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New derivatives of cyclodextrins as chiral selectors for the capillary electrophoretic separation of dichlorprop enantiomers[1]

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Abstract

α-, β- and γ-cyclodextrins (CDs), as well as some of their chemical derivatives, have been tested as chiral resolving agents for the capillary zone electrophoretic resolution of the racemic herbicide dichlorprop, (±)-2-(2,4-dichlorophenoxy)propionic acid, of which only the (+)-isomer is herbicidally active. The complexation constants of the herbicide enantiomers with the cyclodextrin host molecules have been calculated from the electrophoretic migration time data at variable cyclodextrin concentration. The experimental results showed that several of the investigated CDs allowed dichlorprop enantiomer resolution. In particular, a newly synthesised ethylcarbonate derivative of β-CD showed the best enantiomer resolution properties among the tested compounds, while the remaining ones showed inferior or no performances at all. The calculated inclusion constants allowed identification of the best conditions for enantioresolution, and an explanation of the different complexation properties of the investigated compounds has been proposed on the basis of molecular modeling.

Keywords: Enantiomer separation; Chiral selectors; Cyclodextrins; Dichlorprop; Pesticides

1. Introduction

Cyclodextrins (CDs) are nonreducing oligosaccharides which originate from amylose by the action of glucosyltransferase. The cyclic polymers formed by six, seven and eight glucopyranose units are named α-, β- and γ-CD, respectively; they have the shape of truncated cones with hydrophilic outside surfaces, whereas their inner cavities are hydrophobic. They can thus form inclusion complexes with hydrophobic molecules, or with hydrophilic molecules which possess hydrophobic moieties. Due to the optical activity of their structural monomer, CDs are optically active, therefore they can, in principle, distinguish between the optical antipodes of chiral molecules. This capability has made native CDs, as well as their available chemical derivatives, among the most widely used chiral selectors for several separation techniques, including capillary zone electrophoresis (CZE) [1–19,31–39]. The enantioselectivity of complexation depends on several factors, the dimensions of the CD cavity and the presence and properties of functional groups being among the most relevant, but no general rule for linking the

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stereoselectivity of the CDs with their chemical structure has been discovered as yet. For this reason, molecular modeling, an effective interpretative tool widely used in several fields of chemistry, has been proposed for providing an insight into the inclusion complexation phenomena which are of interest in separation science [20].

Few chemically derivatised CDs are commercially available, but new derivatives are being synthesised and their properties investigated, because of the importance of these compounds in several fields of application. Carbonate esters, a specific class of synthetic derivatives of CDs, can provide a wide range of derivatives which are highly soluble in water, with properties which depend on the nature of the substituents. In this work, the enantioresolution properties of newly synthesised carbonate derivatives of CDs have been investigated and compared with those of both native and derivatized CDs commercially available.

Dichlorprop, (±)-2-(2,4-dichlorophenoxy)-propionic acid, was chosen as the test molecule, because of its use as an herbicide. Commercial dichlorprop is racemic, but only its D-(+)-isomer is herbicidally active [21], therefore resolution of the two optical isomers can be of interest in agricultural and environmental applications. Dichlorprop enantiomers have been separated by means of GLC [22], HPLC [23] and CZE [24] techniques. GLC separation required preliminary conversion of nonvolatile dichlorprop into its isopropylamide derivative, but direct separations were obtained by means of HPLC and CZE. These last two techniques were performed by means of chiral mobile phases: optically active metal complexes were used for HPLC separation, while CDs were used for CZE resolution.

In this work, the application to the CD-assisted CZE enantiomer resolution of new carbonate derivatives of CDs has been studied, in order to compare their enantioseparation properties with those of the commercially available CDs, on the basis of electrophoretic and thermodynamic data and with the help of molecular modeling.

2. Materials and methods

α- and γ-CD were purchased from Fluka, β-CD was kindly donated by Roquette (Cassano Spinola, Italy) and methyl-β- and hydroxypropyl-β-CD were kindly supplied by Wacker Chemie (Munich, Germany). C_6-Capped-β-CD [25], ethyl carbonate-β- and ethyl carbonate-γ-CD [26] were synthesised according to the procedure described in the literature. Racemic dichlorprop and its pure D-(+)-enantiomer, (dichlorprop-p) were purchased from Alltech (Sedriano, Milan, Italy).

