Modelling-based optimization of the simultaneous enantiomeric separation of multicomponent mixtures of phenoxy acid herbicides using dual cyclodextrin systems by Capillary Electrophoresis

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Abstract

In this work, the model previously proposed by Dubsky et al. [1] for Capillary Electrophoresis (CE) enantioseparation systems with a mixture of chiral selectors, was applied to the rapid optimization of the simultaneous enantiomeric separation of a multicomponent mixture of six phenoxy acid herbicides using a dual system of two cyclodextrins (CDs), (2-hydroxypropyl)-β-CD (HP-β-CD) and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD). The full experimental optimization of enantiomeric separations using dual systems can be time-consuming since there are many possibilities to be assayed regarding the total concentration of both chiral selectors and the proportion of each one in the mixture for a given total concentration of both. The simultaneous separation of these twelve enantiomers was achieved in a previous work using the procedure of trial and error. Enantiomeric resolutions ranging from 1.1 to 2.7 were obtained for the six phenoxy acid herbicides using a mixture of both CDs of 7 mM HP-β-CD and 20 mM TM-β-CD. The model proposed by Dubsky et al. enables to foresee the results that could be obtained for any possible combination of concentrations and relative proportion of both CDs in the mixture, from a small amount of individual experiments carried out separately with each CD. Results obtained in this work demonstrated that the model was successful by improving the previous results experimentally obtained by the trial and error method for the simultaneous enantiomeric separation of the six phenoxy acid herbicides studied in this work. In fact, the separation was improved in terms of enantiomeric resolutions obtained (from 1.2 to 4.2 for concentrations of CDs of 4 mM HP-β-CD and 16 mM TM-β-CD) and by considerably reducing the time to optimize the separation conditions enabling to find, in a faster and efficient way, the most adequate proportion of both CDs and the concentration of each CD in the mixture in order to obtain the base line separation of the twelve enantiomers.

Additionally, the apparent complexation constants between enantiomers and each CD were calculated. This is the first time that the above-mentioned model was applied to a multicomponent mixture of chiral compounds.

Keywords: simultaneous chiral separation, enantiomers, phenoxy acid herbicides, electrokinetic chromatography, modelling-based optimization, cyclodextrin dual systems.

1. Introduction

The analysis of chiral compounds presents a great interest in different areas of science due to the different properties that the enantiomers of a chiral compound may have. In the environmental field, many of the agrochemicals used are chiral although in many cases only one of the stereoisomers possesses the desired pesticide activity [2]. This is the case of phenoxy acid herbicides, a class of pesticides extensively used in agriculture, for which the R-enantiomer is biologically active, whereas the other isomer is inactive or less active enantiomer [2]. The use of the racemic mixtures of these agrochemicals unnecessarily increases the environmental pollution.

Capillary Electrophoresis (CE) has shown a big potential in the field of chiral separations [3] due to some advantages such as the use of low amounts of reagents and samples, high efficiency and resolution, and simplicity since no chiral columns are needed. In fact, a chiral selector is added to the separation medium in the CE mode named Electrokinetic Chromatography (EKC). Among the chiral selectors that can be used in CE, the native cyclodextrins (CDs) and their derivatives are, undoubtedly, the most used due to their high discrimination power [4]. In spite of this, in some cases, the use of a CD in the separation medium does not enable to achieve the desired enantiomeric separation, so the use of a mixture of two CDs in a dual system is required. However, this increases the difficulty of the optimization procedure due to the high number of possibilities to be tested by varying the total CDs concentration and their proportion in the mixture.

In a previous work of our research group, the chiral separation of a multicomponent mixture of six chiral phenoxy acid herbicides (fenoprop, mecoprop, dichlorprop, 2-(4-chlorophenoxy)propionic acid (4-CPPA), 2-(3-chlorophenoxy)propionic acid (3-CPPA) and 2-phenoxypropionic acid (2-PPA)) was carried out by CE with CDs as chiral

selectors [5]. For this purpose, a trial and error procedure was used. An initial screening of neutral CDs was carried out (α-CD, β-CD, heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD), heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD), (2-hydroxypropyl)-β-CD (HP-β-CD) and (2-hydroxypropyl)-β-CD (HP-γ-CD)) but the complete separation of the twelve enantiomers was not possible. Different combinations of binary CD mixtures were tested being possible the enantiomeric separation of the six compounds studied when using the dual CD system consisting in a mixture of 7 mM HP-β-CD + 20 mM TM-β-CD. Nevertheless, under these conditions, baseline separation was not complete for fenoprop enantiomers and a co-elution was observed between one of the enantiomers of mecoprop and an enantiomer of dichlorprop. Furthermore, in practice, this trial and error procedure does not allow the evaluation of all possible combinations in terms of ratios and concentrations of the two CDs used in the binary mixture.

In this context, different equations have been proposed to describe single and dual CDs systems in EKC. For single CD systems, Wren and Rowe [6] gave an equation to calculate the apparent complexation constants (K_C) and the electrophoretic mobilities of the complexes analyte-CD (μ_C) assuming the stoichiometry of the analyte-CD complexation equilibrium is 1:1,

$$\mu_{A,eff} = \frac{\mu_{A,f} + \mu_C K_C [S]}{1 + K_C [S]} \quad (1)$$

where, $\mu_{A,eff}$ is the effective electrophoretic mobility of the analyte, $\mu_{A,f}$ the electrophoretic mobility of the free analyte, that is, in the absence of CD; and [S] is the concentration of the chiral selector, in this case, the concentration of CD that remains free in the complexation equilibrium with the analyte.

