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Cationic β-cyclodextrin derivative for chiral separations

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Abstract

A novel hepta-substituted β-cyclodextrin bearing the methoxyethylamine group linked to the upper cyclodextrin rim was successfully used as a chiral selector for enantiomeric separation of non-steroidal anti-inflammatory drugs (NSAIDs) and phenoxypropionic acid herbicides (PPAHs). Separation parameters such as pH and concentration were found to have major influences on enantiomeric resolution of the NSAIDs and PPAHs. Results indicate that heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] performs exceptionally well for the enantiomeric resolution of NSAIDs: indoprofen and fenoprofen (R_s=11 and 14, respectively). In addition, baseline enantiomeric separation of a mixture of six pairs of PPAHs was achieved in under 30 min. Compared to other cationic β-cyclodextrins reported in the literature, the β-CD-OMe (VII) showed improved selectivity for both classes of the aforementioned anionic racemates. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral selectors; Cyclodextrins; Non-steroidal anti-inflammatory drugs; Pesticides; Phenoxypropionic acids

1. Introduction

The ability of native and derivatized cyclodextrins (CDs) to form host–guest complexes and to bind stereoselectively, enhances their performance for chiral resolution of enantiomeric species in both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The use of CDs as chiral selectors in CE was pioneered by Fanali [1]. Since that report, there has been a spectacular growth in the use of native (α- [2,3], β- [4–10] and γ- [10–15]) CDs and alkyl modified (hydroxypropyl- [16–20], ethylated- [21] and methylated- [22–26]) CDs in CE. Among the various non-charged derivatized CDs reported to date, β-CD, γ-CD and dimethyl-β-CD are versatile chiral selectors for enantioseparation of a wide range of racemates [1].

The recent widespread applications of charged CDs for chiral separation can be attributed to two important factors. First, neutral racemates that lack electrophoretic mobility, as well as the charged racemates, can be enantioresolved with charged CDs. Secondly, introduction of ionogenic groups on the CD rim, or connected to it via a short alkyl chain, enhances the solubility of charged CDs in aqueous media. Terabe [27] showed the first application of a

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charged β-CD for the resolution of dansylated amino acid enantiomers. Over the last five years, the majority of chiral separations of basic enantiomers has involved the use of anionic (carboxylated [28–30], sulfated [31–38] and phosphated [39]) derivatives of β-CD. However, enantiomeric separation of acidic racemates are not generally feasible with anionic CDs [40], probably due to the strong electrostatic repulsion between a selector–solute pair carrying the same negative charge. Chiral separations with the aid of cationic CDs in CE has been somewhat obscure albeit analytically useful for host–guest complexation. Currently, there are only a few reports concerning the use of cationic CDs as chiral selectors in CE [27,41–51]. Moreover, the commercial availability of cationic CDs is limited. There is only one commercially available cationic CD [52]. In contrast, there are more than 15 anionic CDs commercially available.

It has been suggested that when only one positive charge is present, irrespective of its position near or from the CD rim, the enantioselectivity decreases [45]. The use of polycationic derivatives of CD, not only provide a stable reverse electroosmotic flow (EOF), but also results in a wider separation window. Thus, this provides the possibility of achieving high enantioselectivity for acidic racemates.

In this manuscript, we describe chiral separations by use of the cationic β-cyclodextrin derivative, heptakis(6-methoxyethylamine) herein noted as β-CD-OMe (VII). The 6-methoxyethylamino group provides a derivative which is extensively charged, and is highly soluble in aqueous solution with good buffer capacity. It appears that the positively charged β-CD-OMe (VII) not only covers the inner wall of the bare fused-silica capillary tubes but also counteracts the excess of negatively charged buffer. Thus, a compact layer with a substantial anionic character produces a stable reverse EOF. In order to test the effectiveness of β-CD-OMe (VII) as a chiral selector, we have targeted two classes of anionic chiral racemates. The first class is the aryl propionic acid non-steroidal anti-inflammatory drugs (NSAIDs). The second class of chiral racemates studied is phenoxypropionic acid herbicides (PPAHs).

2. Experimental

2.1. Reagents and chemicals

Analytical grade (GR) sodium phosphate dibasic anhydrous was purchased from EM Science (Gibbstown, NJ, USA). Sodium hydroxide (ACS Reagent) was purchased from J.T. Baker (Phillipsburg, NJ, USA). The racemic mixtures of ketoprofen (Ketop), indoprofen (Indop), ibuprofen (Ibup), flurbiprofen (Flurp), suprofen (Sup), fenoprofen (Fenop) and carprofen (Carp) were purchased from Sigma (St. Louis, MO, USA). The racemic PPAHs including (±)-2-(3- chlorophenoxy)propionic acid (2,3-CPPA), (±)-2-(2-chlorophenoxy)propionic acid (2,2-CPPA), (±)-2-(4- chlorophenoxy)propionic acid (2,4-CPPA), (±)-2-(2,4-dichlorophenoxy)propionic acid 2(2,4-DCPPA), 2(2,4,5-trichlorophenoxy)propionic acid 2(2,4,5-TCPPA) and (±)-2(2-phenoxy)propionic acid (2-PPA) were purchased from Aldrich (Milwaukee, WI, USA). Deionized distilled water (18.3 MΩ/cm) was obtained from a Purelab Polishing System (Lowell, MA, USA). All reagents were used without purification.

