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A Persubstituted Cationic β-Cyclodextrin for Chiral Separations

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The applications of a novel polycationic derivative of β-cyclodextrin (β-CD), heptakis(6-hydroxyethylamino-6-deoxy-β-cyclodextrin) (β-CD-EA), as a chiral host−guest additive for the enantioseparation of various classes of chiral anionic analytes are presented. The cationic β-CD described in this paper is persubstituted with seven ethanolamine side arms at the primary rim of each cyclodextrin (CD) molecule. It is found that the electrophoretic mobility of β-CD-EA can be adjusted to influence the chiral selectivity by changing the pH of the background electrolyte. Most of the observed CD capillary zone electrophoresis (CZE) separations of anionic drugs and herbicides were accomplished in the pH range of 4.0−7.0 with a reverse polarity configuration. At pH 5.0, enantioseparation of a mixture of three structurally related antiinflammatory agents (fenoprofen, flurbiprofen, and ibuprofen) was possible in about 30 min. However, other chiral acids, such as a series of phenoxypropionic acid herbicides and dansylated amino acids (glutamic acid and aspartic acids), were best separated at pH 6.0 or 7.0. An impressive separation of a mixture of six structurally related anionic herbicides [(±)-2-phenoxypropionic acid, (±)-2-(2-chlorophenoxy)propionic acid, (±)-2-(3-chlorophenoxy)propionic acid, (±)-2-(4-chlorophenoxy)propionic acid, (±)-2-(2,4-dichlorophenoxy)propionic acid, and (±)-2-(2,4,5-trichlorophenoxy)propionic acid] was achieved for the first time in about 15 min during a single run with 20 mM β-CD-EA. The analytical applicability of this cationic CD molecule for chiral separations is discussed in detail.

The ability of cyclodextrins (CDs) to engage in chiral recognition through inclusion complexes is well known in high-performance liquid chromatography.[1] With this property in mind, Fanali was the first to show the utility of CDs for chiral separations in capillary electrophoresis (CE).[2] Since that report, there has been an explosive increase in the use of native (α-,[3,4] β-,[5−11] and γ-[11−16]) CDs and neutral derivatized (hydroxypropyl-,[17−20] ethylated,[21] and methylated[22−26]) CDs as chiral additives in CE.

The recently developed applications of charged CDs for chiral separations in CE offer at least two important advantages over those with neutral CDs. First, neutral racemates that lack electrophoretic mobility as well as the charged racemates can be enantioresolved with charged CDs. Second, introduction of ionogenic groups on the CD rim or connected with it via a short alkyl chain enhances the solubility of charged CDs in aqueous media. Although the applications of negatively charge (carboxylated,[27−29] sulfated,[30−37] and phosphated[38]) CDs for the separation of both neutral and charged chiral racemates have currently

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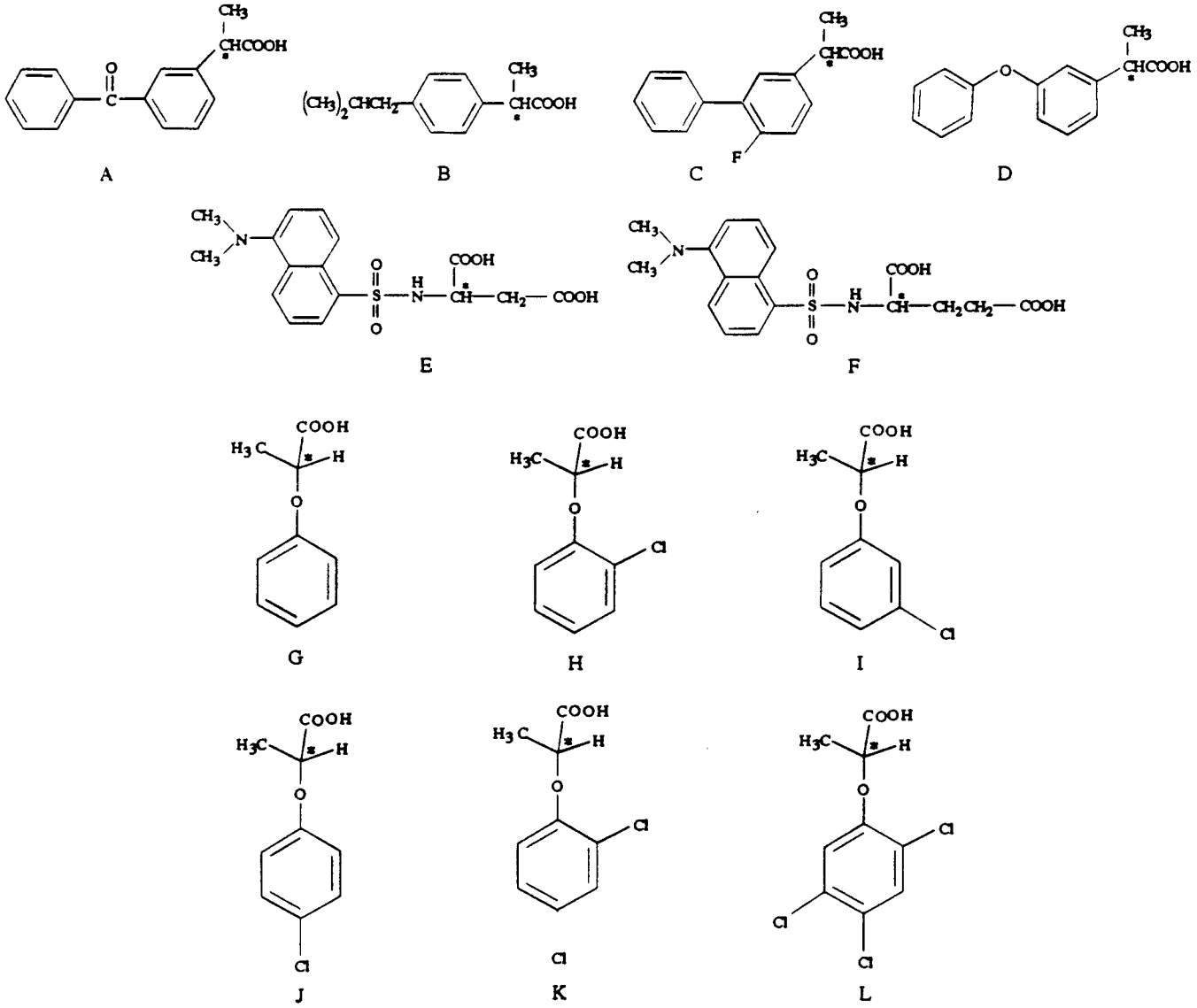

Figure 1. Molecular structures of chiral anionic analytes: A, ketoprofen (Ketop); B, ibuprofen (Ibup); C, flurbiprofen (Flurp); D, fenoprofen (Fenop); E, DNS-aspartic acid (Asp); F, DNS-glutamic acid (Glu); G, 2-phenoxypropionic acid (2-PPA); H, 2-(2-chlorophenoxy)propionic acid (2-CPPA); I, 2-(3-chlorophenoxy)propionic acid (3-CPPA); J, 2-(4-chlorophenoxy)propionic acid (4-CPPA); K, 2-(2,4-dichlorophenoxy)propionic acid (2,4-DCPPA); and L, 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TCPPA).