Based on this equation (Eq. (1)), Dubsky et al. proposed a theoretical model that allows predicting what would happen for any possible combination of concentrations and molar fractions in a dual system of two CDs by performing a few series of individual experiments with each CD separately at different concentrations [1]. This model is based on the following equation:

$$\mu_{\mathrm{A,eff}} = \frac{\mu_{\mathrm{A,f}} + \mu_{\mathrm{C}}^{\mathrm{over}} K_{\mathrm{C}}^{\mathrm{over}} c_{tot}}{1 + K_{\mathrm{C}}^{\mathrm{over}} c_{tot}} \quad (2)$$

where, $\mu_{\mathrm{A,eff}}$ is the effective electrophoretic mobility of the analyte; $\mu_{\mathrm{A,f}}$ is the electrophoretic mobility of the free analyte, in the absence of CDs; c_{tot} is the total concentration of CDs, that is, the sum of the concentrations of the CDs involved; K_C^{over} represents the global apparent complexation constant, which is calculated with the following equation:

$$K_{\mathrm{C}}^{\mathrm{over}} = \sum_i \chi_i \, K_i \quad (3)$$

and μ_C^{over} is the global electrophoretic mobility of the analyte-CD complexes, which is determined with the equation:

$$\mu_{\mathrm{C}}^{\mathrm{over}} = \frac{\sum_i \chi_i \mu_i K_i}{\sum_i \chi_i K_i} = \frac{\sum_i \chi_i \mu_i K_i}{K_{\mathrm{C}}^{\mathrm{over}}} \quad (4)$$

In both Eqs. (3) and (4) χ_i, K_i and μ_i represent the molar fraction of each CD in the mixture, its K_{C} with the analyte and the μ_C of the complex, respectively.

This model assumes that the complexation reaction between an enantiomer and any CD in the mixture is much faster than the separation and interconversion, the concentration of the enantiomers is small enough for not to change the concentration of free CDs, and that enantiomers interact with any of the CDs in a 1:1 ratio [1]. Under these

considerations, the model is valid for single-CD and for multi-CDs enantioseparation systems. To our knowledge, the theoretical model of Dubsky et al., has been applied to predict the chiral separation of lorazepam enantiomers [7], and also, to predict the non-enantiomeric separation of a mixture of ibuprofen and flurbiprofen drugs [8]. However, it has never been applied to the optimization of the simultaneous enantiomeric separation of multicomponent mixtures of chiral compounds.

In the present work, the model proposed by Dubsky et al. [1] for CE enantioseparation systems with a mixture of chiral selectors has been applied, for the first time, to the rapid optimization of the simultaneous enantiomeric separation of a multicomponent mixture of the six phenoxy acid herbicides previously separated by our research group using the trial and error method employing a dual CD system (HP-β-CD + TM-β-CD). The objective was to improve the separation obtained and considerably reduce the optimization time of the separation conditions. In this way, it is possible to find more quickly and efficiently the proportion in which both CDs should be mixed, and the proper concentration of each of them to achieve complete baseline separation of the twelve enantiomers of the mixture.

2. Materials and methods

2.1.Chemicals, reagents and standard solutions

Ortho-phosphoric acid 85% and methanol (MeOH) were purchased from Scharlau Chemie (Barcelona, Spain), sodium hydroxide (NaOH) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany). The water employed was obtained from a Millipore Milli-Q-System (Bedford, MA, USA).

147 TM-β-CD (molecular weight 1429.54 g mol^{-1}) was purchased from Sigma-Aldrich (St.
148 Louis, MO, USA) and HP-β-CD (average degree of substitution ~ 4, molecular weight
149 1380 g mol^{-1}) was bought in Fluka (Buchs, Switzerland).

150 Racemic standards (1:1, v/v) of phenoxy acid herbicides with high purity (> 98%):
151 (R,S)-2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop, molecular weight 269.51 g
152 mol^{-1}), (R,S)-2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop, molecular
153 weight 214.65 g mol^{-1}), (R,S)-2-(2,4-dichlorophenoxy)propionic acid (dichlorprop,
154 molecular weight 235.064 g mol^{-1}), (R,S)-2-(4-chlorophenoxy)propionic acid (4-CPPA,
155 molecular weight 200.618 g mol^{-1}) and (R,S)-2-(3-chlorophenoxy)propionic acid (3-
156 CPPA, molecular weight 200.62 g mol^{-1}) were purchased from Sigma-Aldrich (St.
157 Louis, MO, USA) and (R,S)-2-phenoxypropionic acid (2-PPA, molecular weight 166.17
158 g mol^{-1}) was from Chem Service (West Chester, USA). Stock standard solutions (1000
159 mg L^{-1}) of each analyte were prepared in MeOH and stored at 4 ˚C. Working standard
160 solutions containing a mixture of the analytes at different concentration levels were
161 prepared by appropriate dilution of the stock solutions with Milli-Q water until desired
162 concentration.

163 *2.2. Instrumentation*

164 CE experiments were performed on a HP ^{3D}CE system from Agilent Technologies (Palo
165 Alto, CA, USA) equipped with a diode array detector (DAD) controlled by a HP ^{3D}CE
166 ChemStation software. Separation was achieved using an uncoated fused-silica capillary
167 of 50 μm I.D. with a total length (L_t) of 58.5 cm (50 cm effective capillary length (L_d))
168 from Polymicro Technologies (Phoenix, AZ, USA).