2.2. Synthesis of β-CD-OMe (VII)

The 6-perbromination of β-CD **1** resulted in (6-bromo-6-deoxy)-β-CD **2**. The details of the bromination procedure are reported elsewhere [53]. β-CD-OMe (VII) **3** was prepared from **2** by dissolving the latter in methoxyethylamine and heating at 65°C for 48 h. The reagent and solvent were removed under vacuum, and the resulting residue was dissolved in methanol at 55°C. A precipitate was obtained by slow addition of methanolic solution to stirring analytical reagent acetone and collected by gravity filtration. The precipitate was redissolved in water and treated with a basic ion-exchange resin. Lyophilization of the precipitate yielded **3** (60–65%). Schematics of the complete reaction sequence are shown in Fig. 1. Persubstitution was confirmed by elemental analysis and ^{1}H NMR. A doublet was observed at 5.10 ppm for the anomeric hydrogen (H-1) [54].

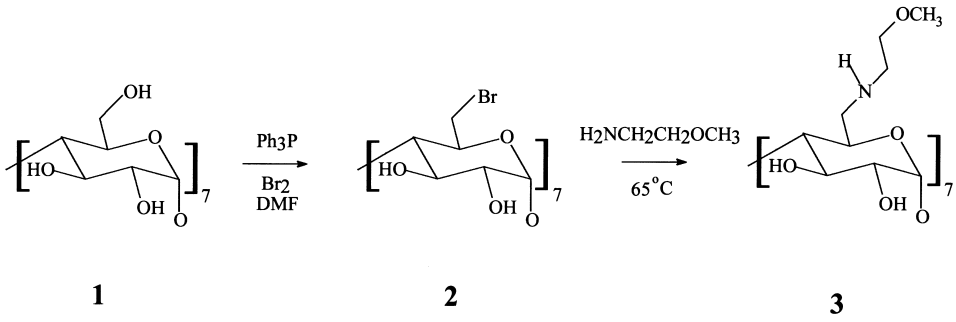

Fig. 1. Schematic synthesis for heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)].

2.3. Preparation of analyte and running buffer solutions

Stock solutions of each analyte were prepared at a concentration of 1.0 mg/ml in a methanol–water (1:1, v/v) solution. A mixture of the analytes was prepared (0.1 mg/ml) in methanol–water (1:1, v/v) from their respective stock solutions. Running buffers used in these separations were prepared by weighing the desired amount of β-CD-OMe (VII) into a volumetric flask which contained 7.5 ml of a 100 m*M* NaH_2PO_4 stock solution as the background electrolyte (BGE). The pH was adjusted with either 1.0 *M* H_3PO_4 or 0.1 *M* NaOH. After adjusting the pH and filling to volume, the solutions were sonicated for 5 min and filtered with a 0.45-μm Nalgene Syringe filter (Rochester, NY, USA).

2.4. Instrumentation

Chiral separations were performed by use of a Beckman System Pace 5510 CE (Fullerton, CA, USA) equipped with: (1) UV lamp operated at 214 nm and (2) System Pace software for system control and data handling. An uncoated fused-silica capillary of 47 cm (40 cm to detector window)×50 μm I.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA). The run voltage ranged from −15 to −30 kV. All separations were conducted at ambient temperature (~23°C).

2.5. Capillary electrophoresis procedure

A new capillary was conditioned by flushing successively with 1.0 *M* NaOH (60 min), 0.1 *M* NaOH (30 min) and triply deionized distilled water (30 min), before use. Between each injection, the capillary was rinsed with 0.1 *M* NaOH (1 min), triply deionized distilled water (1.5 min) and with the respective running buffer (2 min). All samples were introduced into the capillary with an 85 kPa·s pressure injection.