received a great deal of attention, the role of positively charged CDs has been surprisingly ignored. To date, there are only five published papers concerning the use of cationic CDs in CE.[39−43] Terabe was the first to report on the use of positively charged CDs. He and his co-workers investigated the enantioseparation of several dansylated amino acids using mono(6-β-aminoethylamino-6-deoxy-β-CD).[39] Fanali et al. published a paper highlighting the use of mono-and dimethylamino β-CDs for the enantioseparation of racemic mandelic acid and its derivatives.[40] Various neutral and derivatized CDs, such as native β-CD, heptakis(2,6-di-*O*-methyl-β-CD), and 2-hydroxypropyl-β-CD, were also compared for separation of these analytes. Improved chiral recognition with the use of both aminoalkyl derivatives of CD suggested that electrostatic or ion-pairing interactions between amino substituents of CDs and carboxylated analytes is the major factor. For example, dimethylamino-β-CD successfully resolved the enantiomers of *m*-methylmandelic acid and 3,4-dimethylmandelic acid at concentrations as low as 1 mmol/L. In contrast, no chiral separations were observed even when 80 mmol/L concentrations of the neutral CDs were added to the background electrolyte (BGE). Another probable reason for enhanced enantioselectivity of anionic analytes is the electrophoretic mobility of the cationic CDs that is in an opposite direction to the electrophoretic mobility of the anions. Recently, the same research group also compared mono- and hepta-substituted methylamino β-CD for the enantioseparation of several other anionic analytes.[41] They found that the hepta-substituted form is a better chiral resolving agent than the monosubstituted cationic β-CD for resolution of arylpropionic acids (APAs). However, the reverse was true for the separation

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of anticoagulants (warfarin and acenocoumarol) racemates. Lelievre and co-workers have applied the idea of selectivity in CE using a cationic CD molecule, the mono(6-amino-6-deoxy-β-CD), for chiral separations of some neutral and anionic enantiomers.[42,43]

The research described here reports the first application of a novel polycationic derivative of β-CD, heptakis(6-hydroxyethylamino-6-deoxy-β-CD) (β-CD-EA), as a chiral host−guest additive. A systematic approach is outlined for chiral separation of three classes of anionic analytes: (1) APAs, (2) dansylated amino acids (DNS-AAs), and (3) phenoxypropionic acids (PPAs). The concentration of β-CD-EA and the pH of the BGE were found to be the important factors in enhancing the enantiomeric separation of the aforementioned analytes. We believe that this is the first example of a positively charged CD that offers superior chiral resolution over other neutral or charged CDs reported so far in the CE literature. This benefit is clearly illustrated through the separation of anionic enantiomers of APAs and PPAs, which are important for pharmaceutical and agricultural industries, respectively.

EXPERIMENTAL SECTION

Materials. The racemic mixture of APAs [ketoprofen (Keto), fenoprofen (Feno), flurbiprofen (Flurp), and ibuprofen (Ibup)] as well as pure (*S*)-(−)-Ibup was obtained from Sigma (St. Louis, MO). DNS-AAs [DL-glutamic acids (Glu), DL-aspartic acid (Asp)] and their individual L-forms were also obtained from Sigma. The PPA herbicides [including (±)-2-phenoxypropionic acid (2-PPA), (±)-2-(2-chlorophenoxy)propionic acid (2-CPPA), (±)-2-(3-chlorophenoxy)propionic acid (3-CPPA), (±)-2-(4-chlorophenoxy)-propionic acid (4-CPPA), (±)-2-(2,4-dichlorophenoxy)propionic acid (2,4-DCPPA), and (±)-2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TCPPA)] were purchased from Aldrich (Milwaukee, WI). The structures of all anionic racemates used in this study are given in Figure 1. Monobasic sodium dihydrogen phosphate (NaH_2PO_4) and dibasic disodium hydrogen phosphate (Na_2HPO_4), used as BGEs, were of analytical reagent grade and were obtained from Amresco (Solon, OH).

Synthesis of Cationic β-Cyclodextrin. The 6-perbromination of β-CD (**1**) resulted in heptakis(6-bromo-6-deoxy-β-CD) (**2**). The details of the bromination procedure are reported elsewhere.[44] Heptakis(6-hydroxyethylamino-6-deoxy-β-CD) (β-CD-EA, **3**) was prepared from **2** by dissolving the latter in ethanolamine at 65 °C and heating at the same temperature for 48 h. The reagent/solvent was removed under vacuum and the resulting residue dissolved in hot methanol. A precipitate was obtained by the slow addition of the methanolic solution to stirred analytical grade acetone and collected by gravity filtration. The precipitate was redissolved in water and treated with a basic ion-exchange resin. Lyophilization yielded **3** (60−65%). The schematic of the reaction sequence is shown in Figure 2. Persubstitution was confirmed by elemental analysis and by the simple proton NMR, which showed a fine doublet at 5.10 ppm for the anomeric hydrogen (H-1).[45]

Potentiometric Measurements. Potentiometric titrations of β-CD-EA were conducted in water, at 25 °C under argon, using a

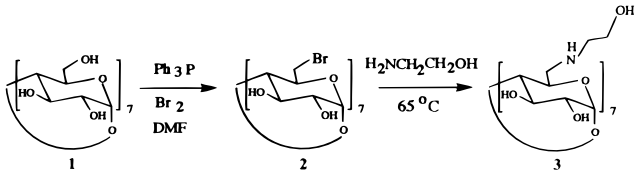

Figure 2. Synthetic route for hepatakis(6-hydroxyethylamino-6-deoxy-β-cyclodextrin) (β-CD-EA).

Mettler DL 25 automatic potentiometric titrator, equipped with a thermostated cell. The concentration of free hydrogen ions, $[H^+]$, was measured with a combined glass electrode (Orion 9103SC) connected to the titrator. The standard filling solution of the external reference of the electrode was replaced by a 0.1 M aqueous solution of NaCl saturated with AgCl. The electrode was standardized in concentrations at pH = $-\log [H^+] = 2$ with a solution of 10^{-2} M HCl (NaCl = 9×10^{-2} M). As the junction potentials vary exponentially with $-\log [H^+]$, the following correction relationship was used:

$$-\log [H^+]_{real} = -\log [H^+]_{measd} + a + b[H^+]_{measd}$$

or

$$pH_{real} = pH_{measd} + a + b \times 10^{-pH_{measd}}$$

The factors a and b were determined by measuring the pH of a 10^{-3} M HCl solution in the presence of 9.9×10^{-2} M NaCl ($pH_{real} = 3$, $I = 0.1$ M). Mean values were $a = 0.087$ and $b = -8.947$. The autoprotolysis constant of water (pK_w) at 25 °C and $I = 0.1$ M NaCl was checked by Gran titration of HCl with NaOH, pK_w being equal to $2V_b$ (volume at neutrality). The mean value was 13.78.

