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Enantiomeric separation of chiral phenoxy acid herbicides by electrokinetic chromatography. Application to the determination of analyte-selector apparent binding constants for enantiomers

The enantiomeric resolution of chiral phenoxy acid herbicides was performed by electrokinetic chromatography using a cyclodextrin as chiral pseudophase (CD-EKC). A systematic evaluation of several neutral and charged cyclodextrins was made. Among the cyclodextrins tested, (2-hydroxy)propyl β-cyclodextrin (HP-β-CD) was found to be the most appropriate for the enantioseparation of phenoxy acids. The influence of some experimental conditions, such as nature and pH of the background electrolyte, chiral selector concentration, and temperature, on the enantiomeric separation of phenoxy acids was also studied. The use of a 50 mM electrolyte solution in ammonium formate at pH 5 and a temperature of 40°C enabled the enantiomeric resolution of four of the six phenoxy acids investigated (2-phenoxypropionic acid, 2-(3-chlorophenoxy)propionic acid, 2-(4-chlorophenoxy)propionic acid, and 2-(2,4-dichlorophenoxy)propionic acid) obtaining migration times ranging from 9 to 15 min. Mixtures of the two phenoxy acids not enantiomerically resolved (2-(4-chlorophenoxy)-2-methylpropionic acid and 2-(2,4,5-trichlorophenoxy)propionic acid) and up to three of the phenoxy acids enantiomerically resolved were separated in about 15 min. Finally, the apparent binding constants for each enantiomer-HP-β-CD pair were calculated at two temperature values (20 and 40°C).

Keywords: Phenoxy acid herbicides / Electrokinetic chromatography / (2-Hydroxy(propyl-β-cyclodextrin / Complex mobility / Enantioseparation / Apparent binding constants EL 4505

1 Introduction

Phenoxy acids are widely used in agriculture as selective herbicides. Due to their solubility in water, they can move in agriculture ecosystems, causing surface and ground waters pollution [1]. Their toxicity and herbicidal effects have been studied in detail, and many efforts have been made to develop reliable and sensitive methods for determining phenoxy acids in various matrices, most of these methods being based on high performance liquid chromatography (HPLC) or gas chromatography (GC) [2–9]. Biological activity in soil or water environments may result in the preferential reactivity of one enantiomer in terms of microbial degradation, biological uptake, metabolism, or toxicity [10, 11]. In fact, several of the phenoxy acid herbicides are optically active, being only one of the enantiomers the active ingredient of the racemic mixtures. Chiral separation of these herbicides are required in order to assess the enantiopurity of formulations and to optimize enantioselective production processes. GC or HPLC with chiral stationary phases have been generally used for this purpose [12, 13].

In recent years, chiral separations by capillary electrophoresis (CE) have witnessed a steady growth, which has been outlined by several recent review articles [14–18]. In this technique, enantiomeric separation can be achieved relatively fast, with high resolution and using a small amount of sample and chiral selector, by means of electrokinetic chromatography (EKC). This mode of CE is based on the partitioning of the solutes between a buffer solution, which migrates with the velocity of the electroosmotic flow (EOF), and a chiral pseudophase [19]. From the different compounds used as chiral pseudophases, the neutral or charged CD derivatives have been the most widely employed [14–18, 20–23]. There is no room

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Abbreviations: CM-β-CD, carboxymethylated β-cyclodextrin; **4-CPMPA**, 2-(4-chlorophenoxy)-2-methylproprionic acid; **3-CPPA**, 2-(3-chlorophenoxy)proprionic acid; **2,4-DCPPA**, 2-(2,4-dichlorophenoxy)proprionic acid; **HP-β-CD**, (2-hydroxy)-propyl β-cyclodextrin; **PM-β-CD**, methylated β-cyclodextrin; **SC**, cholic acid sodium salt; **STC**, taurocholic acid sodium salt; **Succ-β-CD**, succinylated β-cyclodextrin; **2,4,5-TCPPA**, 2-(2,4,5-trichlorophenoxy)propionic acid

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for doubt that despite the major progress in chiral CE, there is still capacity for improvement, as far as the optimization of enantiomeric separations is concerned, so that the resolution of a wider range of racemates could be achieved. In addition, CE has been proved to be suitable for the analysis of a wide variety of chiral and achiral pesticides [24].

In order to achieve the enantiomeric separation of phenoxy acid herbicides by CE, various native and CD derivatives have been employed [25–33], although, in most cases, baseline resolution is only achieved for two or three of the compounds studied. From the different charged CD derivatives used for the enantioseparation of different classes of optically active herbicides, it can be mentioned the anionic sulfobutyl ether β-CD [32, 33] or the cationic β-CD derivative heptakis(6-methoxyethylamine) β-CD [31], although in the last case excessive migration times were obtained (about 30 min). On the other hand, CE has become attractive in the last years not only as a separation technique but also for the determination of dissociation and binding constants. By measuring mobility as a function of selector concentration in CE, analyte-selector binding constants can be obtained. The main advantages of this technique in the binding constants' measurement are the small amounts of acceptors and ligand required, the possibility of studying one acceptor with several ligands simultaneously in a single run, and the investigation of competitive binding. In this context, the most important advantage of CE is that this technique allows the measurement of binding constants exactly under conditions in which enantioseparations are performed. Nevertheless, this technique cannot provide any direct information about molecular mechanisms of selector-selectand interactions [34].

