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Optimization of the cyclodextrin-assisted capillary electrophoresis separation of the enantiomers of phenoxyacid herbicides

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Abstract

An ethylcarbonate derivative of β-cyclodextrin (β-CD) with three substituents per molecule, hydroxypropyl-β-CD and native α-CD have been tested as resolving agents in the capillary zone electrophoresis (CZE) separation of the four enantiomers of the herbicides mecoprop and dichlorprop. The performances of the three compounds have been quantified by means of two-levels full factorial design and the inclusion constants were calculated from CZE migration time data. Possible structure of inclusion complexes have been proposed, on the basis of molecular mechanics simulations.

Keywords: Enantiomer separation; Cyclodextrins; Phenoxy acids; Pesticides

1. Introduction

The peculiar stereo- and enantioselective complexation properties of native cyclodextrins (CDs) and of their synthetic derivatives have been largely exploited for resolving isomers by means of electrophoretic separation techniques, including capillary zone electrophoresis (CZE) [1]. Since the enantioselectivity of complexation is likely to depend on factors such as the number and the chemical properties of functional groups linked to CD rims, experimenting and characterising new CD derivatives is of considerable interest for increasing the field of CZE applications. In a previous study [2], newly synthesised CD carbonate esters were compared with commercially available CDs for CZE enantiomer separation, and the effectiveness in separating the optical antipodes of (±)-2-(2,4-dichlorophenoxy)-propionic acid (dichlorprop) of an ethylcarbonate derivative of β-CD, having an average substitution degree (SD) equal to 2, was found competitive with respect to several commercial CDs. In this work, a different ethylcarbonate derivative of β-CD, with average SD larger than that of the previously experimented one, has been tested. As a test mixture, two congeners racemic compounds, dichlorprop and (±)-2-(2-methyl-4-chlorophenoxy)-propionic acid (mecoprop) have been chosen and the capability of the different CDs in separating the four optical isomers have been investigated.

Experimental design strategies have been successfully applied to the optimisation of CZE separations [3–12], since these statistical techniques can help to minimise the number of experiments while obtaining maximum information. In this work, two level full factorial designs were used to screen the effect of the

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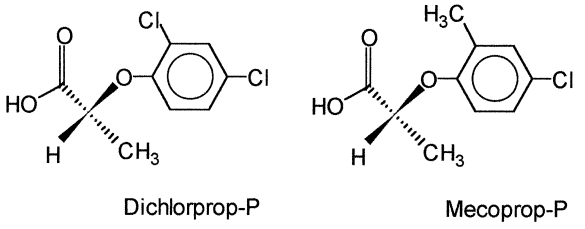
concentration of three different CDs and of the organic modifier concentration on the separation of the four optical antipodes of mecoprop and dichlorprop. Additional information may be provided by the inclusion constants, which can be obtained from migration time data at variable CD concentration. Further, possible structures of the inclusion complexes may be proposed, on the basis of molecular mechanic simulations.

2. Materials and methods

α- and γ-CD were purchased from Fluka, β-CD was kindly gifted by Roquette Italia (Cassano Spinola, Italy). Methyl-β- and hydroxypropyl-β-CD were kindly supplied by Wacker Chemie (München, Germany). Ethylcarbonate-β-CD with average SD equal to 3 was synthesised according to the procedure described in the literature [13]. Ethylcarbonate substituents were randomly distributed on 2 and 3 hydroxyls situated on the larger rim of β-CD cavity. Racemic mecoprop and dichlorprop and their pure D(+) enantiomers, (mecoprop-p and dichlorprop-p) were kindly gifted by BASF (Ludwigshafen, Germany). The structures of mecoprop-p, dichlorprop-p and ethylcarbonate-β-CD are shown in Fig. 1.



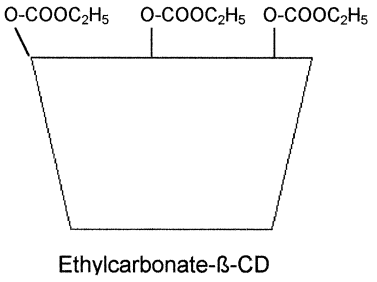


Fig. 1. Structures of mecoprop-p, dichlorprop-p and ethylcarbonate-β-CD.