2.6. Calculation

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

$$R_s = 2[(t_2 - t_1)/(w_2 + w_1)]$$

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

3. Results and discussion

As shown in Fig. 1, β-CD-OMe (VII) possesses a methoxyethylamine group at the 6′ position of every D-glucose unit. The presence of the seven secondary amine (methoxyethylamines) groups makes the CD derivative positively charged at the pH values (4–7) used in our CE separations. The presence of positive charges in the aqueous buffer not only enhances the solubility of β-CD-OMe (VII) in aqueous buffer in comparison with the parent compound, but also

enhances its adsorption to the negatively charged silanol groups on the capillary surface. Thus, the direction of the EOF is reversed. Under conventional reversed polarity conditions, the β-CD-OMe (VII) migrates toward the cathode with a mobility of μCD^{+}. Uncomplexed negatively charged analytes under these conditions migrate along with the EOF toward the anode with mobility μA^{-}. Hence, the analytes and the chiral selector have a tendency to migrate in different directions. However, if an anionic analyte is strongly included into the cavity of β-CD-OMe (VII), the complex will have an overall positive charge and will migrate towards the cathode. Thus, it will elute later than the anionic analyte that is weakly complexed with β-CD-OMe (VII). This is because β-CD-OMe (VII) is multiply charged. Eventually, the CD–analyte ($CD–A^{+}$) complex with lower velocity will be pulled by the EOF towards the anode (detector end) in a reverse polarity CE configuration. The Wren and Rowe theory suggests that chiral selectors which carry a charge opposite that of the analyte will give the greatest apparent mobility difference between two enantiomers [55]. Consequently, for the maximum mobility difference between the two enantiomers to be enhanced, the concentration and pH parameters of β-CD-OMe (VII) must be carefully optimized.

3.1. Separation of NSAIDs

The NSAIDs are nonopioid compounds that have anti-inflammatory, analgesic and antipyretic properties [56]. The chemical structures of the NSAIDs studied are shown in Fig. 2. Each NSAID contains a propionic acid group (chiral center) adjacent to a phenyl group. Since the NSAIDs are often used as active ingredients in over-the-counter drugs and have chiral centers, they fall under the mandate of the US Food and Drug Administration (FDA) regulations for stereoisomeric compounds. FDA regulations stipulate that the stereoisomeric composition of a drug with a chiral center should be studied for its biological activities and toxicological effects [57]. The enantiomers of NSAIDs have different properties. In the case of ibuprofen, (the active ingredient in Motrin, Advil and Nuprin), the *S*-(+) enantiomer has the primary therapeutic activity [58]. Thus, an efficient and rapid method for qualitative analysis of stereoisomeric compounds is necessary.

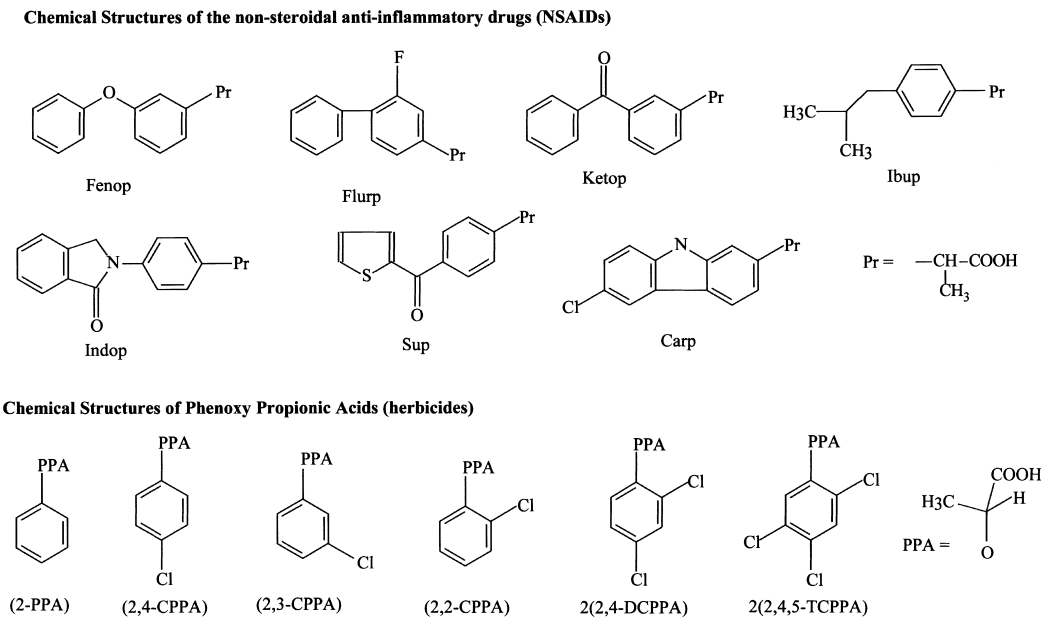

Fig. 2. Chemical structures of the NSAIDs and PPAHs.