169 *2.3. Experimental conditions*

The initial experimental conditions were based on a previous work of our research group [5]. However, in the present work, different concentrations of each CD in single solutions were investigated (0-35 mM) and different combinations of both CDs in dual systems were tested by changing the total CDs concentration and their molar fraction. The background electrolyte (BGE) consisted of 50 mM phosphate buffer (pH 7.0). The stock solution of each chiral selector was prepared by directly dissolving the corresponding CD amount in the phosphate buffer to obtain the highest CD concentration used. BGEs containing a single chiral selector at lower concentrations were prepared by diluting the stock solution of the corresponding CD with phosphate buffer. For dual selector systems, BGEs containing both CDs were prepared by mixing the stock solutions of the single selectors in the required ratio to obtain the desired concentration. Samples contained the six phenoxy acid herbicides and DMSO as electroosmotic flow (EOF) marker (0.01 %, v/v) in Milli-Q water. Samples did not contain any chiral selector. Injections were performed in hydrodynamic mode by applying 50 mbar for 10 s, and the electrophoretic separation was achieved at 15 ˚C in positive-polarity mode (25 kV). Detection was carried out with a response time of 1.0 s and a wavelength of 194 nm for 2-PPA and 4-CPPA, 200 nm for mecoprop, dichlorprop and 3-CPPA, and 210 nm for fenoprop (bandwidth 5 nm). At the beginning of each working day, the capillary was flushed with 0.1 M NaOH, Milli-Q water, buffer solution and BGE during 5, 5, 5 and 10 min, respectively. To ensure repeatability between injections, the capillary was conditioned 2 min with Milli-Q water, 2 min with 0.1M NaOH, 2 min with Milli-Q water and 5 min with BGE.

2.4. Data treatment

The effective electrophoretic mobility (μ_{eff}) was calculated using the following equation:

$$\mu_{\text{eff}} = \frac{L_d\, L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (5)$$

L_d is the effective capillary length; L_t is the total capillary length; V is the voltage; t_m is the migration time; and t_0 is the EOF time (determine with the EOF marker).

The HP ^{3D}CE ChemStation software of Agilent Technologies was used for data collection and acquisition. Data treatment and calculations were performed with the software OriginPro 8.0 and Microsoft office Excel 2016.

3. Results and discussion

3.1. Apparent complexation constants of the phenoxy acid enantiomers with HP-β-CD and TM-β-CD

In order to apply the model proposed by Dubsky et al. [1] using Eq. 2 to the optimization of the simultaneous enantiomeric separation of the multicomponent mixture of phenoxy acid herbicides, the K_C for each enantiomer and the μ_C were firstly determined for each CD (HP-β-CD and TM-β-CD). For this purpose, electrophoretic assays were performed in duplicate with each enantiomeric pair of phenoxy acids at different CD concentrations (0, 5, 10, 15, 25 and 35 mM) using a 50 mM phosphate buffer at pH 7.0 and a temperature of 15 ^{o}C. The type of separation buffer and the working conditions were previously optimized by our research group [5] and the injected concentration of each enantiomer was 5 μg mL^{-1}. From the migration times obtained, the μ_{eff} for each enantiomer was determined for each CD concentration according to Eq. (5) (see Tables S1 and S2 in supplementary material).

Figure 1 shows, by way of example, the variation of the μ_{eff} for fenoprop enantiomers as a function of the concentration of each CD (HP-β-CD and TM-β-CD) in the separation buffer. Results obtained for the other analytes studied in this work are shown in Figures

S1 to S5 in supplementary material. The red lines indicate the non-linear adjustment of the experimental points to the Wren and Rowe equation [6], which enables to obtain K_C and μ_C values by assuming the stoichiometry of the analyte-CD complexation equilibrium is 1:1, according to Eq. (1). Since the CD concentrations used are much higher than the ones of the phenoxy acids, it can be considered that the concentration of free CD is approximately equal to the total concentration set in the separation buffer. The K_C and μ_C values for all the enantiomers were obtained from the parameters provided by the non-linear adjustment of Eq. (1), where $\mu_{A,eff}$ and [S] are the variables involved.

Table 1 collects the values obtained for the K_C and μ_C for each of the enantiomers of the phenoxy acids studied with HP-β-CD and TM-β-CD. Both working temperature and the ionic strength of the separation buffer were kept constant. It was not necessary to perform corrections in the $\mu_{A,eff}$ obtained, since changes in the viscosity of the medium due to the variation of the CD concentration were not appreciable, as evidenced by the fact that currents barely varied with the CD concentration (Tables S1 and S2 in supplementary material) [9].

The values of μ_C corresponding to the enantiomeric pair of 2-PPA with HP-β-CD (Table 1) lack of real physical sense, since in the experimental conditions of pH 7.0 the phenoxy acid-CD complexes should have negative charge, as the CDs used are neutral and the phenoxy acids would be negatively charged due to their carboxylic acid functional groups. The same happens in the case of 3-CPPA and 2-PPA enantiomers with TM-β-CD (Table 1). This may be due to the low values of their K_C (Table 1), thus the analyte fraction (Table S3 in supplementary material) that forms the complex is below 0.2 and variations in the CD concentration lead to small variations in the $\mu_{A,eff}$ of

the analyte that may be lower than the experimental random error. The same arises when the complex fraction of analyte is greater than 0.8. Therefore, the appropriate working range is between 0.2 and 0.8, in terms of the fraction of analyte complexed [10]. Nonetheless, all the data in Table 1 have been used for modelling and theoretical predictions about the best conditions to achieve the simultaneous separation of the multicomponent mixture of phenoxy acids using a dual system based on HP-β-CD and TM-β-CD. Moreover, these data have been useful for the prediction of the experimental behavior observed, as it will be seen in the experimental data shown in section 3.3.