CE was performed using a BioFocus 2000 CE system (Bio-Rad) equipped with 50 μm I.D. untreated fused-silica capillary, 40 cm long (35.4 cm to the detector), thermostatted at 20±0.1°C, with 214 nm UV detection. All separations were carried out by operating in reversed-polarity mode (cathode on the injection side), with 20 kV migration voltage. The running electrolyte was Na_2HPO_4 adjusted to pH 5 with citric acid (total concentration 45 m*M*) to which the different chiral additives tested were added. $1\cdot10^{-4}$ *M* dichlorprop aqueous solutions, containing also 100 mg dm^{-3} potassium bromide, were injected by a 10 kPa pressure for 3 s. Electroosmotic flow was measured by replicate injections of benzyl alcohol, and an average value of $9.8\pm0.3\cdot10^{-4}$ m s^{-1} was observed.

The migration time of the bromide ion, which was assumed to interact nor with CDs neither with analytes, was used for correcting the measured migration times, in order to reduce as much as possible the influence of unwanted effects, such as dependence of the viscosity of the running buffer on the concentration of chiral modifier added [2].

3. Results and discussion

3.1. Preliminary screening

Electrophoretic runs with 8 m*M* of one of the following CDs were performed on a mixture of racemic dichlorprop and mecoprop native α-, β- and γ-CD, hydroxypropyl-β-CD, methyl-β-CD, ethylcarbonate-β-CD (SD=3) and diphenylcarbonate-β-CD (SD=1). Enantioresolution was observed with α-CD, hydroxypropyl-β-CD and ethylcarbonate-β-CD. Methyl-β-CD gave only poor resolution, while β-CD produced retardation of peaks but not enantiomer separation. The poor solubility of diphenylcarbonate-β-CD, either in water or water methanol buffers, limited its application to this study. The choice of a larger d.s. for ethylcarbonate-β-CD was based on the

Table 1
Table of the experiments of the (a) factorial design and (b) factor experimental range

Experiment	[CH_3OH] (%, v/v)	[CD] (m*M*)
(*a*) *Factorial design*		
1	−1	−1
2	+1	−1
3	−1	+1
4	+1	+1
(*b*) *Factor range*		
+1	10	12
−1	0	6

consideration that native β-CD was found ineffective as chiral auxiliary agent, while two ethylcarbonate substituents per molecule conferred to this compound enantiomer discrimination properties. Therefore, it seemed worth to be investigated the effect of a third ethylcarbonate group on the molecule. Solubility in water of this derivative (40 m*M* at 20°C) was large enough to allow the preparation of chiral running buffers in a wide range of concentration. After these preliminary runs, attention was restricted on the three CDs which gave the best results, namely α-CD, hydroxypropyl-β-CD and ethylcarbonate-β-CD.

3.2. Experimental design

For further quantitative screening of the effects of the three chiral auxiliary additives mentioned above, in the meanwhile minimising the number of experiments and therefore time and reagents consumption, two-level factorial design strategy was adopted. The effects of concentrations of CD and of an organic modifier (methanol) in the running buffer were investigated for the three CDs considered. Low levels for CD and methanol concentrations were set at 6 m*M* and 0% (v/v), while high levels were set at 12 m*M* and 10% (v/v), respectively. The variation of experimental responses was presumed approximately linear inside these intervals. The scheme of experimental conditions is reported in Table 1.

The differences in the migration times of the enantiomers and in the migration times of the two congeners were the experimental responses chosen for evaluation. The average between the migration times of each enantiomer pair was used as the migration time of the corresponding congener. The data obtained in the twelve experiments are reported in Tables 2 and 3. The main effects and the interaction between the two experimental factors were calculated by means of the Yates algorithm. The main effects may be obtained by summing the products of corresponding variables (Table 1, part a) and effects (Δt in Tables 2 or 3) and then dividing by the number of variables (2, in this case), while the effects attributable to interaction between variables may be calculated by summing the product of both variables and effect and then dividing by 2. Information contained in experimental data results quite easy to read, since the effect of each factor is expressed by a numeric value, whose sign indicates the direction of the effect.