3.1.1. Effect of concentration on the migration time

Fig. 3 shows the effect of concentration on the migration times of seven enantiomeric pairs of NSAIDs at pH 5. As expected, with an increase in concentration of β-CD-OMe (VII) from 1 to 5 m*M*, an inverse migration time (decrease in apparent mobility) for all the NSAIDs was observed. Using concentrations ≥3 m*M*, noticeable differences in migration times of the enantiomers were observed. The order of migration was established [3 m*M* β-CD-OMe (VII)] as Ibup>Fenop>Indop>Flurp>Sup>Carp>Ketop. However, with concentrations ≥3 m*M* β-CD-OMe (VII), the second enantiomer of Flurp migrates slower than both enantiomers of Fenop whereas Sup migrates ahead of Indop. This shift in the migration of Fenop and Sup is probably due to a change in net charge with increasing ionic strength. Interestingly, β-CD-OMe (VII) offered no chiral discrimination toward Ketop at pH 5; however, enantiomeric separation of this racemate was achieved under different pH conditions. Furthermore, note that no migration data were recorded for (±)-Ibup due to its strong interactions at ≥4 m*M* β-CD-OMe (VII).

The mechanism of complexation between the cationic β-CD-OMe (VII) and the anionic NSAIDs was attributed to a combination of electrostatic, hydrogen bonding and/or sterically favored interactions. In general, hydrogen bonding or ion pair interactions between the methoxyethylamine group substituted at position 6 of the CD molecule versus the anionic or protonated carboxylate group of the analyte seems to be operative. However, steric interactions appear to dictate the chiral recognition ability of NSAIDs with β-CD-OMe (VII). Evidence for a sterically favored mechanism for complexation was demonstrated with Ketop. At pH 5, no chiral discrimination of Ketop was observed. The mechanism in this case could be attributed to either a weak interaction with β-CD-OMe (VII) or an orientation of the phenyl propionic acid group which causes steric hindrance of the analyte. In the case of the NSAIDs, as the pH increases, the negative charge on the analytes increases. The result is a stronger electrostatic interaction with β-CD-OMe (VII). Thus, for electrostatic interaction to prevail as a participant in the separation mechanism, Ketop (pK_a=4.0 [59]) should have been resolved at pH 5. However, since Ketop was not resolved, we postulate that the separation mechanism of the enantiomeric separation of NSAIDs must be attributed to orientation of the phenyl propionic moiety. This mechanism is supported by the enantiomeric separation of Sup which is structurally similar to Ketop and can be enantiomerically separated at pH 5.0. The phenyl propionic acid moiety of Sup is in the *para* position, whereas it is in the *meta* position for Ketop. Sup can orient favorably within the CD cavity. Similar observations were reported by Fanali and Aturki [26] and it has been suggested that using tri-*O*-methyl-β-cyclodextrins, *para*-substituted aromatic rings demonstrated better fit into the CD cavity than that of *meta*-substituted aromatic rings.

3.1.2. Effect of pH on the resolution

The effect of pH on the migration time is more complicated with β-CD-OMe (VII) than with neutral or other derivatized β-CDs. This can be attributed to two major reasons. First, β-CD-OMe (VII) has several protonated amine groups (DS=7) with various pK_a values [53,54]. Secondly, the NSAIDs possess ionizable carboxylate groups. Therefore, pH not only affects the EOF but also the charge and the chiral recognition properties of both the analyte and chiral additives. Fig. 4 shows the effect of pH on the enantiomeric separation of NSAIDs using 3 m*M* β-CD-OMe (VII). The general trend of an initial decrease in migration time from pH 4 to 5, followed by an increase in migration time from pH 5 to 7 was observed. The initial decrease in migration time of NSAIDs was attributed to an increased dissociation of the carboxylic acid groups on the analytes as well as an increase in EOF (see Fig. 4 inset). The pK_a values of the NSAIDs range from 4–5 [59]. Similar trends were observed for Carp, Indop, Sup and Ibup (plots not shown). At higher pH values, the ionic strength of the buffer increases, resulting in a thinner double layer and lower zeta potential. Thus, a corresponding decrease in EOF must occur.

Fig. 5 shows the electropherogram of a mixture of three NSAIDs (Ketop, Fenop and Flurp). The optimum experimental conditions were determined to be pH 6 and a concentration of 3 m*M* β-CD-OMe (VII) due to the combined requirement of baseline resolution and sufficiently low migration times. The