3.2. Prediction of the conditions for the simultaneous separation of the multicomponent mixture of phenoxy acids using the dual system HP-β-CD + TM-β-CD

The determination of K_C and μ_C values of the analytes with HP-β-CD and TM-β-CD previously described in section 3.1, enabled to predict what may happen in a dual system using HP-β-CD and TM-β-CD together in the separation buffer at any total concentration of the sum of both CDs and at any molar fraction of both CDs in the mixture, according to the model proposed by Dubsky et al. [1]. This model predicts the behavior of the dual system through a similar equation (Eq. (2)) to that of Wren and Rowe for a single CD (Eq. (1)) [1]. K_C^{over} and μ_C^{over} parameters were obtained from the K_C and μ_C values calculated for the analytes with HP-β-CD and TM-β-CD in the previous section 3.1 using Eq. (3) and Eq. (4). With these equations, the $\mu_{A,eff}$ was determined depending on the c_{tot} in the range from 0 to 40 mM and on the molar fraction of each CD in the range from 0 to 1 for each total CDs concentration. Table 2 shows, by way of example, the $\mu_{A,eff}$ predicted for each analyte obtained for a molar fraction of 0.2 HP-β-CD and 0.8 TM-β-CD. A Table gathering the predicted $\mu_{A,eff}$ data for each analyte obtained using other molar fractions values for HP-β-CD and TM-β-

CD (0, 0.1, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 y 1) is included in the supplementary material (Table S4 in supplementary material). In this table, data have been sorted according to the expected migration order. According to the results obtained, fenoprop enantiomers would be the first migrating enantiomers, while 2-PPA enantiomers would be the last ones. Nevertheless, it was not possible to identify each enantiomer because pure enantiomer standards of the target analytes were not available and the racemic mixtures were 1:1 (v/v), so the height and area of the peaks of both enantiomers of the same analyte were equivalent, not being possible discrimination among them. Table 2 shows that, for a molar fraction of 0.2 HP-β-CD and 0.8 TM-β-CD, the model only predicts a change in the migration order in the case of 4-CPPA and 3-CPPA for a concentration 0 mM of CDs. In that case, according to the model, 3-CPPA would migrate before 4-CPPA. For other molar fractions and total CDs concentrations, the model also predicts changes in the migration order for these two above-mentioned compounds and others, as indicated in Table S4 in supplementary material.

In the experiments performed in section 3.1, two hypothesis were considered: (i) the enantiomer that migrates first using HP-β-CD as chiral selector also migrates first with TM-β-CD, and (ii) the enantiomer that migrates first using HP-β-CD as chiral selector, migrates second with TM-β-CD. This affects the assignment of K_C and μ_C values to one or the other enantiomer. However, the results obtained for K_C^{over} and μ_C^{over} with the HP-β-CD + TM-β-CD mixture applying the model of Dubsky et al. [1] (Table 2) were identical for both hypothesis, although it was not possible to identify which peak corresponded to each enantiomer. Nonetheless, this limitation does not affect the objective of the present work which is to predict the experimental conditions to achieve the complete separation of a complex mixture of chiral compounds using a dual system of two CDs by just performing few individual experiments with each CD separately.

Experiments carried out in section 3.1 with each pair of enantiomers separately, enabled to conclude that baseline separation among consecutive peaks could be achieved when the difference between their electrophoretic mobilities was around 0.2 x 10^{-9} (m^2 s^{-1} V^{-1}), and even below, between 0.1 x 10^{-9} and 0.2 x 10^{-9} (m^2 s^{-1} V^{-1}), in some instances. The differences between the electrophoretic mobilities obtained for consecutive peaks using the model of Dubsky et al. [1] were calculated by subtracting the consecutive values shown in Table 2. Results obtained from this subtraction are shown in Table 3 for a 0.2 molar fraction of HP-β-CD and in Table S5 (supplementary material) for other molar fractions of this CD. These tables show that minimal values higher than 0.14 for the difference in the electrophoretic mobilities of consecutive peaks for all compounds were obtained for a molar fraction of 0.1 HP-β-CD (for a total CDs concentrations of 30, 35 and 40 mM) and for a molar fraction of 0.2 HP-β-CD (for total CDs concentrations of 20, 25 and 30 mM). Although a molar fraction of 0.8 HP-β-CD at a total CDs concentration of 10 mM enabled to obtain values for the above-mentioned differences higher than 0.14, these values were lower than 0.2 in four cases and experimentally it was observed that high concentrations of HP-β-CD gave rise to worst separations. Therefore, a molar fraction of 0.2 HP-β-CD and a 20 mM total CDs concentration were chosen to be tested experimentally since the complete separation of the mixture should be possible using the lowest CD concentration.

3.3. Simultaneous enantiomeric separation of the multicomponent mixture of phenoxy acids herbicides using a dual HP-β-CD + TM-β-CD system

Different experimental assays were carried out to verify the model predictions. Figure 2a shows that the complete baseline separation of the mixture of the phenoxy acid herbicides was obtained for a molar fraction of 0.2 HP-β-CD and a 20 mM total CDs

315 concentration, as predicted by the model. The resolution values ranged from 1.2 to 4.2
316 for enantiomeric pairs, and from 2.2 to 32.9 for non-enantiomeric consecutive peaks.
317 The identification of the pairs of peaks corresponding to each phenoxy acid was carried
318 out by injecting mixture samples of the six compounds enriched in each of them (see
319 Fig. S6 in supplementary material). It was observed that the migration order matched
320 with the one predicted by the model (Table 2).

321 Different molar fractions of both CDs were also tested keeping constant the total CDs
322 concentration at 20 mM but at low concentrations of HP-β-CD (0.1 and 0.9, and 0 and 1
323 molar fractions of HP-β-CD and TM-β-CD, respectively), as it has been above indicated
324 that high concentrations of HP-β-CD gave rise to worst separations. Figures 2b and 2c
325 show that the simultaneous baseline separation of the mixture was not possible with
326 these molar fractions, as predicted by the model. With a molar fraction of 0.1 HP-β-CD
327 and 0.9 TM-β-CD it was not possible to separate 3-CPPA enantiomers (Fig. 2b),
328 whereas the molar fraction 0.0 HP-β-CD and 1.0 TM-β-CD did not allow the
329 enantiomeric separation of 2-PPA and in the case of 3-CPPA their enantiomers were
330 partially separated (Fig. 2c). Therefore, as predicted by the model, the molar fraction of
331 0.2 HP-β-CD and 0.8 TM-β-CD was the one enabling the complete enantiomeric
332 separation of the six herbicides. In summary, the model predicts a deterioration of the
333 simultaneous enantiomeric separation, at a 20 mM total CDs concentration, when the
334 HP-β-CD molar fraction decreases below 0.2 and increases above 0.2 (Table S5 in
335 supplementary data).