Data of Tables 2 and 3 show that ethylcarbonate-β-CD originates the largest average response both on the separation of optical antipodes and of congeners, while the smallest average response is obtained with hydroxypropyl-β-CD. The highest separation factors, α, were obtained by the use of ethylcarbonate-β-CD as chiral modifier of the running buffer. Table 4 reports the main effects of methanol and CD concentrations on the difference in the migration times of enantiomers. It can be seen that both methanol (X_1) and CD (X_2) concentrations produce the largest effects with ethylcarbonate-β-CD, but while an increase in methanol concentration decreases the experimental response, an increase in CD concentration exerts a positive effect on it. With ethylcarbonate-β-CD, interactions between factors are relevant when dealing with congeners separation, but not with enantiomer resolution. Methanol concentration produces small effects with α-CD and hydroxypropyl-β-CD. Increase of CD concentration were observed to originate positive variations of the responses in all cases except two, of limited significance.

Therefore, it was concluded that the best chiral auxiliary agents, among those considered, for the CZE separation of the mentioned compounds are α-CD and ethylcarbonate-β-CD. The results reported in Table 4 indicate that, for best results, ethylcarbonate-β-CD concentration should be increased, while methanol concentration should be maintained low. The data concerning congeners

Table 2
Experimental data: migration times (minutes, seconds) of the first eluting enantiomer ($t_{L(-)}$ or $t_{D(+)}$); difference in migration times (Δt) of each enantiomer pair, seconds; separation factors (α) of each enantiomer pair. Capillary: 40 cm, 50 μm I.D.; migration voltage 20 kV; run buffer 45 m*M* NaH_2PO_4, pH 5

Factors		α-Cyclodextrin						Ethylcarbonate-β-CD						Hydroxypropyl-β-CD					
$[CH_3OH]$ (%)	[CD] (m*M*)	Mecoprop			Dichlorprop			Mecoprop			Dichlorprop			Mecoprop			Dichlorprop		
		$t_{L(-)}$	Δt	α	$t_{L(-)}$	Δt	α	$t_{D(+)}$	Δt	α	$t_{D(+)}$	Δt	α	$t_{D(+)}$	Δt	α	$t_{D(+)}$	Δt	α
0	6	9.10	15	0.45	9.56	23	0.50	19.48	67	0.69	22.50	148	1.08	6.27	6	0.38	6.39	10	0.54
10	6	8.35	11	0.44	9.00	25	0.44	14.53	20	0.30	16.22	35	0.60	7.13	5	0.28	7.35	0	0.44
0	12	10.50	16	0.34	11.42	18	0.25	24.01	115	1.14	28.09	222	1.12	7.27	10	0.56	7.55	5	0.24
10	12	10.13	19	0.59	10.59	34	0.77	23.25	59	0.69	26.56	118	1.00	8.19	10	0.48	8.46	2	0.33
Average			15			25			65			131			8			4	

Table 3
Differences between the average migration times of dichlorprop and mecoprop, seconds

[CH_3OH] (%)	[CD] (m*M*)	α-Cyclodextrin	Ethylcarbonate-β-CD	Hydroxypropyl-β-CD
0	6	50	223	14
10	6	32	97	38
0	12	53	302	25
10	12	53	265	24
Average		47	221	25

Table 4
Effects (in seconds) of experimental factors on the differences in migration times of each enantiomer pair. X_1, methanol concentration; X_2, CD concentration; $X_1 * X_2$, interaction between factors

	Mecoprop resolution			Dichlorprop resolution			Mecoprop/dichlorprop separation		
	X_1	X_2	$X_1 * X_2$	X_1	X_2	$X_1 * X_2$	X_1	X_2	$X_1 * X_2$
α-Cyclodextrin	−0.5	4.5	3.5	9	2	7	−9	12.5	9
Ethylcarbonate-β-CD	−51.5	43.5	−4.5	−108.5	78.5	4.5	−81.5	123.5	44.5
Hydroxypropyl-β-CD	−0.5	4.5	0.5	6.5	−1.5	3.5	11.5	−1.5	−12.5

resolution by α-CD indicate that efficiency can be improved by increasing CD concentration and decreasing methanol one, but no clear indication can be achieved about enantiomer resolution.