Ten milliliters of a solution of β-CD-EA ($C \approx 10^{-3}$ M, $I = 0.1$ M NaCl, 7 equiv of HCl) was titrated with NaOH ($C \simeq 4 \times 10^{-2}$ M) in the thermostated cell. Several titrations were performed. The pH values of the titration curves were corrected, and the data were treated with the Hyperquad program.[46−48] This program is based on a Gauss−Newton weighed least-squares method. It refines separately the free concentrations of the various species and their formation constants (stability of complexes or, in this case, protonation). The data to be introduced in the potentiometric data file are (1) the concentration of the various constituents (ligand, proton), expressed as the number of millimoles present in the cell at the begining of the titration, (2) the analytical concentration (mol/L) of the titrant reagent, and (3) the data of the experimental titration curve (volumes of the titrant added in milliliters, corrected pH). The pK_w, the stoichiometry of the different species that are potentially formed, and the estimation of their global formation constant ($\log \beta_i$) are entered in a model file. The calculations are realized by an iterative process that mimizes the sum of the squares of the differences between experimental and calculated values of the variable considered. Statistic tests demonstrate the quality of the refinement.

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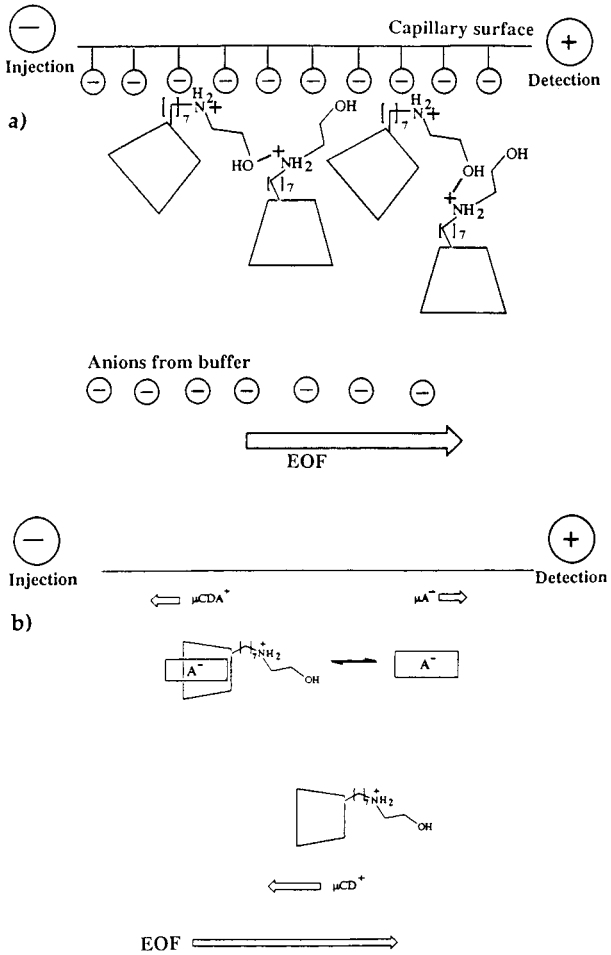

Figure 3. (a) Schematic representation for reversal of EOF by the addition of β-CD-EA to the buffer. (b) Schematic representation indicating direction of moblilities of the components in the system.

Protonation of the ligand LH_n is described by the following equilibria:

$$L^{n-} + iH^+ \rightleftharpoons LH_i^{(i-n)} \qquad \text{overall constant } \beta_i$$

$$LH_{i-1}^{(i-n-1)} + H^+ \rightleftharpoons LH_i^{(i-n)} \qquad \text{stepwise constant } K_i$$

$$K_i = [LH_i^{(i-n)}]/[LH_{i-1}^{(i-n-1)}][H^+] \qquad 1 \le i \le n$$

These global and stepwise apparent protonation constants are defined for particular conditions of temperature and ionic strength:

$$\beta_n = \pi_{i=1}{}^n K_i \quad \text{or} \quad \log \beta_n = \sum_{i=1}{}^n \log K_i$$

$$pK_{a_1} = \log K_n$$

$$pK_{a_n} = \log K_1$$

$$pK_{a_1} = \log K_{n-i-1}$$

The stepwise protonation constants log K_i for β-CD-EA were obtained from the calculation of the global protonation constants log β_i. The mean values are LH, 8.75; LH_2, 7.98; LH_3, 7.46; LH_4, 7.19; LH_5, 6.64; LH_6, 6.62; LH_7, 5.49. The percentage of formation

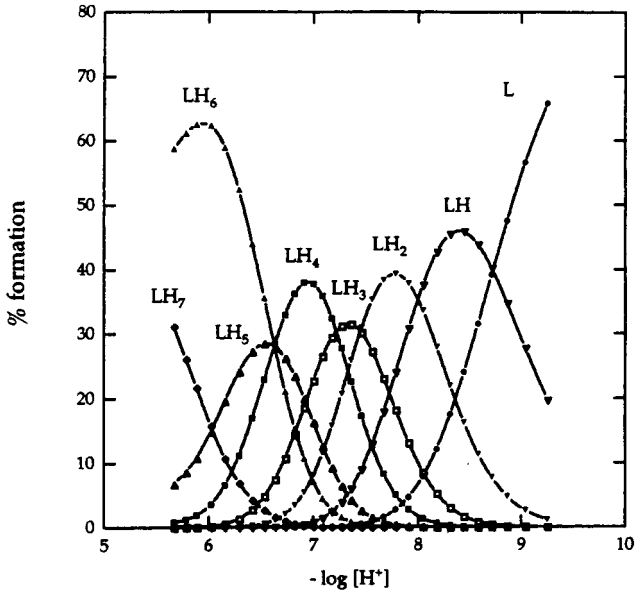

Figure 4. Plot of potentiometric titration studies showing the distribution of protonated species for β-CD-EA as a function of pH.

of the successive protonated species can be calculated by dividing the calculated free concentrations by the total concentration of the ligand.

Instrumentation. A Beckman PACE 5510 CE instrument was used. This instrument was equipped with (1) 0−30 kV high-voltage built-in power supply, (2) 214 nm selectable wavelength filter, and (3) software System Gold for system control and data handling. The fused-silica capillary, 57 cm × 75 μm i.d. × 320 μm o.d. (50 cm to the detector), was obtained from Polymicro Technologies (Phoenix, AZ). The capillary was fitted into a 200 μm aperture cartridge. The temperature of the cartridge holding the capillary column was thermostated at 23 ± 0.1 °C by use of a fluoroorganic fluid provided by Beckman. The run voltage was −15 kV in all experiments.

Capillary Electrophoresis Procedure. Before first use, a new capillary was subjected to a standard wash cycle for 1 h using 1 M NaOH. As a daily routine procedure, the capillary was conditioned with 1 M NaOH for 10 min. Between introduction of samples, the capillary was rinsed with operating buffer for 2 min. However, for herbicides, the rinsing system was altered so that there was a 2 min water rinse, 2 min 0.1 M NaOH rinse, 2 min water flush, and finally a rinse with the operating buffer for 2 min. The separation was initiated by applying a voltage (−15 kV) between two capillary ends, each of which was immersed in 4 mL vials containing the operating buffers.

Preparation of Analyte and Electrolyte Solutions. All analyte stock solutions were prepared in a 50/50 (v/v) methanol/water mixture at a concentration of 1 mg/mL, except for the stock solution of 2,4,5-TCPPA prepared in 80/20 (v/v) methanol/water mixture. Appropriate dilutions of stock solutions were obtained with a 50/50 (v/v) methanol/water mixture.