336 Table 4 shows the agreement between the effective electrophoretic mobilities
337 experimentally obtained for the mixture 4 mM HP-β-CD + 16 mM TM-β-CD ($\mu_{A,eff(E)}$)

(that is, a molar fraction of 0.2 for HP-β-CD at a total CDs concentration of 20 mM), and those theoretically foreseen by the model ($\mu_{A,eff(T)}$).

Therefore, the application of the model proposed by Dubsky et al. [1] enabled with only a few experiments to foresee the best experimental conditions to achieve the complete simultaneous baseline separation of the twelve enantiomers studied in this work. Moreover, the model allowed improving the previous results experimentally obtained by the trial and error procedure for the simultaneous enantiomeric separation of the six phenoxy acid herbicides [5] since, although the analysis time slightly increased, the resolution values between enantiomers and consecutive compounds were improved, disappearing the coelution problem. Additionally, the concentration of CDs used is lower and the time to optimize the separation conditions is considerably reduced in contrast to the previous work [5]. Therefore, this model enables to achieve separations in a more cost-effective and quicker way.

4. Concluding remarks

The model previously proposed by Dubsky et al. for CE enantioseparation systems with a mixture of chiral selectors was applied for the first time to the optimization of the simultaneous enantiomeric separation of a multicompoment mixture using a dual system of two neutral CDs. Results showed that this model enabled the rapid optimization of the simultaneous enantiomeric separation of a mixture of six phenoxy acid herbicides using a dual system of two CDs, HP-β-CD and TM-β-CD. The model enables to foresee the results that could be obtained for any possible combination of concentrations and relative proportion of both CDs in the mixture, from a small amount of individual experiments carried out separately with each CD. This considerably simplifies the experimental optimization of enantiomeric separations using dual

systems, which can be time-consuming due to the high number of possibilities to be assayed regarding the total concentration of both chiral selectors and the proportion of each one in the mixture for a given total concentration of both. Results obtained in this work demonstrated that the model was successful by improving the previous results experimentally obtained by the trial and error method for the simultaneous enantiomeric separation of the six phenoxy acid herbicides studied in this work. In fact, the separation was improved in terms of resolution values obtained between enantiomers and consecutive compounds, by decreasing the concentration of CDs employed and by considerably reducing the time to optimize the separation conditions enabling to find, in a faster and efficient way, the most adequate proportion of both CDs and the concentration of each CD in the mixture in order to obtain the base line separation of the twelve enantiomers.

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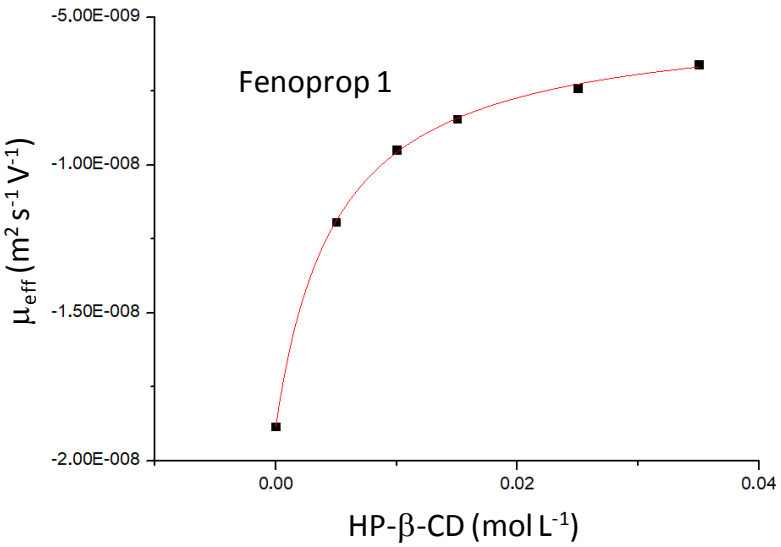
406 **Figure captions**

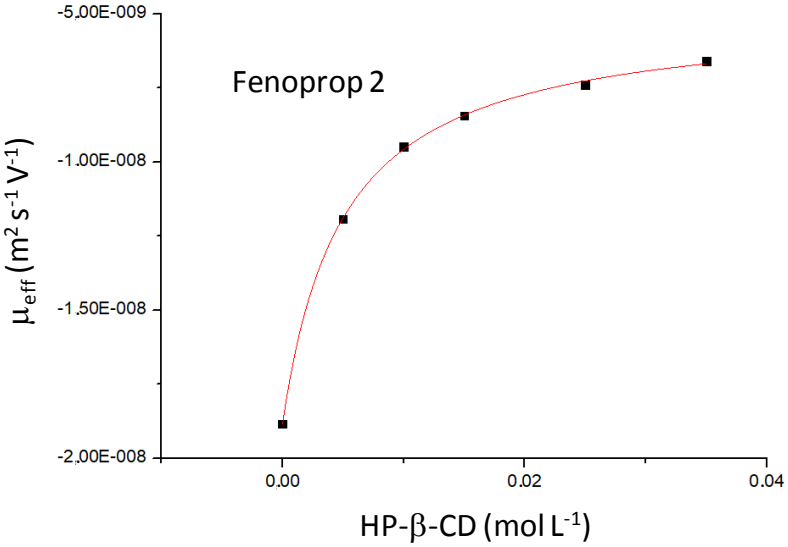
407 **Fig. 1** Variation of the μ_{eff} for fenoprop enantiomers as a function of the concentration
408 of each CD (HP-β-CD and TM-β-CD) in the separation buffer. Experimental
409 conditions: BGE 50 mM phosphate buffer (pH 7.0), capillary (50 μm I.D., Lt of 58.5 cm
410 (50 cm Ld), hydrodynamic injection 50 mbar x 10 s, temperature 15 ˚C, voltage 25 kV,
411 UV detection at 210 nm.