Thus, the aim of this work was the selection of the optimal experimental conditions enabling the enantiomeric separation of a group of chiral phenoxy acid herbicides. A systematic evaluation of several chiral selectors was made. The influence of other experimental conditions on the enantiomeric separation as nature and pH of the background electrolyte, separation temperature, and concentration of chiral selector, was also studied. In addition, the apparent binding constants for each enantiomer with a neutral CD at two temperatures were calculated.

2 Materials and methods

2.1 Apparatus

Two capillary electrophoresis instruments have been used: (i) a Prince model from LauerLabs (Emmen, The Netherlands), equipped with a Lambda 1000 UV detector, and an acquisition data system Model Star 4.5 from Varian Associates (Sugar Land, TX, USA); and (ii) a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) and an HP 3D-CE Chemstation software. A 50 μm inner diameter (ID) and 375 μm outer diameter (OD) fused-silica capillary with an effective length of 50 cm (65 cm total length for the LauerLabs instrument and 58.5 cm for the HP^{3D} system) was employed (Polymicro Technologies, Phoenix, AZ, USA). Injection was performed hydrodynamically at 50 or 30 mbar for 2 s. Usual CE conditions for separations of the phenoxy acid compounds were: applied voltage, 20 kV; detection wavelength, 220 nm; and the capillary temperature was set at 20 or 40°C. Electrolytic solutions were degassed in an ultrasonic bath KM from Raypa (Barcelona, Spain). A 654 pH-meter from Metrohm (Herisau, Switzerland) was employed to measure the pH of the separation solutions.

2.2 Chemicals

All reagents were of analytical grade. Taurocholic acid sodium salt (STC) and cholic acid sodium salt (SC) were purchased from Sigma (St. Louis, MO, USA); sodium dihydrogen phosphate, tris(hydroxymethyl)aminomethane hydrochloride (Tris), 2-morpholinoethanesulfonic acid (MES), sodium dodecyl sulfate (SDS), and sodium hydroxide were supplied from Merck (Darmstadt, Germany); ammonium formate, ammonium acetate, β-CD, γ-CD, and urea were from Fluka (Buchs, Switzerland); (2-hydroxy)propyl-β-CD (HP-β-CD, averaged degree of substitution (DS)~3), methylated β-CD (PM-β-CD, DS~12–13), carboxymethylated β-CD (CM-β-CD, DS~3), carboxymethylated γ-CD (CM-γ-CD, DS~3), succinylated β-CD (Succ-β-CD, DS~3.5), and succinylated γ-CD (Succ-γ-CD, DS~3) were obtained from Cyclolab (Budapest, Hungary). Water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA). All solutions were filtered through 45-μm pore size disposable nylon filters from Scientific Resources (Eatontown, NJ, USA). The phenoxy acid herbicides 2-phenoxypropionic acid (2-PPA), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DCPPA), and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TCPPA) were purchased from Chem Service (West Chester, PA, USA). 2-(4-Chlorophenoxy)-2-methylpropionic acid (4-CPMPA), 2-(3-chlorophenoxy)-propionic acid (3-CPPA), and 2-(4-chlorophenoxy)propionic acid (4-CPPA) were obtained from Aldrich (Milwaukee, WI, USA). Figure 1 shows the structures of the herbicides studied. Methanol of HPLC grade (Labscan Ltd., Dublin, Ireland) was used for the preparation of phenoxy acid herbicides solutions.

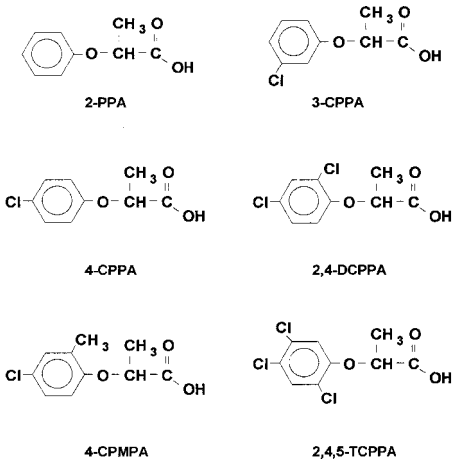

Figure 1. Structures of the phenoxy acid herbicides studied.

2.3 Procedure

Electrolyte solutions were prepared by dissolving the appropriate amount of sodium dihydrogen phosphate, ammonium formate, ammonium acetate, Tris or MES in Milli-Q water, and adjusting to the desired pH value with a 1 M solution of sodium hydroxide (for phosphate, Tris or MES), formic acid (for formate), or acetic acid (for acetate). The chiral selector (a bile salt or a CD) was dissolved in the corresponding electrolyte solution. Urea and SDS were added to the background electrolyte prior to adjusting the pH. When methanol was used as additive, it was added after electrolyte solution preparation. Stock solutions of phenoxy acids (5 mg/mL) were prepared by dissolving 10 mg of compound in 2 mL of methanol. The working solutions were obtained at 0.2 mg/mL by dilution of the stock solutions in methanol. In order to obtain good peak shapes and reproducible retention data, the capillary was conditioned at the beginning of the day, prior to each injection and at the end of the day. In all cases, the conditioning run included the following steps: (i) 2-min rinse with Milli-Q water; (ii) 2-min rinse with 0.1 M sodium hydroxide; and (iii) 2-min rinse with Milli-Q water at 1000 mbar. Before sample injection the capillary was also rinsed with the running separation solution for 2 min.