For all CE experiments, the BGE consisted of either 50 mM NaH_2PO_4 or Na_2HPO_4 buffer. Before adjusting the pH of these buffers, an appropriate amount of β-CD-EA (1−20 mM) was added to the BGE. The desired pH adjustments were made using either 0.12 and 1.2 M HCl or 0.5 M NaOH. After the pH was adjusted, the final CZE running buffers were filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY), by creating a vacuum

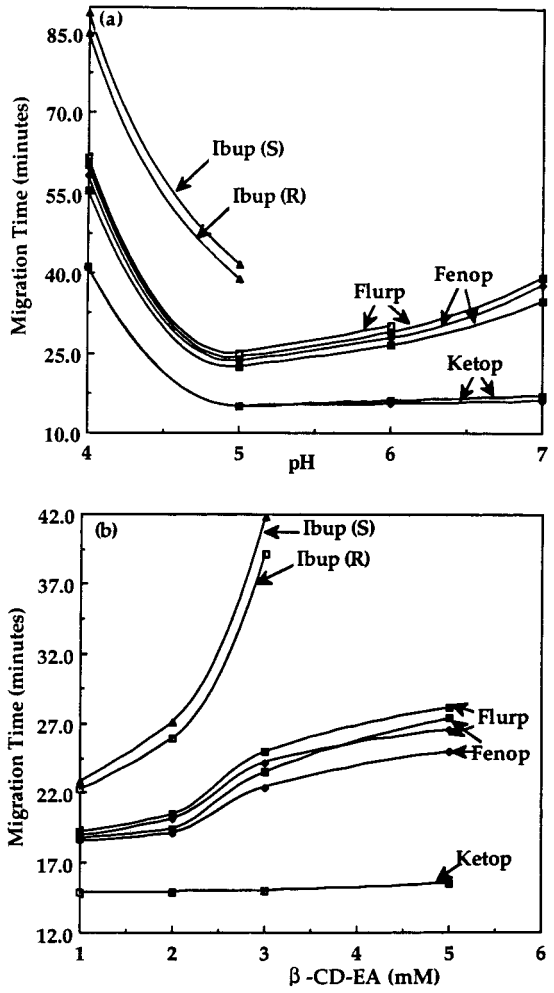

Figure 5. Plots of migration time of APAs enantiomers (a) as a function of pH at a fixed concentration of 3 mM β-CD-EA, (b) as a function of β-CD-EA concentration at a fixed pH 5.0. The BGE in both (a) and (b) contains 50 mM NaH_2PO_4. Pressure injection for 3 s (0.1 mg/mL) for all analyte anions. Separation voltage was −15 kV, current 20−30 μA depending on the pH and concentration of β-CD-EA. UV detection at 214 nm.

inside the syringe. This was followed by ultrasonication for about 10 min to ensure appropriately degassed CE buffers.

RESULTS AND DISCUSSION

The scheme for the synthesis of β-CD-EA is described in the Experimental Section. As can be seen in Figure 2, the β-CD-EA (**3**) possesses seven modified hydroxyl groups at position 6. This modification results in substitution of seven secondary amine (ethanolamine) groups that make it possible to have the CD derivative positively charged. The presence of positive charges not only enhances the solubility of β-CD-EA in an aqueous buffer in comparison with that of the parent compound, but also its adsorption on the capillary wall reverses the direction of the electroosmotic flow (EOF). Figure 3a represents schematically what we believe to occur at the capillary surface. After the initial flushing with NaOH, the free silanol groups on the capillary surface are ionized to the anionic form (SiO^-). This is countered by the cationic groups of the modified cyclodextrin (β-CD-EA) molecule. The β-CD-EA exists in differing orientations at the capillary surface, some ion-pairing with the silanol groups and some hydrogen-bonding with CD molecules. Thus, the cationic groups present a positively charged surface layer, enabling the solvated anions from the buffer solution, rather than the usual cations, to migrate toward the anode. This produces a reversal in the direction of the EOF.

Table 1. Recorded Resolution (Rs) of Profens Enantiomers with Varying pH[a]

	pH			
profens	4.0	5.0	6.0	7.0
Fenop	5.00	4.83	5.50	6.48
Flurp	1.40	2.20	2.40	
Ibup	1.24	1.52		
Ketop	0.00	0.00	0.50	0.80

[a] Using 50 mM NaH_2PO_4, 3 mM β-CD-EA; −15 kV applied for separation. Current varied from 22 to 30 μA. Analyte concentration, 0.1 mg/mL. Pressure injection for 1.5 s.

Table 2. Recorded Resolution (Rs) of Profens Enantiomers with Varying β-CD-EA Concentrations[a]

	β-CD-EA concn (mM)			
profens	1.0	2.0	3.0	5.0
Fenop	1.54	3.60	4.83	7.70
Flurp	1.00	1.84	2.20	3.10
Ibup	1.20	0.70	1.52	

[a] Using 50 mM NaH_2PO_4, pH 5.0; −15 kV applied for separation. Current varied from 20 to 28 μA. Analyte concentration, 0.1 mg/mL. Pressure injecton for 1.5 s.

The schematic illustration of the migration of a cationic CD and an anionic analyte under the conditions of reverse EOF is shown in Figure 3b. The free cationic CD moves with a mobility μCD^+ toward the cathode, whereas the free anionic analyte moves toward the anode with mobility μA^- and is in an equilibrium state with the CD−analyte complex. If an anionic analyte is strongly included into the cavity of cationic CD, it will migrate toward the cathode and will elute later than the anionic analyte complexed weakly with the cationic CD. This is because the CD is multiply charged. However, the CD−analyte ($CD\text{-}A^+$) complex has lower velocity toward the cathode as compared to the EOF. Consequently, the $CD\text{-}A^+$, with a mobility μCDA^+, will eventually be pulled toward the anode by the EOF. Since the electrophoretic mobility of chiral selector and the EOF are in opposite directions, maximum resolution is achieved for anionic racemates. Note that, for faster elution of anionic enantiomers, it was necessary to reverse the polarity of the power supply in order to counter the observed reversed EOF. This is because, in a preliminary experiment (using a positive polarity on the power supply), we observed that even 1 mM of β-CD-EA did not permit detection of any analyte peaks.

Figure 4 shows the numerous protonated species of β-CD-EA that exist at changing pH. No single species is exclusively present, except under conditions not studied, such as strongly acidic or basic pH, but rather several species of protonation coexist at a given pH. Thus, the electrophoretic mobility of β-CD-EA is expected to be very much pH-dependent.