3.3. Complexation constants

Further information about the characteristics of the two chiral auxiliary agents which showed the best performance can be obtained from the values of their complex formation constants with the analytes:

$$K_{A-CD} = \frac{[A–CD]}{[A][CD]} \quad (1)$$

where K_{A-CD} is the formation constant of the complex A–CD, [A] and [CD] are the guest compound and cyclodextrin concentrations, respectively.

The electrophoretic mobilities of enantiomers, corrected for the contribution of electroosmotic flow and for the variation in the viscosity of the eluent, were used for calculating the inclusion constants by least square fitting them to the following equation:

$$\mu_A = \frac{\mu_f + \mu_{compl} K_{A-CD}[CD]}{1 + K_{A-CD}[CD]} \quad (2)$$

where μ_A is the corrected electrophoretic mobility of the enantiomer, μ_f is the mobility of the free enantiomer and μ_{compl} is the electrophoretic mobility of the complexed enantiomer [14]. Cyclodextrin concentrations into the running buffer ranged 0–15 m*M* and Sigmaplot 3.0 was used for mathematical calculations.

Table 5 shows the inclusion constants calculated

Table 5
Inclusion constants: standard deviation±10%

	α-Cyclodextrin		Ethylcarbonate-β-CD	
	0% Methanol	10% Methanol	0% Methanol	10% Methanol
D(+)-Mecoprop	234	97	87	61
L(−)-Mecoprop	228	92	93	63
D(+)-Dichlorprop	601	102	120	71
L(−)-Dichlorprop	578	99	131	78

for α- and ethylcarbonate-β-CD. It can be seen that 10% methanol in the running buffer reduces the inclusion constants, without producing positive effects on separation. Optimum concentration of chiral additives can be calculated from the values of inclusion constants [15]:

$$[C]_{opt} = l/(K_1K_2)^{1/2} \quad (3)$$

where $[C]_{opt}$ is the additive concentration which gives the maximum selectivity, K_1 and K_2 are the formation constants. According to this relationship, a twofold increase in concentration of chiral additive is required to compensate the halving of inclusion constants. Water solubility of these compounds allow to reach these concentrations without the need for an organic solvent, therefore the use of methanol can be avoided. Optimum concentration of ethylcarbonate-β-CD in aqueous running buffer are 9.2 m*M* and 8.5 m*M* for resolving enantiomers of mecoprop and dichlorprop, respectively. The average between these two concentration may be chosen for performing the best possible separation of the four enantiomers. An electropherogram showing complete separation of all four optical isomers is reported in Fig. 2. Fig. 3 shows a possible structure of the inclusion complexes of mecoprop and dichlorprop with ethylcarbonate-β-CD. Steric hindrance of ethylcarbonate substituents could explain of the lower values of inclusion constants of ethylcarbonate-β-CD with respect to those of α-CD.

4. Conclusions

Three different cyclodextrins, namely α-CD, hydroxypropyl-β-CD and ethylcarbonate-β-CD have been compared as chiral additives in the CZE separation of the four enantiomers of the chiral herbicides mecoprop and dichlorprop. Comparison was conducted with the aid of experimental design techniques and by comparing the values of the inclusion constants, obtained from migration times data at variable CD concentration. A newly synthesised ethylcarbonate derivative of β-CD with an average SD of 3 showed the largest effect on both the separation of the enantiomers and of the two congener herbicides and was found capable of giving baseline resolution of all the four enantiomers.

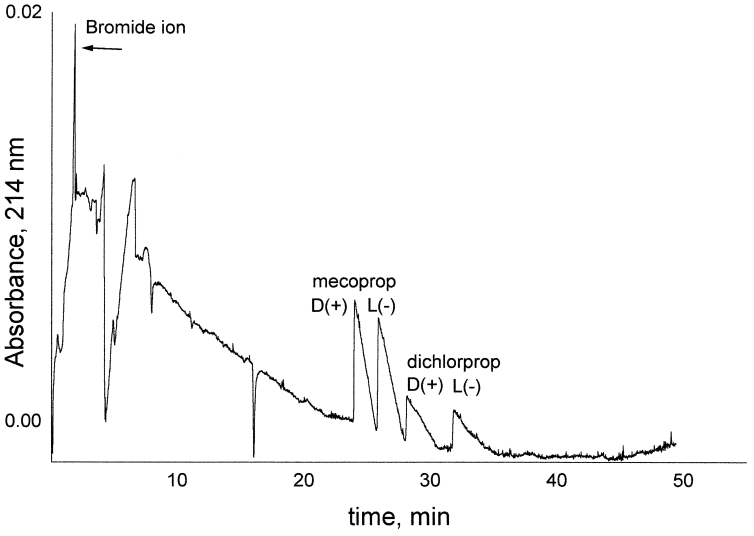


Fig. 2. Complete resolution of the four optical isomers, obtained with ethylcarbonate-β-CD in the running buffer.