2.4 Calculations

Enantiomeric resolution was calculated by Eq. (1):

$$R_s = 1.18\,(t_2 - t_1) / (w_1 + w_2) \quad (1)$$

where t_1 and t_2 are the two enantiomers migration times and w_1, w_2 are the peak widths at half-height of the corresponding peaks. The apparent binding constants for each enantiomer-HP-β-CD pair were calculated from electrophoretic mobilities using linear plotting approaches by least squares methods. From the experimental data, the electrophoretic mobility of each enantiomer (μ_i) was determined by Eq. (2):

$$\mu_i = \frac{lL}{V}\left(\frac{1}{t_i} - \frac{1}{t_0}\right) \quad (2)$$

where L and l are the total capillary length and the length to the detector, respectively, V is the run voltage, t_i is the enantiomer migration time, and t_0 is the migration time of methanol, used as neutral marker to correct changes in solution viscosity caused by variations in CD concentration. The electrophoretic mobilities of free phenoxy acids, μ_f, were determined from injections using only the background electrolyte solution as separation solution, *i.e.*, under conditions in which the chiral selector concentration was zero. Experimental data were manipulated and parameters were calculated using Excel 7.0 from Microsoft Office software. The uncertainties in the binding constants and mobilities were calculated by error propagation methods using the errors in the slopes and intercepts obtained by least squares methods.

3 Results and discussion

3.1 Enantiomeric separation of phenoxy acids

In preliminary experiments, the enantiomeric resolution of phenoxy acid herbicides was tried by using different chiral selectors in the separation solution. Neutral CDs and charged CD derivatives were employed in EKC; bile salts and mixtures of SDS and neutral and charged CDs were used in micellar EKC (MEKC). The effect of nature, concentration, and pH of the background electrolyte, the chiral selector nature and concentration, as well as the use of additives, was evaluated. Table 1 summarizes some of the conditions investigated. The neutral CDs β-CD, HP-β-CD, PM-β-CD, and γ-CD were used under different experimental conditions. The native CD β-CD was tested using an ammonium formate solution at pH 6.5 at two concentrations, 2 and 20 mM. For the lower concentration, no enantioseparation was achieved, although the peaks corresponding to the phenoxy acids 2,4-DCPPA and 3-CPPA exhibited a little shoulder. An increase in the β-CD concentration led to the partial enantioseparation of herbicides 2-PPA and 3-CPPA. The use of 10 mM β-CD in acetate buffer at pH 4.6 only enabled the enantioseparation of 3-CPPA.

In the evaluation of HP-β-CD as chiral selector, Tris (pH 8.3), ammonium formate (pH 5 or 6.5), phosphate (pH 6.5), and ammonium acetate (pH 4.6) solutions were tested as background electrolytes. The concentration of

Table 1. Experimental conditions tested for the enantiomeric separation of phenoxy acid herbicides

Chiral selector	Concentration of chiral selector	Background electrolyte	Additives	Phenoxy acids partially separated enantiomerically
HP-β-CD	10 mM	50 mM Acetate/pH 4.6	–	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
	10 mM	50 mM Acetate/pH 4.6	50 mM SDS	None
	10 mM	50 mM Tris/pH 8.3	–	None
	10 mM	50 mM Tris/pH 8.5	50 mM SDS	None
	5 mM	50 mM Phosphate/pH 6.5	–	2,4-DCPPA, 3-CPPA, 4-CPPA
	10 mM	50 mM Phosphate/pH 6.5	50 mM SDS	None
	10, 40, 50 mM	50 mM Formate /pH 6.5	–	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
β-CD	10 mM	50 mM Acetate/pH 4.6	–	3-CPPA
	2 mM	50 mM Formate/pH 6.5	–	2,4-DCPPA, 3-CPPA
	20 mM	50 mM Formate/pH 6.5	–	2-PPA, 3-CPPA
	10 mM	50 mM Phospate/pH 6.5	50 mM SDS	None
PM–β-CD	10 mM	50 mM Tris/pH 8.3	–	None
	10 mM	50 mM Acetate/pH 4.6	–	None
γ-CD	10 mM	50 mM Phosphate/pH 6.5	50 mM SDS	None
10 mM HP-β-CD + 10 mMβ-CD		50 mM Acetate/pH 4.6	–	2-PPA, 3-CPPA
Succ-β-CD	10 mM	50 mM Phosphate/ pH 6.5	–	2-PPA, 3-CPPA, 4-CPPA
	10 mM	50 mM MES/pH 6.5	–	None
	15, 20 mM	100 mM Phosphate/ pH 6.5	–	2-PPA, 3-CPPA, 4-CPPA
	15 mM	100 mM Phosphate/ pH 6.5	5% Methanol	2-PPA, 3-CPPA, 4-CPPA
	10 mM	50 mM Phosphate/ pH 6.5	50 mM SDS	None
	10 mM	50 mM Phosphate/ pH 6.5	2%, 5% Methanol	2-PPA, 3-CPPA, 4-CPPA
	10 mM	50 mM Phosphate/ pH 6.5	5% Methanol	2-PPA, 3-CPPA, 4-CPPA
Succ-γ-CD	10 mM	50 mM Phosphate/ pH 6.5	–	None
CM-β-CD	10, 20 mM	50 mM Phosphate/ pH 6.5	–	None
	10 mM	50 mM Acetate/ pH 4.6	–	None
CM-γ-CD	10 mM	50 mM Phosphate/ pH 6.5	–	None
10 mM HP-β-CD + 10 mM CM-γ-CD		50 mM Phosphate/ pH 6.5	–	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
		50 mM MES /pH 6.5	–	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
		50 mM Formate /pH 5	–	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
		50 mM Formate /pH 6.5	0, 5, 10% Methanol	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
		50 mM Formate /pH 6.5	1 M Urea	2-PPA, 2,4-DCPPA, 3-CPPA, 4-CPPA
Sodium cholate	10 mM	50 mM Phosphate /pH 6.5	–	None
Sodium cholate	100 mM	50 mM Phosphate /pH 6.5	–	None
Sodium taurocholate	10 mM	50 mM Phosphate /pH 6.5	–	None
Sodium taurocholate	100 mM	50 mM Phosphate /pH 6.5	–	None

