
	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs
Isoprenaline	6.64/6.94	0.95	6.93/7.32	1.14	7.26/7.68	1.19	7.73/8.22	1.83
Metoprolol	5.88/6.08	0.65	6.23/6.40	0.78	6.61/7.01	0.95	7.01/7.26	0.90
Salbutamol	6.01/6.22	0.77	6.36/6.60	1.00	6.66/6.89	0.91	6.96/7.21	0.89
Ketamine	6.19/6.42	0.83	6.50/6.87	1.12	6.81/7.20	1.12	7.14/7.45	0.94
Mexiletine	9.43/9.50	0.15	9.82/9.96	0.34	10.34/11.03	1.05	10.69/11.14	0.97
Lobeline	8.86/9.04	0.67	9.24/9.52	1.01	9.67/10.54	2.12	10.13/10.59	1.87
Bupivacaine	9.10/9.21	0.58	9.51/9.71	0.69	10.02/10.24	0.70	10.58/10.87	0.86

The electrophoretic conditions are the same as Table 2.

Table 4
Effect of the concentration of HP-β-CD concentration on Rs

Chiral compounds	Concentration of HP-β-CD (mmol/l)							
	10		20		40		60	
	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs
Propranolol	7.83/8.08	0.57	8.24/8.61	0.96	8.70/9.11	1.04	9.20/9.62	1.04
Isoprenaline	5.20/5.31	0.74	5.47/5.63	1.00	5.81/5.99	1.11	6.20/6.41	1.17
Metoprolol	5.80/5.97	0.45	6.01/6.21	0.69	6.35/6.61	0.86	6.54/6.79	0.72
Salbutamol	4.71/4.79	0.85	4.98/5.08	1.00	5.50/5.51	1.00	6.02/6.15	0.97
Ketoconazole	8.81/8.99	0.69	9.00/9.22	0.88	9.27/9.57	1.19	9.56/9.92	1.53
Benzhexol	7.47/7.57	0.33	7.72/7.89	0.68	8.01/8.37	1.05	8.36/8.77	1.15

The electrophoretic conditions are the same as Table 2.

Table 5
Effect of the concentration of 2-AHP-β-CD concentration on Rs and migration time

Chiral compounds	Concentration of 2-AHP-β-CD (g/100 ml buffer)							
	0.62		1.23		1.85		2.46	
	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs	t_1/t_2 (min)	Rs
Benzhexol	7.89/8.01	0.41	8.33/8.58	0.69	8.91/9.32	1.09	8.63/8.89	1.00
Lobeline	7.52/7.67	0.67	8.12/8.35	0.79	8.83/9.23	1.09	9.51/10.25	1.75
Salbutamol	5.14/5.23	0.29	5.44/5.67	0.95	5.83/6.10	1.02	6.23/6.49	0.89
Propafenone	6.04/6.34	1.15	6.52/7.02	1.88	7.02/7.69	2.20	7.82/8.43	2.14
Verapamil	7.31/7.41	0.43	7.84/8.00	0.64	8.43/8.66	0.98	8.98/9.18	0.89
Ketamine	5.23/5.33	0.68	5.78/5.94	0.97	6.23/6.44	1.19	6.74/7.00	1.53
Mexiletine	9.01/9.07	0.25	9.12/9.22	0.58	9.31/9.56	2.01	9.75/10.04	0.98
Econazole	8.91/9.06	0.59	9.31/9.56	0.96	10.10/10.36	0.73	10.85/11.05	0.65
Ketocanazole	9.24/9.36	0.35	9.41/9.68	0.55	9.61/9.98	1.01	9.80/10.25	2.11
Adrenaline	11.01/11.26	0.15	11.31/11.52	0.49	11.74/12.11	0.91	12.23/12.79	2.01
Properanolol	7.63/7.78	0.39	8.12/8.42	0.73	8.79/0.17	1.00	9.35/9.76	1.00
Metoprolol	6.09/6.17	0.14	6.38/6.54	0.42	6.73/6.98	0.93	7.11/7.49	1.01
Isoprenaline	4.62/4.69	0.57	4.80/4.89	0.84	5.37/5.44	1.29	6.02/6.13	1.08
Pemoline	7.01/7.46	1.67	7.60/8.14	2.00	8.21/8.73	1.94	8.90/9.41	1.41

The electrophoretic conditions are the same as Table 2.