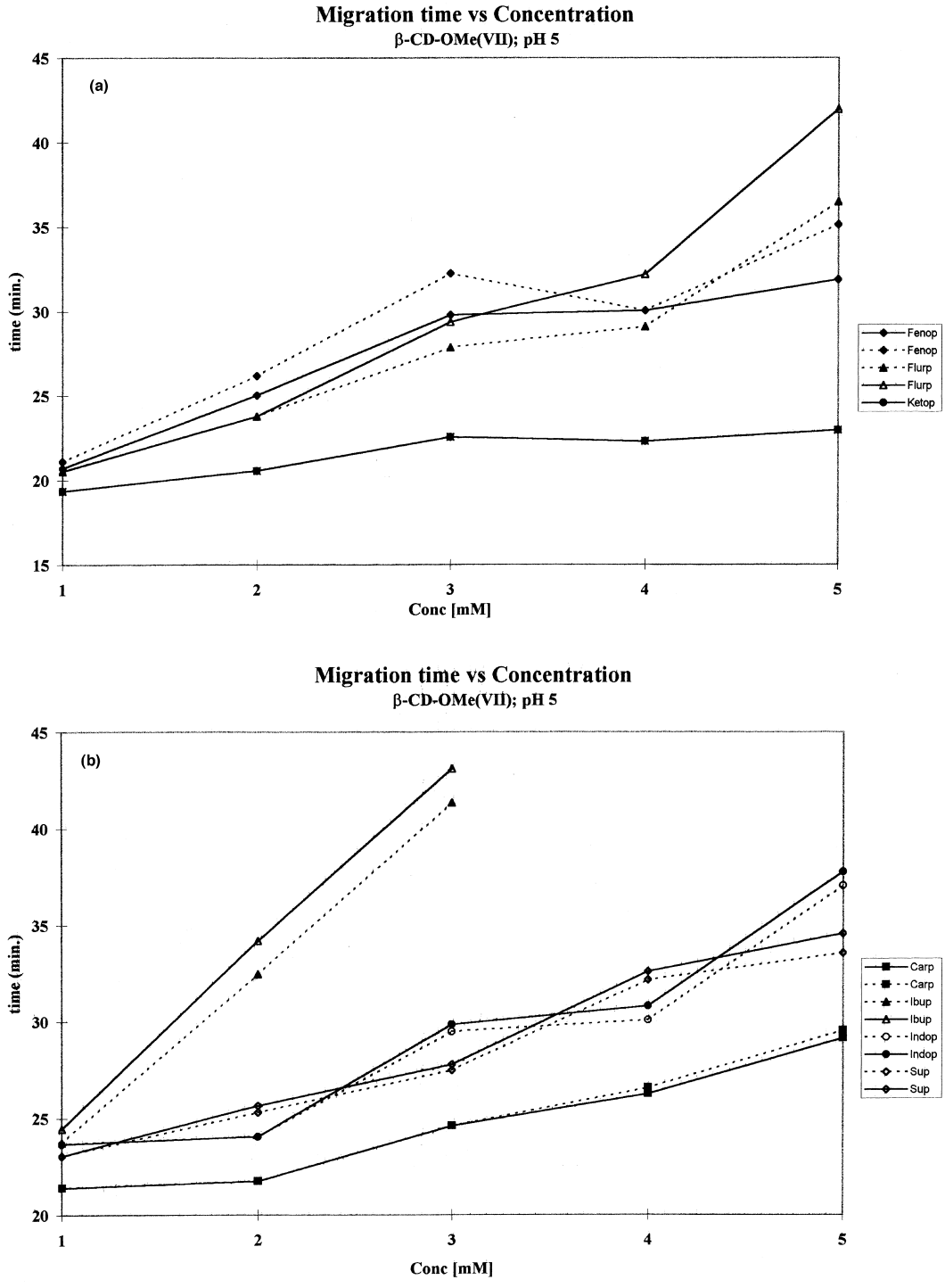

Fig. 3. (a,b) Effect of β-CD-OMe (VII) concentration on the migration times of the NSAIDs studied. The BGE contains 50 m*M* NaH_2PO_4 adjusted to pH 5. Applied voltage was −15 kV, −22~27 μA depending on the concentration of β-CD-OMe (VII); pressure injection 85 kPa·s; sample concentration 0.1 mg/ml in methanol–water (1:1, v/v).