Separation of Arylpropionic Acids. APAs or profens such as Fenop, Flurp, Ibup, and Ketop are an important class of nonsteroidal antiinflammatory drugs (NSAIDs). Thus, they have been used for the treatment of several antiinflammatory diseases and are often prescribed as racemic mixtures. However, it has been recently reported that the physiological activity

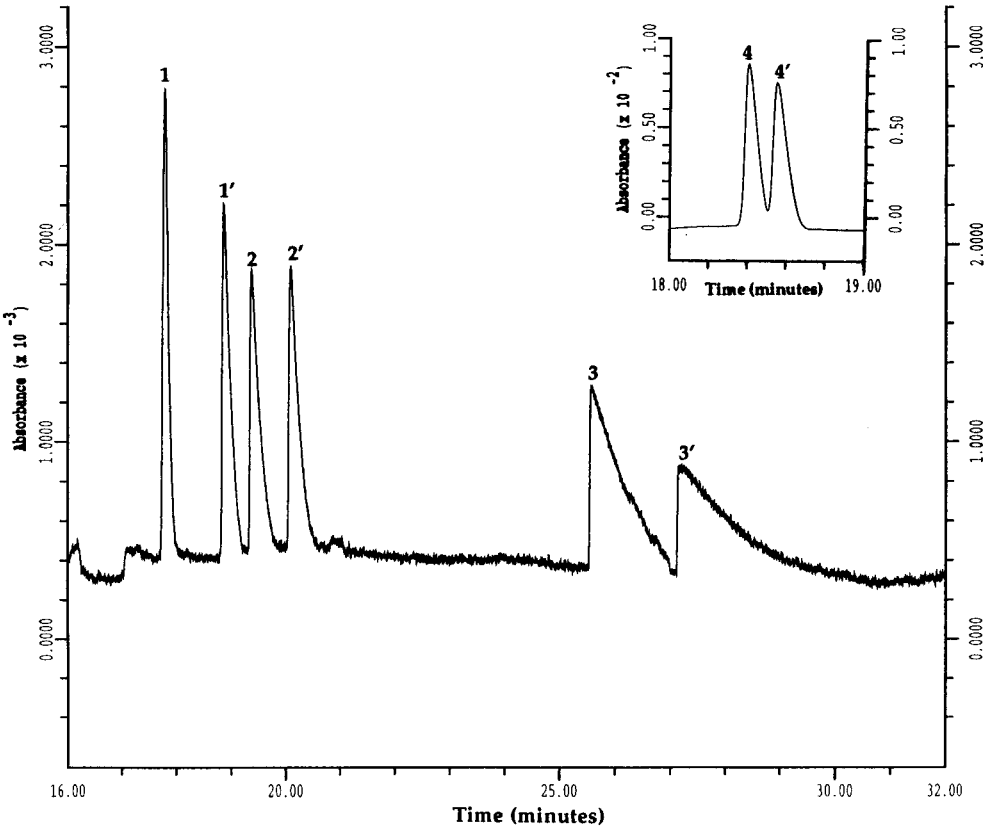

Figure 6. Separation of a standard mixture of six APA enantiomers. Electrolyte, 3 mM β-CD-EA, 50 mM NaH_2PO_4 adjusted to pH 5.0 with 0.5 M NaOH. Peak identification: 0.1 mg/L each of 1,1′ = (±)-Fenop; 2,2′ = (±)-Flurp; 3,3′ = (±)-Ibup. The inset shows a separation of 4,4′ = (±)-Ketop using 20 mM β-CD-EA, 50 mM Na_2HPO_4 adjusted to pH 7.0 with 0.1 M HCl. Other conditions are the same as in Figure 5.

of these NSAIDs resides mainly in the enantiomers with the *S*-configuration.[49] To obtain the best possible CE conditions for the simultaneous and individual enantioseparation of APAs, both the pH and the β-CD-EA concentration need to be carefully optimized.

The effect of pH on the migration time and resolution (Rs) is more complicated with β-CD-EA than with other neutral or derivitized β-CD. This is due to two major reasons. First, as mentioned earlier, β-CD-EA has several protonated amine groups (DS = 7.0) with various pK_as. Second, the analytes studied in this work (APAs, PPAs; see Figure 1 for structures) also possesses ionizable carboxylate groups. Therefore, pH affects not only the EOF but also the charge and the chiral recognition properties of both the analyte and the chiral additives. Figure 5a shows the dependence of the observed migration times of APAs on the pH of the separation electrolyte composed of 50 mM NaH_2PO_4 as BGE and 3 mM β-CD-EA as a chiral selector. Initially, at pH 4.0, the APAs show lower mobilities (longer migration times) due to partial ionization of the carboxylate group. However, by increasing the pH to 5.0 [encompassing the pK_a values of the APAs (4.03−4.50)],[50] the mobilities of the anionic form of each of these acids are increased. Consequently, a significant drop in migration time is observed between pH 4.0 and 5.0. Between pH 5.0 and 7.0, all profens show a general trend of increasing migration time because of the decrease in EOF. However, the degree of increase in migration times was different for each solute. For example, it could be found from Figure 5a that the increment of migration time was largest for Flurp and smallest for Ketop. In addition, note that, because of the strong complexation of (±)-Ibup, and the later eluting enantiomer of Flurp with the β-CD-EA at the respective pHs of 6.0 and 7.0, no migration times for these analytes were recorded at such pH values.

To clearly describe the effect of pH on the separation characteristics, the Rs of each enantiomeric pair of APAs was then calculated as the criterion for optimization. The results are tabulated in Table 1. According to this table, each enantiomeric pair has a different pH for optimum Rs. Fenop and Ketop are best separated (Rs = 6.48 and 0.80, respectively) at pH 7.0. In contrast, Ibup and Flurp at pH 5 and 6 showed best Rs of 1.52 and 2.40, respectively. It appears that, at any pH, these differences in Rs values arise from the differences in host−guest complexation of the APA/β-CD-EA ion pairs. The net effects are differences in apparent electrophoretic mobilities of APAs. Concerning the various APAs, the following order of Rs was observed in most cases irrespective of pH: Fenop > Flurp > Ibup. The observation that Fenop is the best-resolved analyte among the profen series is expected since Fenop has an ether linkage (oxygen atom connected to two phenyl rings) that can serve as a proton acceptor site for hydrogen bonding (see Figure 1D). Thus, it is reasonable to conclude that this additional interaction provided better chiral discrimination. It was also found that Ketop showed very little change in migration time and was only partially resolved as a function of pH when compared to other members of the profen family. In addition, Ibup and Flurp, due to their comigration with the neutral marker, showed no resolution at pH 6 and 7, respectively. However, it could be suggested from Figure 5a and Table 1 that simultaneous separation of the three enantiomeric

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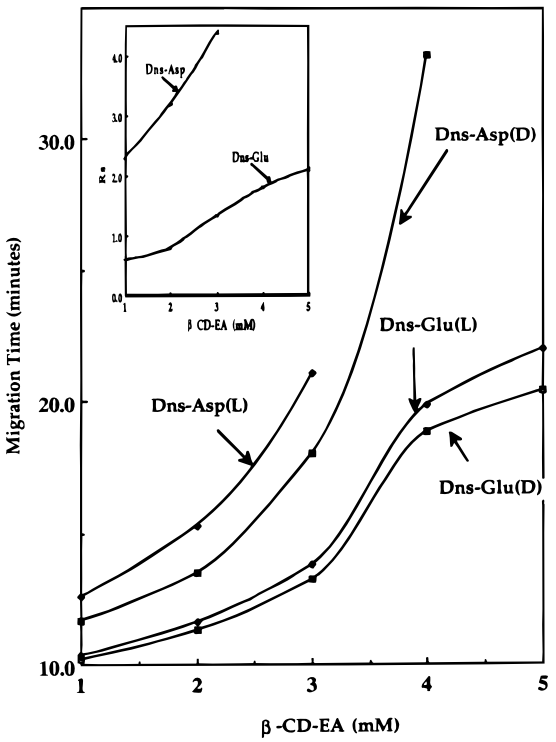

Figure 7. Plot of migration time of DNS-DL-amino acid enantiomers as a function of concentration of β-CD-EA added to 50 mM NaH_2PO_4 adjusted to pH 6.0 with 0.5 M NaOH. The inset shows Rs of enantiomers as a function of β-CD-EA concentration. Other conditions are the same as in Figure 5.

pairs with complete baseline resolution is possible around pH 5.0. Such conditions correspond to a system with predominantly LH_7 or fully protonated β-CD-EA and some LH_6 present (see Figure 4).