the background electrolyte was in all cases 50 mM. The use of phosphate as background electrolyte allowed the partial separation of three phenoxy acids (2,4-DCPPA, 3-CPPA, and 4-CPPA), while ammonium formate and ammonium acetate provided acceptable resolution for four of the herbicides studied (2-PPA, 2,4-DCPPA, 3-CPPA, and 4-CPPA). When acetate buffer at pH 4.6 was used, the resolutions achieved were similar, but migration times were longer than those obtained with an ammonium formate solution at pH 6.5. The use of Tris at pH 8.3 did not allow the separation of any phenoxy acid. The employment of methanol as additive in an ammonium formate solution improved the resolution for the phenoxy acids partially separated. However, an increase

in the migration times was also obtained (between 30 and 60 min for 10% methanol) (results not included in Table 1).

The use of PM-β-CD and γ-CD alone as chiral selectors in the background electrolyte did not enable chiral discrimination for any phenoxy acid (see Table 1). Since mixtures of CDs may originate changes in selectivity, a mixture of 10 mM in β-CD and 10 mM in HP-β-CD in 50 mM acetate buffer at pH 4.6 was also used, but only the partial separation of 2-PPA and 3-CPPA was observed (resolution 1.31 and 0.72, respectively). The anionic CD derivatives Succ-β-CD, Succ-γ-CD, CM-β-CD, and CM-γ-CD, and mixtures HP-β-CD/CM-γ-CD were also evaluated. The use of CM-β-CD, CM-γ-CD, or Succ-γ-CD in phosphate buffer at pH 6.5 did not produce enantioseparation. However, when the Succ-β-CD was used under the same conditions, the partial resolution of 2-PPA, 3-CPPA, and 4-CPPA was obtained, and, in some cases, the peaks corresponding to 2,4,5-TCPPA and 2,4-DCPPA showed a little shoulder. These results reveal the complexity of the enantiomer-CD interactions, since the overall selector-selectand interactions seem to be stronger than the electrostatic repulsion between the anionic CD and the anionic form of the phenoxy acids. On the other hand, when mixtures of HP-β-CD and CM-γ-CD were tested under the conditions grouped in Table 1, the resolutions obtained were worse than those achieved when HP-β-CD was used as a single chiral selector.

Phenoxy acids were also injected in several MEKC systems in which different background electrolytes, 50 mM in SDS and 10 mM in HP-β-CD, β-CD, γ-CD or Succ-β-CD were used. Under all of these conditions, no chiral resolution was observed for the herbicides studied. These results could be explained according to the hypothesis of the competitive interaction of phenoxy acids and surfactant molecules for incorporation into the CD cavity proposed by Gilar *et al.* [35]. When two bile salts, SC and STC, were used as chiral selectors (two concentrations tested in 50 mM phosphate buffer at pH 6.5), no chiral recognition was observed. Under any of the conditions tested, enantioresolution of the phenoxy acids 4-CPMPA and 2,4,5-TCPPA was achieved. This fact could be due to the greater size of these herbicides (see Fig. 1), that prevents the inclusion complex formation with the CDs employed.

Among the different chiral selectors considered, HP-β-CD was found to be the most appropriate for the enantioseparation of phenoxy acids probably due to the fact that this CD is a neutral hydrophilic derivative with a hydroxypropyl group in the CD ring, which enables the complexation required to achieve the enantiomeric separation of phenoxy acids which are acidic hydrophilic compounds. Then, a more exhaustive study on the influence of several parameters on the enantiomeric resolution when the HP-β-CD is used as chiral selector was performed in order to improve the resolution of the compounds studied.

3.2 Influence of chiral selector concentration, background electrolyte pH, and temperature on the enantiomeric separation of phenoxy acids with HP-β-CD

A 50 mM solution in ammonium formate with HP-β-CD as chiral selector was chosen to study the influence of several experimental parameters such as the CD concentration, pH of the background electrolyte, and temperature on the enantiomeric resolution of phenoxy acids. Figures 2 and 3 show the variation of the enantiomeric reso-