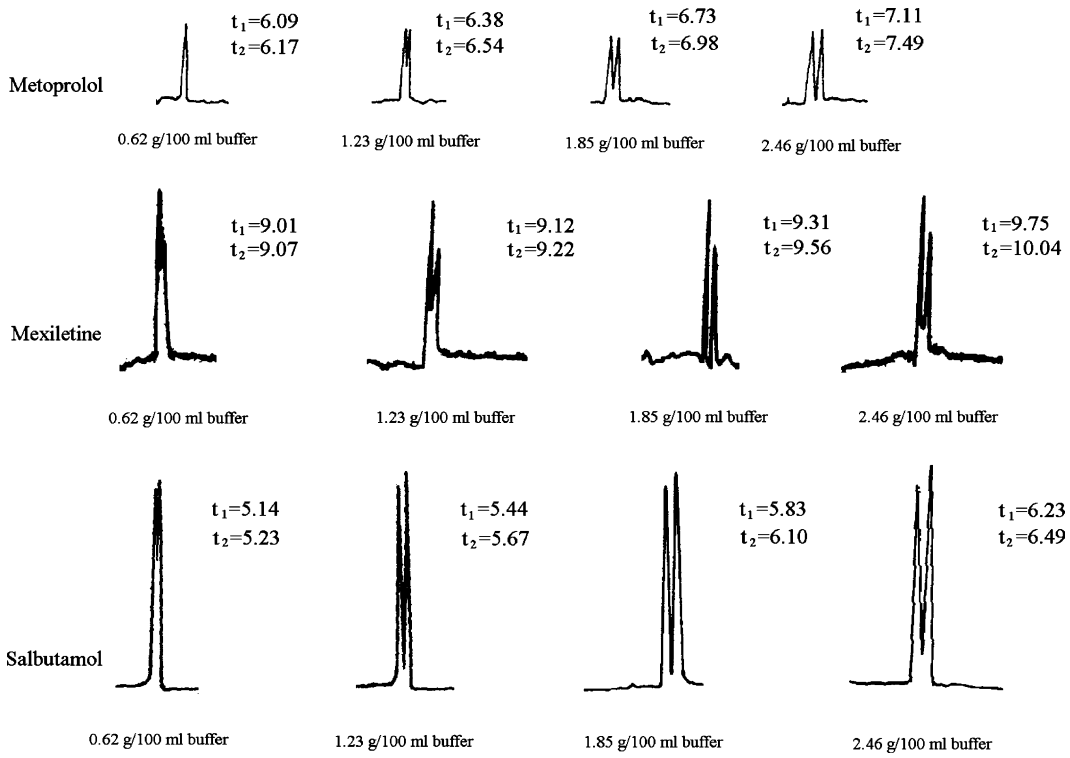


Fig. 5. Enantionmeric separation of metoprolol, salbutamol and mexiletine at different concentration of 2-AHP-β-CD. The electrophoretic conditions are the same as Table 2.

HP-β-CD flexible, accounting perhaps for its better resolution ability than β-CD.

Effect of 2-AHP-β-CD concentration on chiral resolution and migration times of basic compounds were showed in Table 5. We can see that among the 16 basic compounds studied, twelve compounds were separated and two were partly separated. Enantionmeric separation of metoprolol, salbutamol and mexiletine at different concentration of 2-AHP-β-CD were showed in Fig. 5. Compared with β-CD, DM-β-CD and HP-β-CD, more compounds can be separated by 2-AHP-β-CD. It is known that the formation of inclusion complexes between enantiomers and CDs is strongly influenced not only by the hydrophobic interaction in the cavity but also by bonding between the hydroxyl groups (or other substituting groups) on the rim of CDs and substituting groups of the asymmetric center of the analytes. Therefore complex formation is dependent on the CD conformation as well as the character of the guest molecule [27]. Specific interactions between the glucose functional groups on the CD rim and the analytes must take place for chiral separation to occur. For 2-AHP-β-CD, in addition to the interactions between the groups of the analytes and the inner portion of the CD cavity, specific hydrogen bonding transfer interactions between certain group on the analytes chiral center and the rim hydroxyls of the CD may exist. Van der waals interactions, dipole–dipole, and charge interactions can also exist. The existence of all the interactions provide more opportunity for the different interaction between 2-AHP-β-CD and the two enantiomers. So 2-AHP-β-CD possess good enantioselectivity.

4. Conclusions

We have demonstrated that the 2-AHP-β-CD can be used as a chiral selector for enantiomeric separation of some acidic and basic compounds with CE. Chiral resolution in most cases was largely enhanced compared to the commonly used β-CD, DM-β-CD and HP-β-CD. This new system provides a fast and effective way for chiral separation.

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