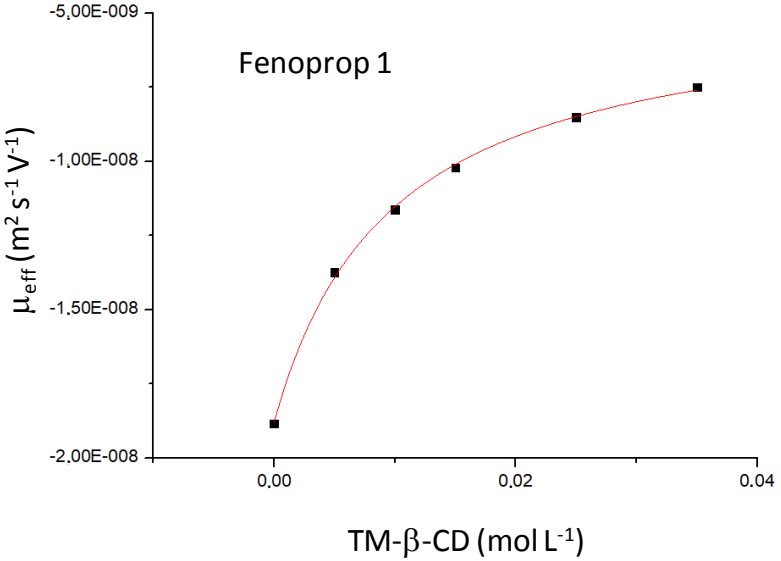
412 **Fig. 2** Electropherograms obtained for the enantiomeric separation of the mixture of six
413 phenoxy acid herbicides enriched in fenoprop (the concentration of each enantiomer
414 was 5 μg mL^{-1}, except in the case of fenoprop which was 10 μg mL^{-1} for each
415 enantiomer) using different concentrations of the dual system HP-β-CD + TM-β-CD.
416 Experimental conditions as in Fig. 1 except UV detection at 200 nm. Resolution values
417 between enantiomeric pairs for **(a)** 4 mM HP-β-CD + 16 mM TM-β-CD: fenoprop (Rs
418 = 2.5), mecoprop (Rs = 4.2), dichlorprop (Rs = 3.0), 4-CPPA (Rs = 2.6), 3-CPPA (Rs =
419 1.2) and 2-PPA (Rs = 1.6), **(b)** 2 mM HP-β-CD + 18 mM TM-β-CD: fenoprop (Rs =
420 3.5), mecoprop (Rs = 4.9), dichlorprop (Rs = 3.2), 4-CPPA (Rs = 2.8), 3-CPPA (Rs =
421 0.0) and 2-PPA (Rs = 1.3), and **(c)** 20 mM TM-β-CD: fenoprop (Rs = 4.6), mecoprop
422 (Rs = 4.8), dichlorprop (Rs = 2.8), 4-CPPA (Rs = 3.1), 3-CPPA (Rs = 0.7) and 2-PPA
423 (Rs = 0.0). 1: First-migrating enantiomer, 2: Second-migrating enantiomer. Resolution
424 values between non-enantiomeric pairs for **(a)** 4 mM HP-β-CD + 16 mM TM-β-CD:
425 fenoprop 2 and mecoprop 1 (Rs = 19.6), mecoprop 2 and dichlorprop 1 (Rs = 2.2),
426 dichlorprop 2 and 4-CPPA 1 (Rs = 3.6), 4-CPPA 2 and 3-CPPA 1 (Rs = 8.4) and 3-
427 CPPA 2 and 2-PPA 1 (Rs = 32.9), **(b)** 2 mM HP-β-CD + 18 mM TM-β-CD: fenoprop 2
428 and mecoprop 1 (Rs = 19.3), mecoprop 2 and dichlorprop 1 (Rs = 3.0), dichlorprop 2
429 and 4-CPPA 1 (Rs = 4.3), 4-CPPA 2 and 3-CPPA (Rs = 8.7) and 3-CPPA and 2-PPA 1

(Rs = 29.7), and **(c)** 20 mM TM-β-CD: fenoprop 2 and mecoprop 1 (Rs = 16.7), mecoprop 2 and dichlorprop 1 (Rs = 3.1), dichlorprop 2 and 4-CPPA 1 (Rs = 4.5), 4-CPPA 2 and 3-CPPA 1 (Rs = 10.8) and 3-CPPA 2 and 2-PPA (Rs = 19.2).