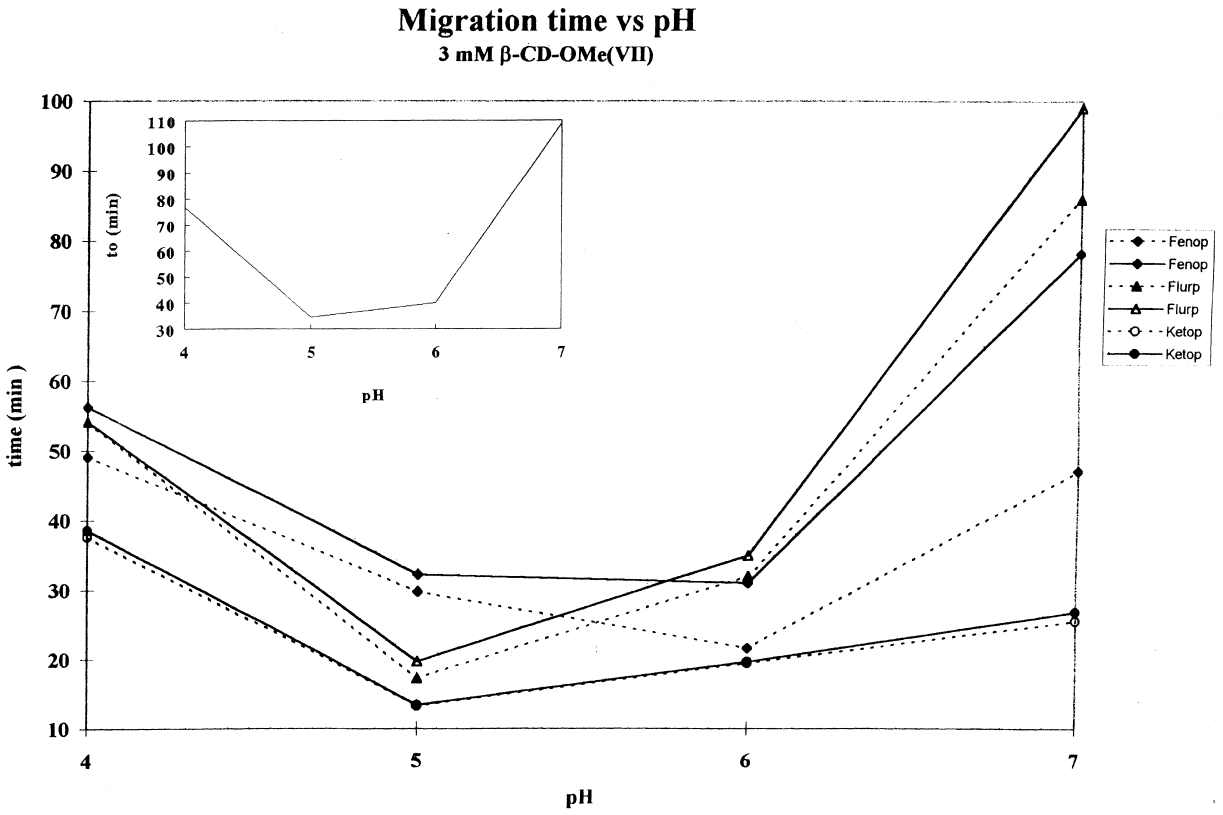

Fig. 4. Effect of β-CD-OMe (VII) pH on the migration times of the NSAIDs studied. Applied voltage was −15 kV, −22–30 μA, Other conditions as in Fig. 3.

individual enantiomers of Ketop, Fenop and Flurp were not available. Thus, the elution order of the enantiomers could not be confirmed. Nevertheless, the migration order of NSAIDs was: Ketop< Fenop<Flurp. Table 1 shows the optimum conditions for resolution of each NSAID racemate. A major advantage of using β-CD-OMe (VII) was the baseline resolution (R_s) obtained for all racemates of NSAIDs. Nielen [25] has investigated the enantioseparation of NSAIDs using various types of modified (heptakis-2,6-di-*O*-methyl-, heptakis-2,3,6-tri-*O*-methyl- and 6^A-methylamino-) β-CD in CZE. He concluded that tri-*O*-methyl-β-CD was the best chiral selector because it allowed chiral resolution of all NSAIDs. Furthermore, among various NSAIDs, (±)-Fenop provided the best possible R_s of 6.0 using 25 m*M* tri-*O*-methyl-β-CD. In our studies, the best resolution factor (R_s=14) was observed for Fenop (Fig. 6b), followed by Indop (R_s=11) (Fig. 6a). The R_s of Flurp (R_s=4.0) and Sup (R_s=2.0) was higher than those reported by Fanali et al. In contrast, the R_s of Ibup and Ketop (R_s of 1.0 and 1.5, respectively) obtained by β-CD-OMe (VII) is lower than those reported by the same research group [26]. Nevertheless, the R_s values obtained for Ibup and Ketop are quite good especially when one considers the low concentration of β-CD-OMe (VII) (3 m*M*) used in this study. Recently, Fillet et al. [60] used a binary cyclodextrin system (e.g. a mixture of 5 m*M* sulfobutyl ether+10–40 m*M* tri- or dimethyl-β-CD) to obtain high resolution values of NSAIDs (4.9–30.6). Our results clearly indicate the excellent chiral R_s ability of β-CD-OMe (VII) for the NSAIDs that is comparable to any other cationic β-CD reported to date in the literature.