The effects of β-CD-EA concentration at a fixed pH of 5.0 on the migration time and Rs of APAs enantiomers are shown in Figure 5b and Table 2, respectively. Both migration time and Rs increased with increasing concentration of β-CD-EA from 1 to 5 mM. Again, note that no migration and Rs data were recorded for (±)-Ibup due to its strong interactions at concentration ≥4.0 mM with β-CD-EA. Considering the increase of migration time in the presence of 1−3 mM β-CD-EA (Figure 5b) as a parameter of complexation, the following order of complexation can be established: Ibup > Flurp > Fenop > Ketop. This is because the difference in the migration time in the absence and presence of 3 mM β-CD-EA (Δt) is largest (i.e., 19 min for Ibup) and smallest (i.e., 0.5 min for Ketop). As shown in Table 2, chiral Rs of APAs increases in the following order irrespective of the concentration of β-CD-EA: Fenop > Flurp > Ibup. It is interesting to note that, except for Ketop, this Rs trend is opposite to the order of the complexation ability of APAs. Baseline separations (Rs ≥ 1.5) of the APAs enantiomers were obtained at different β-CD-EA concentrations: Fenop, 1 mM; Flurp, 2.0 mM; and Ibup, 3.0 mM. However, the optimum Rs of 7.70 and 3.10 for Fenop and Flurp, respectively, were obtained at almost the same concentration (~5 mM β-CD-EA). In contrast, Ibup showed an optimum Rs value of 1.52 at 3.0 mM β-CD-EA.

Based on the pH and the β-CD-EA concentration studies, it appears that the best experimental conditions for the simultaneous separation of the three APAs enantiomers can be obtained around pH 5.0 with 3 mM β-CD-EA. Figure 6 shows the electropherograms for the separation of a standard mixture of Fenop, Flurp, and Ibup into their enantiomers at optimized conditions. In this

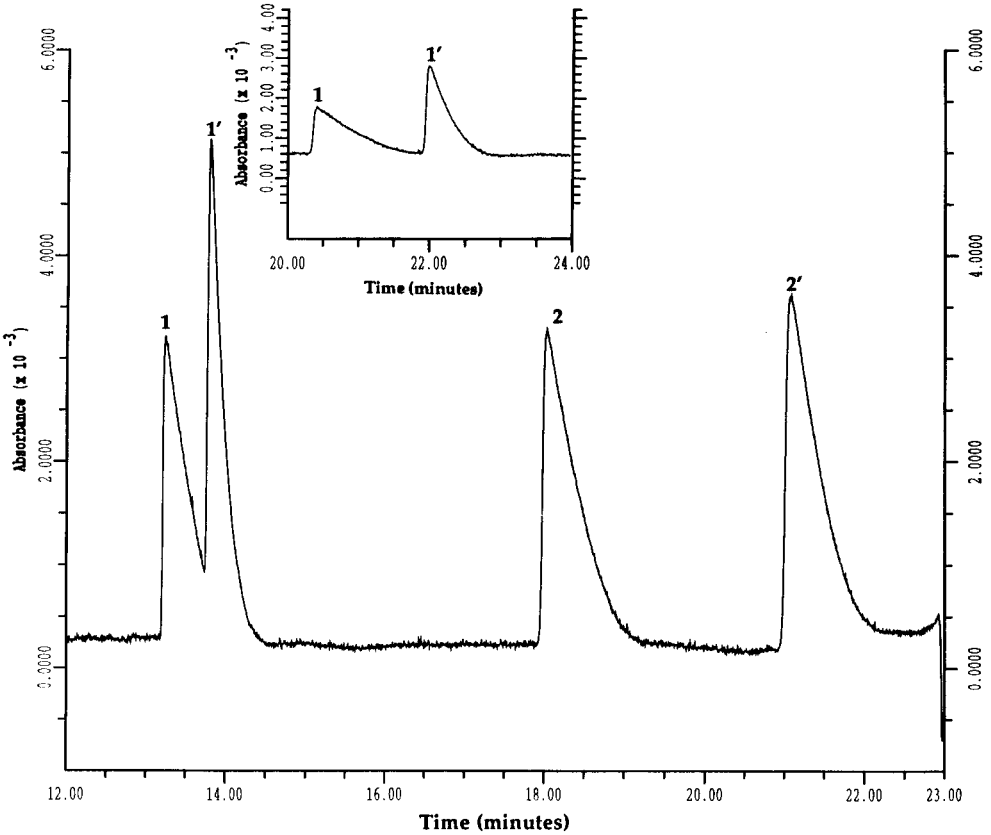

Figure 8. Separation of a mixture of two DNS-DL-amino acids. Electrolyte, 3 mM β-CD-EA, 50 mM NaH_2PO_4 adjusted to pH 6.0 with 0.5 M NaOH. Peak identification: 0.1 mg/L each of 1 = DNS-D-Glu; 1′ = DNS-L-Glu; 2 = DNS-D-Asp; 2′ = DNS-L-Asp. The inset shows a baseline resolution of DNS-DL-Glu at 5 mM β-CD-EA using the same pH and BGE concentration. Other conditions are the same as in Figure 5.

mixture, only the migration order of the enantiomers of (±)-Ibup was verified by spiking the racemic samples with the pure optical isomers. The (*R*)-(+)-Ibup eluted faster than the (*S*)-(−) form. This suggests that the (*S*)-(−) isomer has higher affinity than its optical antipode for β-CD-EA. The elution order of other optical isomers of APAs could not be established due to the nonavailability of pure optical standards. Moreover, as indicated in Table 1, the enantiomers of Ketop showed some chiral resolution only at pH 7.0. Further increases in pH did not improve the separation of this enantiomer. However, when the concentration of β-CD-EA was increased to 20 mM at pH 7.0, an Rs of ∼1.3 was obtained. The inset in Figure 6 shows the separation of Ketop under such conditions.

Several recent papers have also dealt with the chiral separations of APAs using various neutral CDs (β-CD, hydroxypropyl-β-CD, dimethyl-β-CD, trimethyl-β-CD).[26,50] However, the baseline Rs of APAs enantiomers was only achieved with trimethyl-β-CD. Furthermore, among various APAs, (±)-Fenop provided the best possible Rs of 6.0 using 25 mM trimethyl-β-CD.[26] In our work, the same enantiomer provided an Rs of 7.7, but at much lower concentration of 5 mM β-CD-EA. This clearly indicates the superior chiral Rs ability of β-CD-EA for APAs as compared to that of any other neutral or derivitized CD reported so far in the literature.