Figure 1







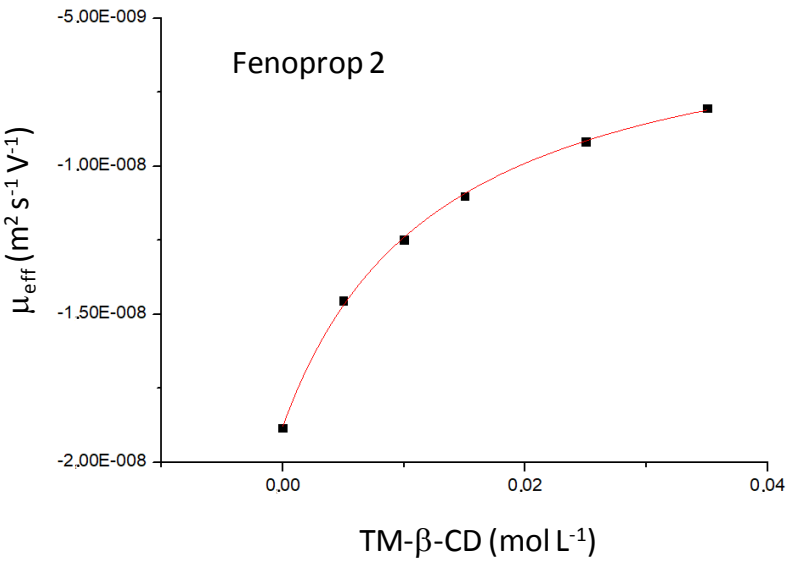


Figure 1

Figure 2

Fig. 2

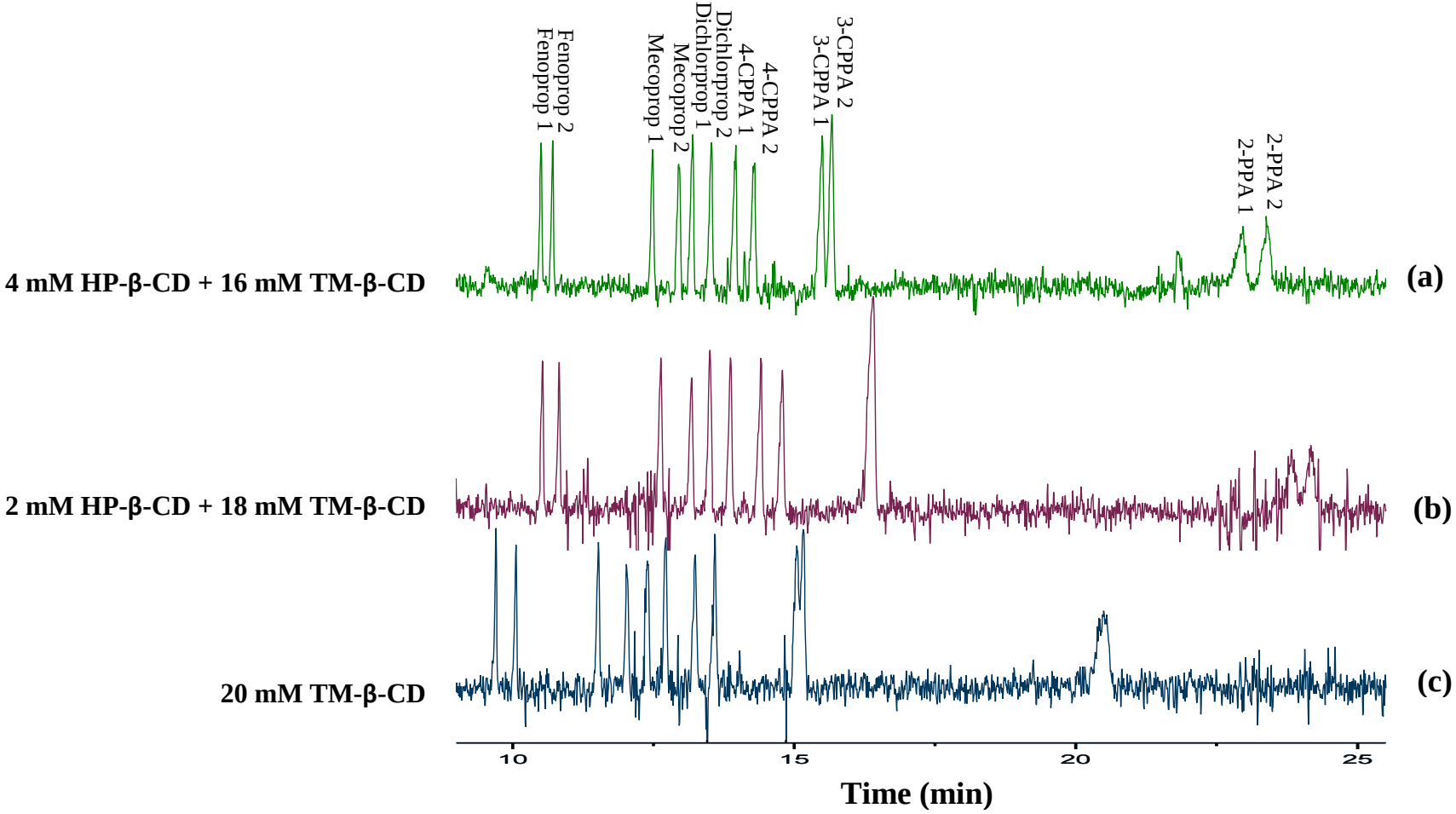


Table 1

Table 1. Apparent complextion constants (K_C) and effective mobilities (μ_C) of the phenoxy acid herbicides complexes with HP-β-CD ($K_{C(HP)}$ and $\mu_{C(HP)}$) and TM-β-CD ($K_{C(TM)}$ and $\mu_{C(TM)}$), respectively, in 50 mM sodium phosphate buffer (pH = 7.0).