3.2. Separation of herbicides (phenoxyalkanoic acids)

The PPAHs are the oldest group of synthetic

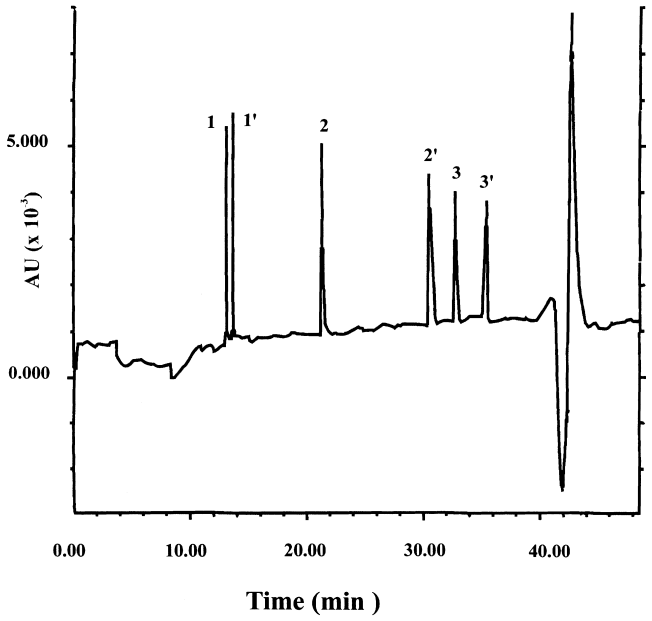

Fig. 5. Enantiomeric separation of a standard mixture of six (±) NSAID enantiomers. The BGE contains 50 m*M* NaH_2PO_4 adjusted to pH 6; 3 m*M* β-CD-OMe (VII); applied voltage was −30 kV, −25 μA; pressure injection 85 kPa·s; sample concentration 0.1 mg/ml in methanol–water (1:1, v/v); (1,1′)=Ketop, (2,2′)=Fenop, (3,3′)=Flurp.

herbicides and are employed in agriculture for the control of weeds in cereal crops [61]. Gas chromatography (GC) is the method of choice for the analysis and detection of achiral PPAHs. However, the disadvantage of using GC for the separation and detection of PPAHs is that (1) they cannot be directly determined by GC at residue levels and (2) they have to be derivatized to be detected [61]. The structures of the PPAHs studied are shown in Fig. 2. PPAHs are composed of mono-, di- and tri-substituted chloro groups in either the *ortho*, *meta* or *para* positions of the phenyl group attached to the chiral propionic acid moiety.

Through a series of concentration and pH studies, it was determined that 20 m*M* β-CD-OMe (VII) and pH 5 were the optimum conditions for achieving enantiomeric separation of a mixture of all six herbicides in a single run (Fig. 7). The migration order of PPAH enantiomers in a mixture of six herbicides was verified by injecting each PPAH

Table 1
Optimum conditions for the separation of NSAIDs using β-CD-OMe (VII)

Analyte	R_s	pH[a]	Separation voltage (kV)	Concentration (m*M*)
Fenop	14	7	−15[b]	5
Flurp	4	7	−30[c]	5
Ketop	1.5	6	−30[c]	3
Carp	3	6	−30[c]	5
Ibup	1	5	−30[c]	3
Indop	11	5	−30[c]	20
Sup	2	5	−30[c]	2

[a] 50 m*M* NaH_2PO_4, injection size=85 kPa·s.
[b] Current~−22 μA.
[c] Current~−32 μA.

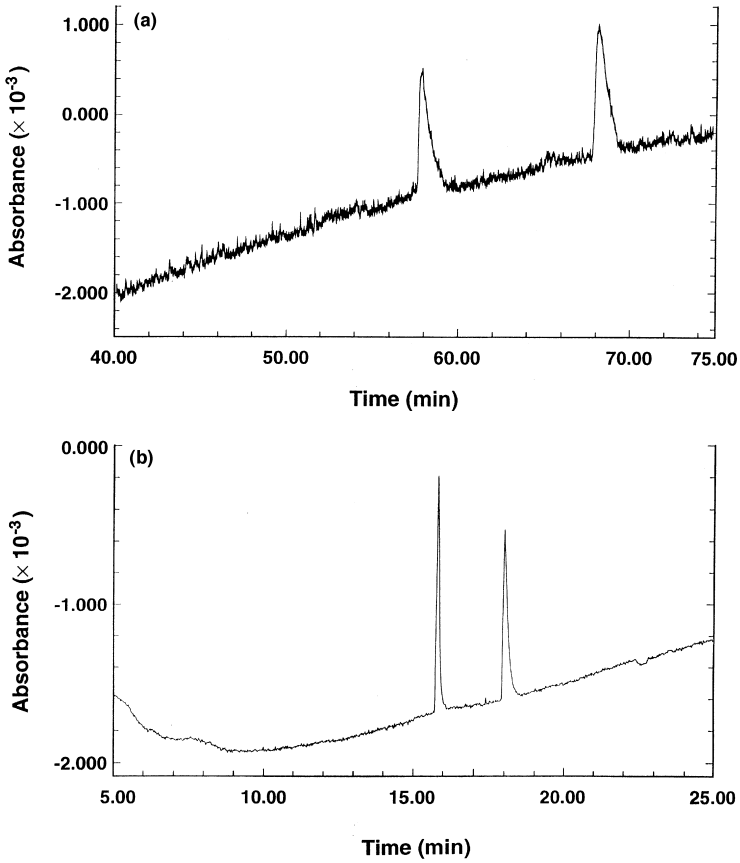

Fig. 6. (a) Enantiomeric separation of indoprofen. The BGE contains 50 m*M* NaH_2PO_4 adjusted to pH 5; 20 m*M* β-CD-OMe (VII); applied voltage was −15 kV, −30 μA; pressure injection 85 kPa·s; sample concentration 0.1 mg/ml in methanol–water (1:1, v/v). (b) Enantiomeric separation of fenoprofen. The BGE contains 50 m*M* NaH_2PO_4 adjusted to pH 7; 5 m*M* β-CD-OMe (VII); applied voltage was −30 kV, −32 μA; pressure injection 85 kPa·s; sample concentration 0.1 mg/ml in methanol–water (1:1, v/v).