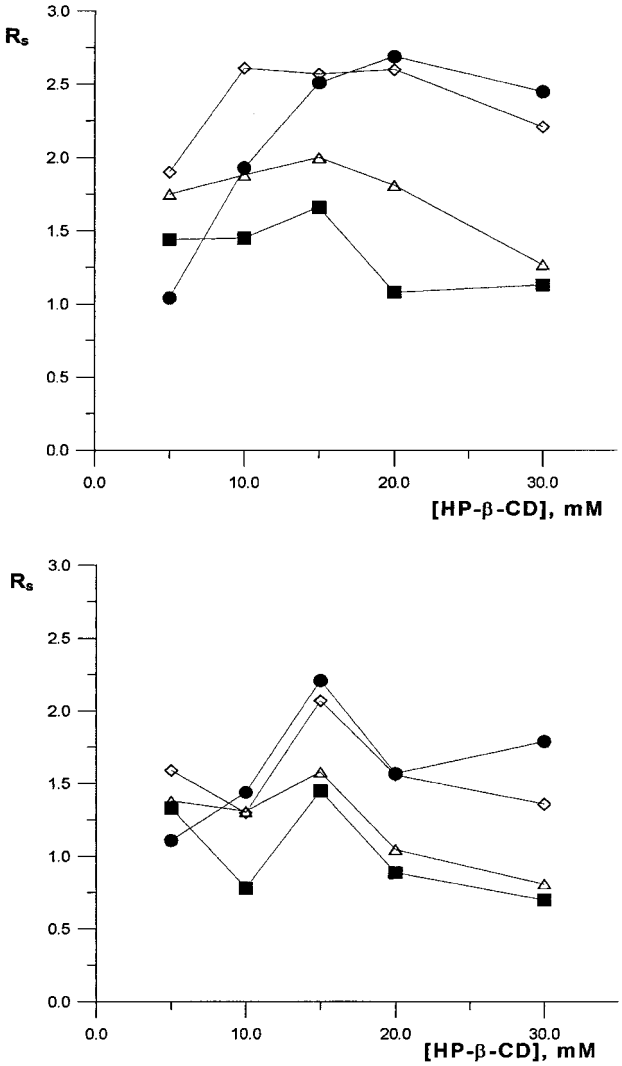

Figure 2. Effect of the HP-β-CD concentration on the resolution of the enantiomers. Background electrolyte, 50 mM ammonium formate at pH 5. Upper part, 20°C; lower part, 40°C. Symbols: (●) 2-PPA; (◇) 3-CPPA; (■) 4-CPPA; (□) 2,4-DCPPA.

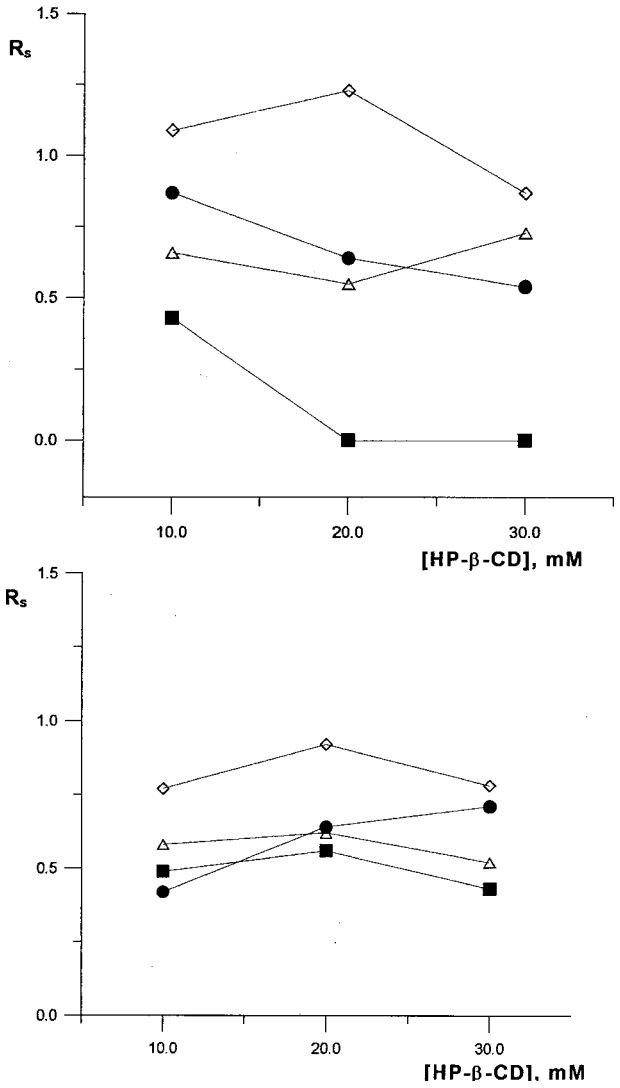

Figure 3. Effect of the HP-β-CD concentration on the resolution of the enantiomers. Background electrolyte, 50 mM ammonium formate at pH 6.5. Upper part, 20°C; lower part, 40°C. Symbols as in Fig. 2.

lution obtained for the phenoxy acids 2-PPA, 2,4-DCPPA, 3-CPPA, and 4-CPPA as a function of the HP-β-CD concentration using a 50 mM solution in ammonium formate at pH 5 and 6.5, respectively, as background electrolyte. The results were obtained at two temperatures, 20°C (Figs. 2 and 3, upper part) and 40°C (Figs. 2 and 3, lower part). In all cases, resolutions were better at pH 5 probably due to the increase obtained in the electroosmotic flow when increasing the pH which shortens the analysis time decreasing in turn the separation selectivity [34]. For both temperatures, at pH 5, the enantioresolution is maximum at intermediate concentrations of HP-β-CD in the range studied. When a 15 mM concentration of HP-β-CD is used, the two enantiomers of the four above-mentioned phenoxy acids studied were baseline-resolved. Resolutions obtained at pH 5 were higher than those at pH 6.5 although an increase in the migration times was also observed with decreasing pH values. On the other hand, the variation of the enantioresolution as a function of the chiral selector concentration at pH 6.5 did not show a clear optimum at any of the concentrations employed.