Separation of DNS-DL-Amino Acids. Chiral separations of two anionic forms of DNS-DL-AAs (i.e., DNS-DL-Asp and -Glu) were achieved by optimizing the pH and the concentration of β-CD-EA. Resolution of both acids was optimum at pH 6.0 (data not shown). At this pH, the β-CD-EA species is predominantly present as a mixture of LH_6 and LH_7, in the ratio of ∼65:35, with an effective charge of +6 and +7, respectively (see Figure 4). Under this optimum pH condition, the concentration of β-CD-EA was varied. As shown in Figure 7, steady increases in the migration times of both enantiomers of DNS-DL-Asp and -Glu were observed with an increase in concentration of β-CD-EA from 1 to 3 mM. Further increase in concentration of β-CD-EA to 5 mM improves the separation of DNS-DL-Glu (Rs = 2.1). However, this occurs at the expense of comigration of DNS-Asp with the EOF marker. Therefore, the simultaneous separation of DNS-DL-Asp and -Glu is only possible at 3 mM. Figure 8 shows the electropherogram of both enantiomers of DNS-DL-AAs under such conditions. Note that the strongly included DNS-DL-Asp in the β-CD-EA cavity provided higher chiral recognition (Rs = 4.5) than the weakly included DNS-DL-Glu (Rs = 1.1). This is in contrast to the Rs trend obtained for APAs enantiomers, in which weakly retained enantiomers showed better chiral resolution (see Tables 1 and 2 and Figure 5). Furthermore, the D-enantiomer of both DNS-AAs migrates faster than the L-enantiomer, suggesting that the L-isomer forms a stronger complex with the cationic CD molecule. In addition, the DNS-DL-Asp that forms stronger ion pairs and has longer migration times gives relatively broader peaks than DNS-DL-Glu with a shorter migration time. The same phenomenon of peak broadening also occurred for DNS-DL-Glu at a concentration of 5 mM β-CD-EA. However, at this concentration of the chiral selector, DNS-DL-Glu was baseline resolved (see Figure 8 inset). Currently, research is underway in our laboratory to investigate the effect of various amines as comodifiers to β-CD-EA. The use of such cationic competitors may help reduce the strong ion-pair interactions between the multicharged anionic analytes such as DNS-Asp or -Glu with cationic β-CD-EA.

Table 3. Recorded Migration Time (t_m) and Resolution (Rs) of Phenoxypropionic Acids (PPAs) Enantiomers as a Function of pH[a]

	pH			
PPAs	5.0	6.0	7.0	8.0
2-PPA				
t_{m1}	17.5	11.1	12.1	17.2
t_{m2}	19.6	11.6	12.5	18.2
Rs	7.80	3.10	3.00	5.00
4-CPPA				
t_{m1}	18.4	11.9	13.3	18.1
t_{m2}	19.8	12.3	14.1	19.7
Rs	6.36	3.90	3.50	5.30
2-CPPA				
t_{m1}	19.6	12.1	13.9	21.0
t_{m2}	25.5	13.2	14.8	22.5
Rs	9.35	7.60	6.40	7.60
3-CPPA				
t_{m1}	24.2	12.8	14.5	22.0
t_{m2}	27.1	13.4	15.3	23.6
Rs	8.40	4.90	4.70	6.20
2,4-DCPPA				
t_{m1}	20.8	12.5	14.5	22.2
t_{m2}	*b*	13.8	15.8	25.2
Rs	*b*	8.10	7.20	9.60
2,4,5-*T*CPPA				
t_{m1}	23.5	14.1	16.2	35.8
t_{m2}	*b*	15.2	16.7	38.8
Rs	*b*	8.20	3.50	5.50

[a] Using 50 mM Na_2HPO_4, 20 mM β-CD-EA; −15 kV applied for separation. Current varied from 20 to 35 μA. Analyte concentration, 0.10 mg/mL. Pressure injection for 3.0 s. [b] The peak was not observed due to comigration with the EOF marker.

Separation of (±)-Phenoxypropionic Acids. PPAs are important agrochemicals used in the control of weeds in cereal crops.[51] The chemical structures of the six PPA herbicides under investigation are shown in Figure 1G−L. All the derivatives of PPA herbicides contain a phenolic ring with a chloro substituent. The oxygen of the phenolic ring is linked to an asymmetric carbon atom, leading to two optical isomers. It is believed that the *R* form of the PPAs possesses the major herbicidal activity, while the *S* form is herbicidally inactive.[52] For chiral separations of most racemates of PPAs, β-CD-EA concentrations of ≥10 mM were necessary to obtain baseline separation.

The effects of pH on the migration time and enantiomeric resolution of the PPAs were studied in 20 mM β-CD-EA, containing 50 mM Na_2HPO_4. The results are summarized in Table 3, and electropherograms are compared in Figure 9. The chiral separations of the first four PPA herbicides (i.e., 2-PPA, 4-CPPA, 2-CPPA, and 3-CPPA) with Rs ≥6.4 were achieved at pH 5.0. However, under such pH conditions, not only longer migration times and broad peaks were observed, but also the later eluting enantiomer of the last two PPAs (i.e., 2,4-DCPPA and 2,4,5-TCPPA) comigrated with the EOF (Figure 9a). A significant drop in the migration velocities of all PPAs was seen as the pH was increased to 6.0 (see Figure 9b and Table 3). This decrease in migration times of PPAs cannot be attributed solely to an increase in reverse EOF because similar larger shifts in the migration times of APAs

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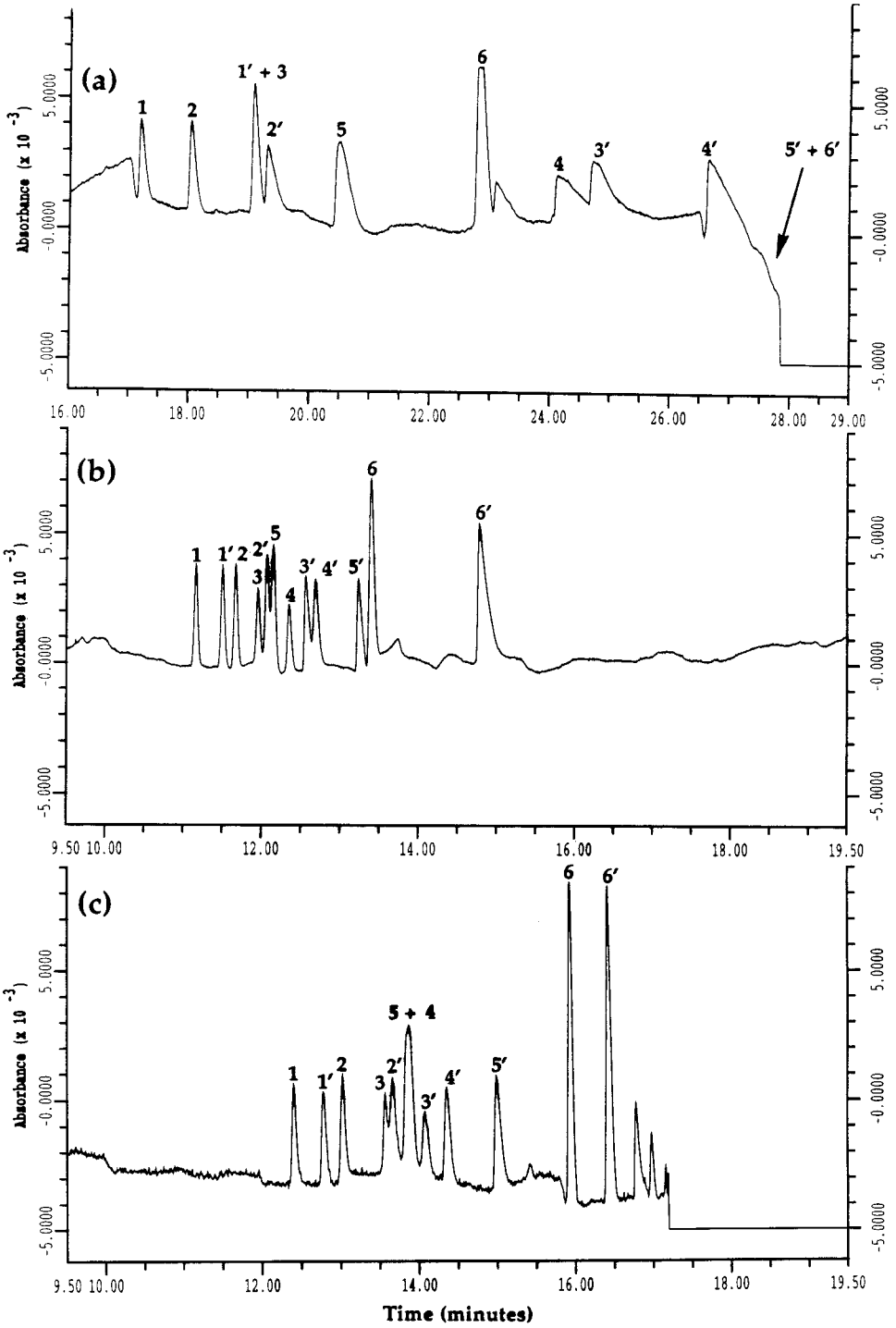