individually, followed by a mixture of two, three, four, five and six PPAHs. The migration order of monochloro-substituted PPAHs corresponds with the position of the chloro groups (*meta*, *ortho* and *para*). It can be clearly observed that enantiomeric separation of the multi-chloro substituted PPAHs was dependent on the charge-to-mass ratio. The two most substituted herbicides [2(2,4-DCPPA) and 2(2,4,5-TCPPA)] were best resolved. One of the faster migrating antipodes of 2(2,4,5-TCPPA) co-eluted with one slower antipode of 2(2,4-DCPPA). However, they were both baseline resolved when run separately. The baseline enantiomeric separation of all six pairs of herbicides further strengthens the case for the implementation of polycationic CDs in CE. Our β-CD-OMe (VII) offers a greater separation window for PPAHs due to the larger difference in electrophoretic mobilities of the uncomplexed β-CD-OMe (VII) with the complex carrying a multiple positive charge.

4. Conclusions

We have demonstrated that the cationic β-cyclodextrin, β-CD-OMe (VII), can be used as a chiral selector for enantiomeric separation of two classes of compounds with CE. Enantiomeric separations were achieved at concentrations as low as 2 m*M*. In accordance with Wren and Rowe theory, resolution

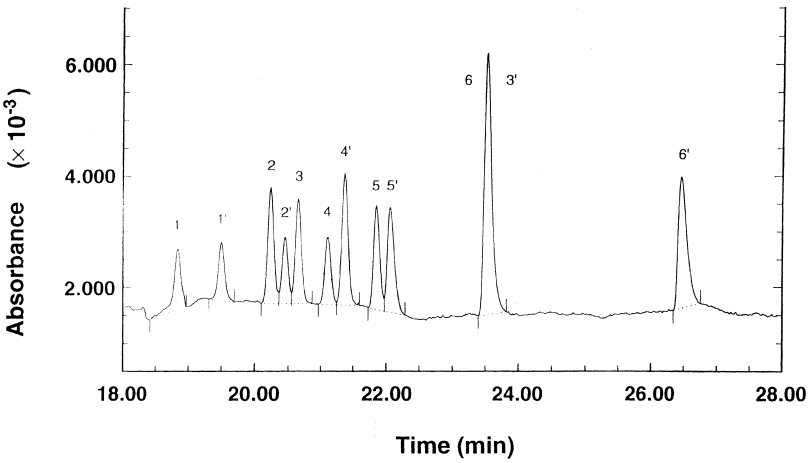

Fig. 7. Enantiomeric separation of a standard mixture of 12 (±) PPAH enantiomers. The BGE contains 50 m*M* NaH_2PO_4 adjusted to pH 6; 3 m*M* β-CD-OMe (VII); applied voltage was −15 kV, −25 μA; pressure injection 85 kPa·s; sample concentration 0.1 mg/ml in methanol–water (1:1, v/v). 1,1′=2-PPA, 2,2′=2,4-CPPA, 3,3′=2(2,4-DCPPA), 4,4′=2,2-CPPA, 5,5′=2,3-CPPA, 6,6′=2(2,4,5-TCPPA).

was strongly influenced by concentration and pH. The β-CD-OMe (VII) provided enantiomeric resolution over a wide range of pH values and concentrations. In some cases, the number of NSAIDs in a mixture that could be enantiomerically resolved in a single run was limited by pH since each racemate of NSAID had different optimum separation conditions. However, β-CD-OMe (VII) has shown excellent resolution of Fenop and Indop in comparison to any other derivatized cyclodextrin that we have found to date in literature [26]. In addition, β-CD-OMe (VII) provided a large window of separation for the herbicides. Baseline resolution of six racemates of chiral PPAHs was achieved simultaneously in less than 30 min with a single electrophoretic run. Future studies will focus on understanding the mechanism of binding of Fenop, Indop and Ibup with β-CD-OMe (VII) using fluorescence spectroscopy, ^{1}H NMR studies and molecular modeling. Such studies can provide a better understanding of the binding mechanism and this information can be used to target other anionic chiral compounds for separation. Furthermore, this work advances separations involving cationic α- and γ-cyclodextrins which could offer a wider range of selectivity for smaller and larger charged and uncharged chiral compounds.

Acknowledgements

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