Temperature is considered as one of the key parameters in the optimization process of CE separations. A general behavior when changing temperature is not observed for a chiral separation. A decrease or an increase in the enantiomeric resolution can be obtained for different analytes being possible to explain these differences through the effects that temperature may have on peak efficiency, buffer viscosity, or selector-selectand interactions [34]. Figures 2 and 3 show that a slightly increase in the enantiomeric resolution was generally obtained for the chiral phenoxy acids herbicides studied when temperature was decreased. These results can be explained by two effects: (i) a decrease in the buffer viscosity when increasing the temperature (a decrease in the analysis time was obtained at 40°C) which favors the diffusional band broadening and (ii) a more similar interaction between each enantiomer of a given compound and the CD at 40°C than at 20°C. An exception to this general above-mentioned behavior was observed for 4-CPPA at pH 6.5, for which at 20°C, partial enantiomeric separation was only achieved at a HP-β-CD concentration of 10 mM, and no separation was reached when the chiral selector concentration was increased. However, partial enantioresolution could be observed at all concentrations tested when the temperature was increased (40°C). Figure 4 shows the separation of the phenoxy acids studied at 40°C, using a solution of 50 mM in ammonium formate at pH 5 and 15 mM in HP-β-CD. Although under these conditions the phenoxy acids 4-CPMPA and 2,4,5-TCPPA were not enantiomerically resolved, they could be separated in multicomponent mixtures as it is shown in these figures. At 20°C separations obtained were similar but implied migration times close to 20 min (results not shown).

3.3 Determination of analyte-selector binding constants for enantiomers

The determination of the constants that characterize the interactions of enantiomers with chiral selectors is very important in both the research of chiral separations and in their practical utilization. In CE the determination of analyte-selector binding constants can be made using a theoretical model which relate the effective mobilities of the enantiomers as a function of the concentration of the chiral selector in the separation media according to Eq. (3) [34]:

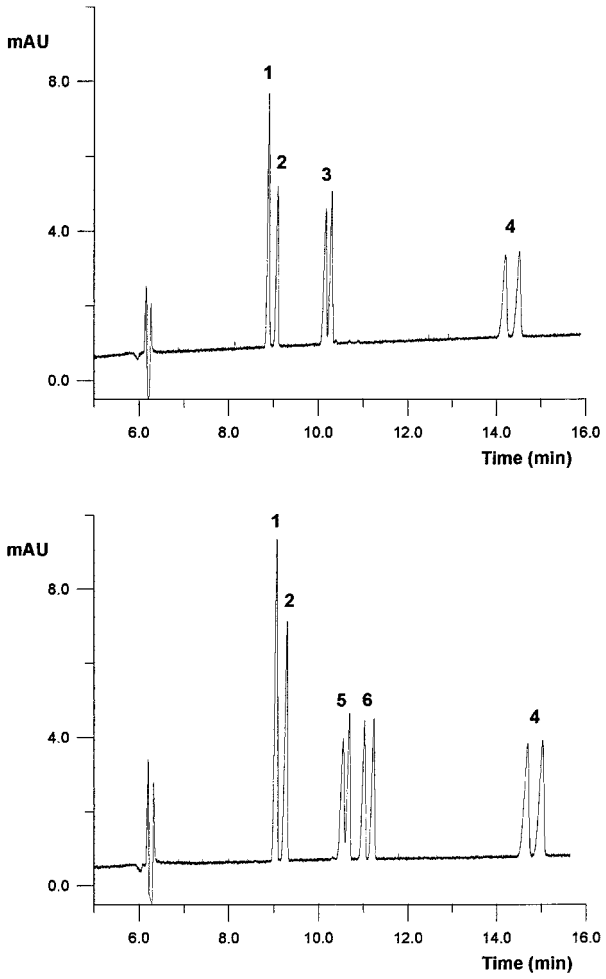

Figure 4. Separation of phenoxy acid herbicides. (1) 4-CPMPA; (2) 2,4,5-TCPPA; (3) 2,4-DCPPA; (4) 2-PPA; (5) 4-CPPA, and (6) 3-CPPA. Background electrolyte: 50 mM ammonium formate at pH 5 containing 15 mM HP-β-CD. Capillary, 50 μm × 50 cm uncoated fused-silica; injection, 50 mbar for 2 s; run voltage, 20 kV; temperature, 40°C; detection wavelength, 220 nm.

$$K = \frac{1}{[L]} \frac{\mu_f - \mu_i}{\mu_i - \mu_c} \quad (3)$$

where K is the binding constant, [L] is the equilibrium concentration of the uncomplexed ligand, μ_f and μ_c are the electrophoretic mobilities of the free and complexed solute and μ_i is the solute mobility at the ligand concentration [L]. Although binding constants calculated in this manner lack of thermodynamic value, they can be useful in the study on structure-affinity relationships between the analytes and the chiral selector used. The mobility of the free solute, μ_f, and the solute mobility, μ_i, can be determined experimentally at concentration zero and at the different concentrations of HP-β-CD considered, respectively. Nevertheless, although an estimation of the mobility of the complex, μ_c, can be made at very high concentrations of CD, sometimes its measurement is difficult or impossible due to the difficulty to find suitable CD markers and to reach saturating conditions. As a consequence, we have used in this work the three plotting forms (Table 2), obtained by rearranging Eq. (3), which do not require the direct measurement of μ_c [34].