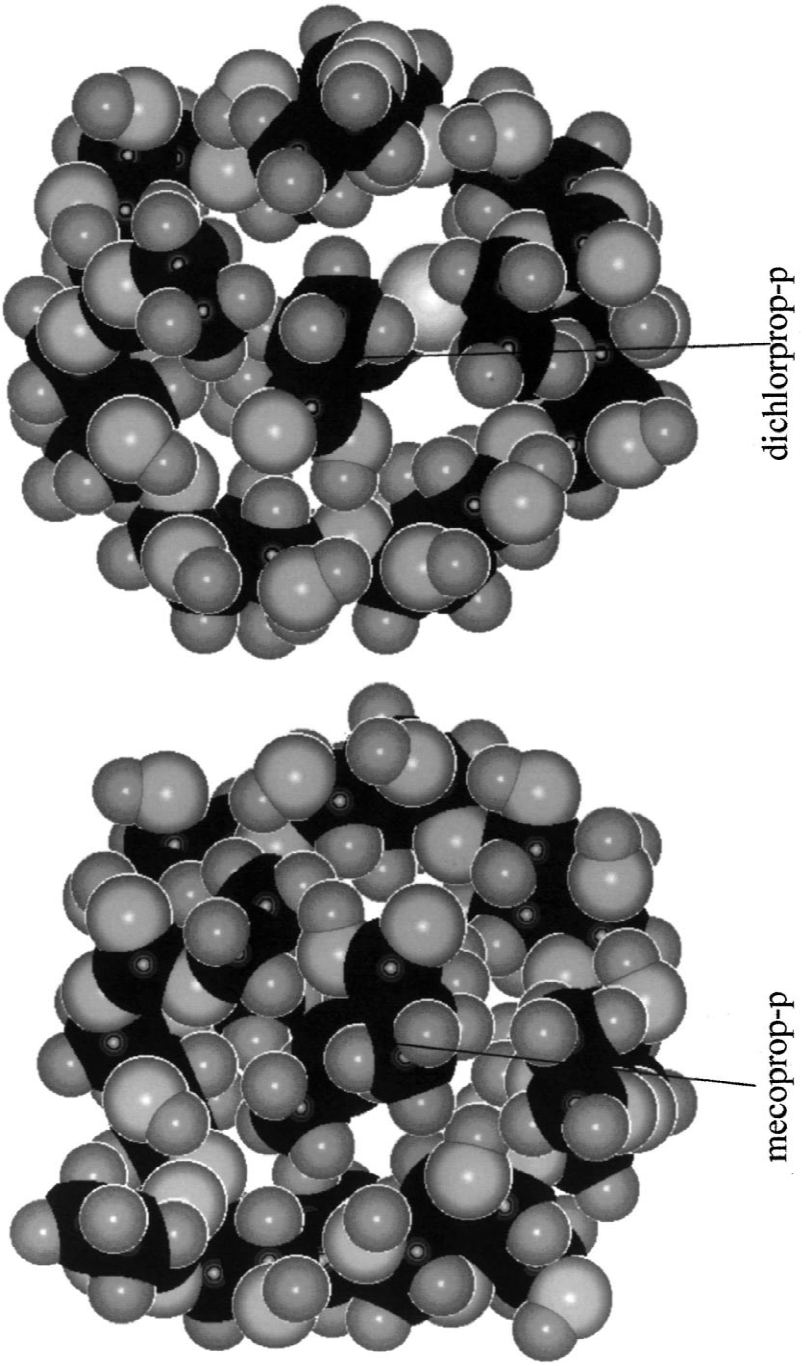


Fig. 3. Possible structures of the inclusion complexes of mecoprop and dichlorprop with ethylcarbonate-β-CD.

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