	$K_{C(HP)}$ (M^{-1})	$\mu_{C(HP)}$ x 10^{-9} ($m^2\ s^{-1}\ V^{-1}$)	$K_{C(TM)}$ (M^{-1})	$\mu_{C(TM)}$ x 10^{-9} ($m^2\ s^{-1}\ V^{-1}$)
Fenoprop 1	210 ± 7	-4.9 ± 0.2	108 ± 4	-4.5 ± 0.2
Fenoprop 2	210 ± 7	-4.9 ± 0.2	82 ± 3	-4.2 ± 0.3
Mecoprop 1	82 ± 8	-3.9 ± 0.8	37 ± 5	-2 ± 2
Mecoprop 2	74 ± 8	-3.7 ± 0.9	29 ± 6	-2 ± 3
Dichlorprop 1	88 ± 2	-4.4 ± 0.2	25 ± 4	-1 ± 2
Dichlorprop 2	79 ± 2	-4.2 ± 0.2	23 ± 5	-0.5 ± 3
4-CPPA 1	77 ± 6	-3.6 ± 0.7	25 ± 5	-2 ± 3
4-CPPA 2	71 ± 6	-3.4 ± 0.8	22 ± 5	-1 ± 3
3-CPPA 1	68 ± 1	-3.9 ± 0.1	13 ± 2	2 ± 4
3-CPPA 2	62 ± 1	-3.8 ± 0.2	13 ± 2	1 ± 4
2-PPA 1	11 ± 4	26 ± 17	5 ± 7	57 ± 337
2-PPA 2	8 ± 4	43 ± 35	6 ± 7	24 ± 108

Table 2. Effective electrophoretic mobility for each analyte ($\mu_{A,eff}$ x 10^{-9}) provided by the model for a molar fraction of 0.2 HP-β-CD and 0.8 TM-β-CD and various total CDs concentrations (0 to 40 mM).

	Total concentration of HP-β-CD + TM-β-CD (mM)								
	0*	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
Fenoprop 1	-19.0	-13.4	-11.0	-9.6	-8.7	-8.1	-7.6	-7.3	-7.0
Fenoprop 2	-19.0	-14.0	-11.5	-10.1	-9.1	-8.5	-8.0	-7.6	-7.3
Mecoprop 1	-19.4	-16.4	-14.3	-12.7	-11.6	-10.6	-9.9	-9.3	-8.8
Mecoprop 2	-19.4	-16.7	-14.8	-13.3	-12.1	-11.2	-10.4	-9.8	-9.2
Dichlorprop 1	-19.7	-17.0	-15.0	-13.5	-12.3	-11.4	-10.6	-9.9	-9.4
Dichlorprop 2	-19.7	-17.2	-15.3	-13.8	-12.7	-11.7	-10.9	-10.2	-9.7
4-CPPA 1	-20.7	-18.4	-16.3	-14.7	-13.5	-12.4	-11.6	-10.9	-10.3
4-CPPA 2	-20.7	-18.6	-16.6	-15.0	-13.8	-12.7	-11.9	-11.1	-10.5
3-CPPA 1	-21.2	-18.7	-17.1	-15.7	-14.6	-13.7	-12.8	-12.1	-11.5
3-CPPA 2	-21.2	-18.8	-17.2	-15.9	-14.8	-13.9	-13.1	-12.4	-11.8
2-PPA 1	-22.0	-21.1	-20.2	-19.5	-18.7	-18.0	-17.3	-16.7	-16.1
2-PPA 2	-22.0	-21.1	-20.3	-19.6	-18.9	-18.2	-17.6	-17.0	-16.4

***** For a total concentration 0 mM of CDs the model predicts a change in the migration order in the case of 4-CPPA and 3-CPPA, which is represented by the different shading color.

Table 3

Table 3.- Differences (x 10^{-9}) between effective electrophoretic mobilities of consecutive peaks for a molar fraction of 0.2 HP-β-CD and 0.8 TM-β-CD and various total CDs concentrations (0 to 40 mM) provided by the model. Those values equal to or greater than 0.2 x 10-9 are marked in color.

Total concentration of HP-β-CD + TM-β-CD (mM)								
0	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
0.00	0.54	0.55	0.50	0.44	0.38	0.33	0.29	0.26
0.38	2.40	2.75	2.65	2.44	2.19	1.95	1.73	1.52
0.00	0.37	0.52	0.57	0.57	0.55	0.52	0.49	0.45
0.30	0.26	0.22	0.20	0.18	0.16	0.15	0.13	0.12
0.00	0.21	0.31	0.35	0.36	0.36	0.35	0.33	0.32
0.96	1.21	1.02	0.88	0.78	0.71	0.66	0.62	0.58
0.00	0.19	0.28	0.31	0.32	0.32	0.30	0.28	0.26
0.56	0.09	0.47	0.70	0.84	0.91	0.95	0.97	0.97
0.00	0.09	0.15	0.20	0.22	0.24	0.26	0.26	0.27
0.71	2.29	3.01	3.52	3.87	4.11	4.25	4.33	4.35
0.00	0.04	0.07	0.11	0.14	0.18	0.21	0.24	0.27

Table 4

Table 4. Empirical effective electrophoretic mobilities ($\mu_{A,eff(E)}$) and effective electrophoretic mobilities provided by the model ($\mu_{A,eff(T)}$) for a 4 mM HP-β-CD + 16 mM TM-β-CD mixture.

	$\mu_{A,eff(T)}$ x 10^{-9} (m^2 s^{-1} V^{-1})	$\mu_{A,eff(E)}$ x 10^{-9} (m^2 s^{-1} V^{-1})
Fenoprop 1	-8.7 ± 0.1	-8.8 ± 0.1
Fenoprop 2	-9.1 ± 0.1	-9.2 ± 0.1
Mecoprop 1	-11.6 ± 0.2	-11.8 ± 0.1
Mecoprop 2	-12.1 ± 0.2	-12.3 ± 0.1
Dichlorprop 1	-12.3 ± 0.1	-12.5 ± 0.3
Dichlorprop 2	-12.7 ± 0.1	-13.0 ± 0.2
4-CPPA 1	-13.5 ± 0.1	-13.4 ± 0.1
4-CPPA 2	-13.8 ± 0.1	-13.7 ± 0.1
3-CPPA 1	-14.6 ± 0.1	-14.8 ± 0.2
3-CPPA 2	-14.8 ± 0.1	-14.9 ± 0.2
2-PPA 1	-18.7 ± 0.1	-18.9 ± 0.2
2-PPA 2	-18.9 ± 0.1	-19.1 ± 0.2

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