To calculate the CD-analyte binding constants, the mobilities were measured at different concentrations of chiral selector. Tables 3 and 4 list the binding constants, enantioselectivities of complexation [34], and electrophoretic mobilities of the complexed solute, μ_c, calculated using the three plotting forms of Table 2 for the chiral phenoxy acids enantioseparated and HP-β-CD. The enantioselectivities of complexation (α) were calculated from the binding constants as $\alpha = K_1/K_2$ being K_1 and K_2 the binding constants for the first and second-migrating enantiomers with HP-β-CD. In all cases, acceptable error limits and good correlation coefficients were achieved although, the correlation coefficients obtained by double- and *y*-reciprocal methods were, in general, slightly higher than those obtained by the *x*-reciprocal method. The values of the binding constants and the mobilities μ_c obtained by

Table 2. Plotting forms of Eq. (3)

Plotting method	Equation	K	μ_c–μ_f
Double reciprocal $\frac{1}{\mu_i - \mu_f}$ *vs.* $\frac{1}{[L]}$	$\frac{1}{\mu_i - \mu_f} = \frac{1}{(\mu_c - \mu_f)K} \frac{1}{[L]} + \frac{1}{(\mu_c - \mu_f)}$	$\frac{\text{Intercept}}{\text{Slope}}$	$\frac{1}{\text{Intercept}}$
y-Reciprocal $\frac{[L]}{\mu_i - \mu_f}$ *vs.* [L]	$\frac{[L]}{\mu_i - \mu_f} = \frac{1}{(\mu_c - \mu_f)}[L] + \frac{1}{(\mu_c - \mu_f)K}$	$\frac{\text{Slope}}{\text{Intercept}}$	$\frac{1}{\text{Slope}}$
x-Reciprocal $\frac{\mu_i - \mu_f}{[L]}$ *vs.* $(\mu_i - \mu_f)$	$\frac{\mu_i - \mu_f}{[L]} = -K(\mu_i - \mu_f) + K(\mu_c - \mu_f)$	−Slope	$-\frac{\text{Intercept}}{\text{Slope}}$

Table 3. Binding constants, enantioselectivities of complexation and complex mobility for the phenoxy acid herbicides studied with HP-β-CD in 50 mM ammonium formate (pH 5) at 20°C

Plotting method	K_1 [a]	K_2 [a]	α [b]	μ_c (1) [c]	μ_c(2) [c]	r (1) [d]	r (2) [d]
2-PPA							
Double-reciprocal	14.73 ± 1.02	11.99 ± 0.20	1.23	–	–	0.9998	0.9997
y-Reciprocal	14.05 ± 0.96	11.60 ± 0.94	1.21	–	–	0.996	0.99
x-Reciprocal	14.15 ± 1.17	11.64 ± 1.13	1.22	–	–	0.99	0.99
4-CPPA							
Double-reciprocal	77.61 ± 0.87	57.62 ± 7.63	1.35	−4.52E-9 ± 0.04E-9	−5.92E-9 ± 0.69E-9	0.9999	0.99
y-Reciprocal	78.90 ± 0.98	59.15 ± 7.38	1.33	−4.66E-9 ± 0.03E-9	−6.47E-9 ± 0.63E-9	0.9999	0.99
x-Reciprocal	78.37 ± 1.31	61.57 ± 9.96	1.27	−4.60E-9 ± 0.33E-9	−6.81E-9 ± 2.67E-9	0.9996	0.98
3-CPPA							
Double-reciprocal	72.06 ± 0.89	58.16 ± 2.80	1.24	−4.35E-9 ± 4.43E-9	−3.37E-9 ± 1.42E-9	0.9999	0.999
y-Reciprocal	73.70 ± 1.03	63.04 ± 2.50	1.17	−4.53E-9 ± 3.82E-9	−4.18E-9 ± 1.10E-9	0.9999	0.999
x-Reciprocal	73.08 ± 1.38	60.98 ± 3.89	1.20	−4.45E-9 ± 3.71E-9	−3.86E-9 ± 1.28E-9	0.9990	0.999
2,4-DCPPA							
Double-reciprocal	92.77 ± 3.21	86.52 ± 1.00	1.07	–	−5.12E-9 ± 0.05E-9	0.999	0.999
y-Reciprocal	88.74 ± 2.92	86.07 ± 1.42	1.03	–	−5.09E-9 ± 0.04E-9	0.999	0.999
x-Reciprocal	90.13 ± 4.31	86.63 ± 1.63	1.04	–	−5.13E-9 ± 0.35E-9	0.99	0.997

a) Binding constants (M^{-1}) for the first and second-migrating enantiomers
b) Enantioselectivities of complexation (α) were calculated as $\alpha = K_1 / K_2$
c) Electrophoretic mobilities of the enantiomer-CD complex in $m^2 V^{-1} s^{-1}$ for the first and second-migrating enantiomers
d) Squared correlation coefficient obtained by least squares method