Figure 9. Effect of BGE pH on the separation of a standard mixture of 12 PPAs enantiomers. Electrolyte, 20 mM β-CD-EA, 50 mM Na_2HPO_4 adjusted to (a) pH 5.0, (b) 6.0, and (c) 7.0 with 0.1 M HCl. Peak identification: 0.1 mg/L each of 1,1′ = (±)-2-PPA; 2,2′ = (±)-4-CPPA; 3,3′ = (±)-2-CPPA; 4,4′ = (±)-3-CPPA; 5,5′ = (±)-2,4-DCPPA; 6,6′ = (±)-2,4,5-TCPPA. Pressure injection for 3.0 s. Separation voltage was −15 kV, current 20−30 μA depending on the pH. UV detection at 214 nm.

(see Figure 5a) were also observed. However, such shifts were seen in a different pH range of 4−5, which is closer to the pK_a values (all around 4.0−4.5) of APAs. Although most of the PPAs have pK_as between 3.0 and 3.5, it is possible that the pK_a values of the complexed PPAs are increased when higher concentrations of β-CD-EA (~20 mM) are used for such separations, as opposed to an optimum concentration of 3mM β-CD-EA used for resolution of profen enantiomers. Moreover, it is interesting to note that, at pH 6.0, the decrease in migration times is accompanied by reduced enantiomeric resolutions of the first four racemic herbicides (Table 3). In contrast, at the same pH, both of the eluting enantiomers of the last two herbicides provided an impressive enantiomer resolution of ≥8.0. Increasing the pH to 7.0 seems to slightly increase the migration time, with a concurrent drop in enantiomeric Rs for all PPAs herbicides. Optimum separation (with higher Rs and efficiency) for each individual PPA was best achieved at pH 8.0. At this pH, the predominant species present are the mono- (LH) and diprotonated (LH2) forms of β-CD-EA. While all individual enantiomers of PPAs were well resolved without difficulty at this pH, some peak overlap with longer

analysis time was observed in separating the mixture of 12 enantiomers. A study of Figure 9 clearly shows that all 12 enantiomers of a mixture of six PPAs can be simultaneously separated best in about 15 min at pH 6.0. Additionally, pH 6.0 was reasonable in terms of obtaining satisfactory enantioresolution and analysis time as well as achieving highest peak capacity compromise of PPA herbicides.

With regard to Figure 9 and Table 3, the electrophoretic mobilities of β-CD-EA/PPAs complexes are lower than those of the free (uncomplexed) anionic PPAs with reversed polarity configuration and reverse EOF. Therefore, the stronger the complexation, the slower the anionic analyte migrated toward the detector end, i.e., the anodic end with the same direction as the EOF. Under all pH conditions, the migration times and, therefore, the binding of the first eluting enantiomers with β-CD-EA seem to increase in the following order: 2-PPA < 4-CPPA < 2-CPPA < 2,4-DCPPA < 3-CPPA < 2,4,5-TCPPA. This migration order obtained for PPAs (with negative polarity) is exactly the opposite of that obtained by El Rassi and co-workers for the CE separation of the same group of herbicides (using positive polarity), in which native and methylated CDs[18] as well as alkylglucoside chiral surfactants[53] served as the chiral pseudostationary phases. The only exception to this trend is the migration order of 3-CPPA, which was last to elute after 4-CPPA and 2-CPPA. Perhaps the pK_a value of the complexed form of 3-CPPA increased relative to those of the complexed form of 2-CPPA, resulting in a decrease in its mobility toward the positive electrode (i.e., detector end in reverse polarity configuration). Based on similar assumptions, one would expect that 4-CPPA, being least acidic, should elute even later than 3-CPPA. In contrast, the reverse occurred. This seems puzzling at first. However, it can be explained by the weaker electrostatic interaction in the β-CD-EA/4-CPPA ion pair. It should be noted that the β-CD-EA used in this work can resolve all six PPAs with a much higher enantioresolution (see Table 3) than other CDs or derivatives of CDs so far employed for chiral separations of PPA herbicides.[18,53,54] Additionally, to the best of our knowledge, this is the first report of the resolution of 2-CPPA enantiomers by a CD molecule.

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CONCLUSIONS

A novel polycationic β-CD-EA reported in this paper appears to be a superior chiral host−guest additive for the separation of a variety of negatively charged enantiomeric mixtures. Chiral separation of various classes of anionic enantiomers was found to be heavily dependent on pH because the degree of protonation of β-CD-EA can alter the shape of the CD cavity by charge repulsion, altering complexation, aiding selectivity, and enabling better enantiomeric resolution. Among the profen series, irrespective of pH, the least retained pair of enantiomers (e.g., Fenop, Table 1) provided the best chiral selectivity. In contrast, an increase in analyte binding with β-CD-EA (translated as a decrease in analyte mobility) was found in many cases [e.g., DNS-Asp (Figure 8), 2,4-DCPPA, and 2,4,5-TCPPA (Table 3)] to improve the enantioselectivity. As indicated earlier and demonstrated experimentally, the Rs values for APA and PPA enantiomers using β-CD-EA are superior to even that for trimethyl-β-CD.[18,26,50] The latter CD derivative is the best example of a CD selector reported so far in the literature for the enantioseparation of the aforementioned anionic racemates. The discovery of this enhanced enantioselectivity in a class of cationic CD opens up new opportunities in the screening of other positively charged CD molecules. For example, interaction with various charged and uncharged chiral analytes can be studied by altering the nature of positively charged substituents and/or the position as well as the degree of substitution on native α-, β-, or γ-CD molecules.

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