Table 4. Binding constants, enantioselectivities of complexation and complex mobility for the phenoxy acid herbicides studied with HP-β-CD in 50 mM ammonium formate (pH 5) at 40°C

Plotting method	K_1 [a]	K_2 [a]	α [b]	μ_c (1) [c]	μ_c(2) [c]	r (1) [d]	r (2) [d]
2-PPA							
Double-reciprocal	58.09 ± 7.08	51.59 ± 2.82	1.13	−15.26E-9 ± 1.62E-9	−13.02E-9 ± 0.64E-9	0.995	0.999
y-Reciprocal	59.86 ± 6.01	56.71 ± 2.47	1.06	−15.86E-9 ± 1.41E-9	−14.03E-9 ± 0.43E-9	0.99	0.999
x-Reciprocal	60.12 ± 3.33	54.62 ± 3.85	1.10	− 8.18E-9 ± 1.49E-9	−13.65E-9 ± 1.42E-9	0.997	0.99
4-CPPA							
Double-reciprocal	97.52 ± 3.29	72.83 ± 3.48	1.34	− 8.69E-9 ± 0.21E-9	− 9.08E-9 ± 0.36E-9	0.999	0.999
y-Reciprocal	93.30 ± 3.02	67.08 ± 2.78	1.39	− 8.26E-9 ± 0.14E-9	− 8.14E-9 ± 0.22E-9	0.9996	0.999
x-Reciprocal	94.83 ± 4.44	69.08 ± 4.37	1.37	− 8.41E-9 ± 1.21E-9	− 8.46E-9 ± 1.59E-9	0.997	0.99
3-CPPA							
Double-reciprocal	78.75 ± 0.98	77.39 ± 3.39	1.02	−14.25E-9 ± 0.14E-9	− 8.60E-9 ± 0.30E-9	0.999	0.9999
y-Reciprocal	78.72 ± 1.26	71.65 ± 2.77	1.10	−14.25E-9 ± 0.13E-9	− 7.74E-9 ± 0.18E-9	0.999	0.9999
x-Reciprocal	79.02 ± 1.53	73.76 ± 4.34	1.07	−14.28E-9 ± 0.36E-9	− 8.05E-9 ± 0.15E-9	0.9950	0.999
2,4-DCPPA							
Double-reciprocal	95.20 ± 2.98	89.39 ± 3.17	1.06	− 8.56E-9 ± 0.19E-9	− 8.61E-9 ± 0.23E-9	0.999	0.999
y-Reciprocal	91.24 ± 2.72	85.36 ± 2.84	1.07	− 8.16E-9 ± 0.13E-9	− 8.16E-9 ± 0.15E-9	0.9996	0.9995
x-Reciprocal	92.68 ± 4.02	86.70 ± 4.22	1.07	− 8.29E-9 ± 0.11E-9	− 8.30E-9 ± 1.18E-9	0.997	0.996

(a) – (d) see Table 3

the different linear plotting methods did not differ significantly, and they are similar to those reported in literature [34] for the complex formation of 2-phenoxypropionic acid and 2-(4-chlorophenoxy)propionic acid with HP-β-CD in 50 mM phosphate buffer at pH 7.

Similar values for the binding constants were obtained for three of the phenoxy acids enantiomerically resolved in this work (3-CPPA, 4-CPPA, and 2,4-DCPPA), these constants being lower for 2-PPA. This result could be explained by similarities in the molecular structures of 3-CPPA, 4-CPPA, and 2,4-DCPPA (Fig. 1) compared with 2-PPA, of which the aromatic ring is not chlorinated. On the other hand, although binding constants for phenoxy acids herbicides generally increased with the temperature, the difference between binding constants for the two enantiomers of a given compound decreased when increasing the temperature. In fact, enantioselectivities of complexation, which ranged from 1.02 to 1.39, were similar at 20 and 40°C for 4-CPPA and 2,4-DCPPA whereas decreasing values were obtained for 2-PPA and 3-CPPA when the temperature increased from 20 to 40°C.

4 Concluding remarks

As a result of the screening of various chiral selectors (native CDs, neutral and anionic CD derivatives, and bile salts) under several experimental conditions, HP-β-CD has demonstrated to be the most useful chiral selector for the baseline resolution of the enantiomers of the phenoxy acids studied in this work. Thus, from the six phenoxy acids studied, four of them (2-PPA, 3-CPPA, 4-CPPA, and 2,4-DCPPA) have been enantiomerically separated using HP-β-CD. In addition, a study on the influence of the chiral selector concentration, background electrolyte, pH and temperature revealed that a concentration of 15 mM HP-β-CD in 50 mM ammonium formate at pH 5 and 40°C enabled to obtain the baseline resolution of the enantiomers of four phenoxy acids. In addition, separation of multicomponent mixtures was also possible.

Finally, CE has been applied to the determination of the apparent analyte-selector binding constants for the four phenoxy acids enantioseparated. Similar values for the binding constants were obtained for three of the phenoxy acids enantiomerically resolved in this work (3-CPPA, 4-CPPA, and 2,4-DCPPA), these constants being lower for 2-PPA of which the aromatic ring is not chlorinated. On the other hand, enantioselectivities of complexation were similar at 20 and 40°C for 4-CPPA and 2,4-DCPPA whereas decreasing values were obtained for 2-PPA and 3-CPPA when the temperature increased from 20 to 40°C due to the lower difference between binding constants for the two enantiomers of these compounds when increasing temperature.

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