CE was performed using a BioFocus 2000 CE system (Bio-Rad) equipped with a polyacrylamide-coated 24 cm, 25 μm I.D. capillary thermostated at 20±0.1°C, with 206 nm detection. All separations were carried out by operating in reversed-polarity mode (cathode on the injection side), with 7 kV migration voltage. Such a relatively low potential was presumed to produce negligible Joule heating of the capillary and longer migration times, therefore allowing better reproducibility and smaller relative errors on the measured migration times data. The running electrolyte was 100 m*M* acetic acid–sodium acetate buffer (pH 5.0) to which the different chiral additives tested were added. Such a running buffer was preferred as it was reported to give the best performances with the chosen analytes [24]. Aqueous $1 \cdot 10^{-5}$ *M* dichlorprop solutions, containing a low concentration of potassium bromide, were prepared immediately before injection. The migration time of the bromide ion, which was assumed not to interact with the CD or with the analyte, was used for correcting the measured migration times of dichlorprop, 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. If the addition of chiral additives to the running electrolyte modifies its viscosity, the consequent migration time can be expressed by means of the following relationship:

$$\mu_c = \mu_{obs} \frac{\eta_c}{\eta_0} \quad (1)$$

where μ_c is the corrected mobility, μ_{obs} is the observed mobility, η_c is the viscosity of the running buffer at the concentration of added modifier, η_0 is the viscosity of the buffer without modifier. The observed electrophoretic mobility can be corrected by separate measurements of η_c and η_0 or, alter-

natively, by multiplying μ_{obs} by the mobility of a reference analyte, measured at the same concentrations of chiral modifier, and then dividing by the mobility of the reference analyte, measured at zero concentration of additive. If bromide ion is the reference analyte, its mobility at concentration C of modifier is related to its mobility at zero concentration by the following relationship:

$$\mu_c^{Br} = \mu_0^{Br}\frac{\eta_c}{\eta_0} \quad (2)$$

Therefore:

$$\frac{\mu_c^{Br}}{\mu_0^{Br}} = \frac{\eta_c}{\eta_0} \quad (3)$$

and:

$$\mu_{obs}\frac{\mu_c^{Br}}{\mu_0^{Br}} = \mu_{obs}\frac{\eta_c}{\eta_0} = \mu_c \quad (4)$$

The method can correct observed mobility for viscosity, with some advantages with respect to measuring viscosity in separate experiments: the viscosity ratio is measured under exactly the same conditions as the electrophoretic run (capillary diameter, temperature), the time required is much less than performing separate measurements, the correction factor μ_c^{Br}/μ_0^{Br} also compensates for other unwanted effects [e.g. random variation of the migration potential, electroosmotic flow (EOF)].

Electropherograms performed by pressure injection of dimethylformamide and mesityl oxide showed no EOF within 180 min. CD concentrations in the running buffer ranged from 0 up to 25 m*M*, depending on the solubility of the compound, and the corrected migration times of dichlorprop enantiomers were used for calculating the inclusion constants by least square fitting them to the following equation:

$$\mu_A = \frac{\mu_f + \mu_{compl}K_{A-CD}[CD]}{1 + K_{A-CD}[CD]} \quad (5)$$

where μ_A is the corrected electrophoretic mobility of the enantiomer, μ_f is the mobility of the free enantiomer, μ_{compl} is the electrophoretic mobility of the complexed enantiomer, K_{A-CD} is the formation constant of the complex and [CD] is the cyclodextrin concentration [27–29]. Graphical calculations of the unknown parameters were also performed [28] in order to validate the obtained data; SIGMAPLOT 2.0 was used for mathematical calculations.

The molecular models were produced by means of the build function of HYPERCHEM 2.0 software. Electrostatic charges on atoms were calculated by means of the AM1 algorithm, and the geometry of the resulting model was optimised by using Amber forcefield.

3. Results and discussion

Eight cyclodextrins were examined, namely the α-, β- and γ- native CDs, the commercially available methyl- and hydroxypropyl-β-CD derivatives and the newly synthesised C_6-capped-β-CD, ethylcarbonate-β-CD and ethylcarbonate-γ-CD. The C_6-capped-β-CD differs from native β-CD molecules in having an hexyl-1,6-dicarbonate bridge while ethylcarbonate-β- and γ-CD bear ethyl chains covalently linked to the cyclodextrin rim by means of carbonate esterification of the hydroxy groups of the CD. In these last two molecules, substituents are randomly distributed and the average degree of substitution (D.S.) is about 3. Mainly primary hydroxyls were esterified in the C_6-capped derivative, while the ethylcarbonate groups were attached principally to secondary hydroxyls. A typical electropherogram of racemic dichlorprop in the presence of ethylcarbonate-β-CD is shown in Fig. 1, while Fig. 2 shows the effect of increasing concentrations of this CD derivative, where the effect of CD complexation on both

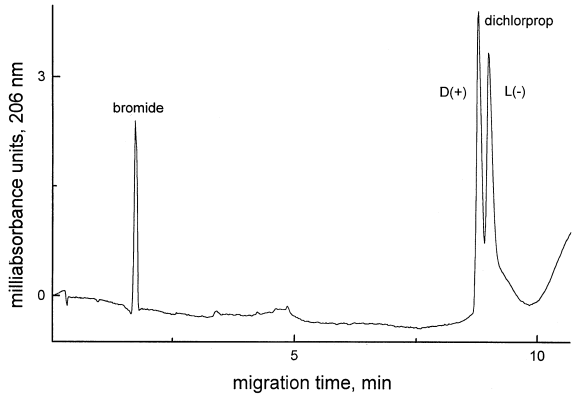

Fig. 1. Electropherogram of racemic dichlorprop obtained with 5 m*M* ethylcarbonate-β-CD added to the running buffer.

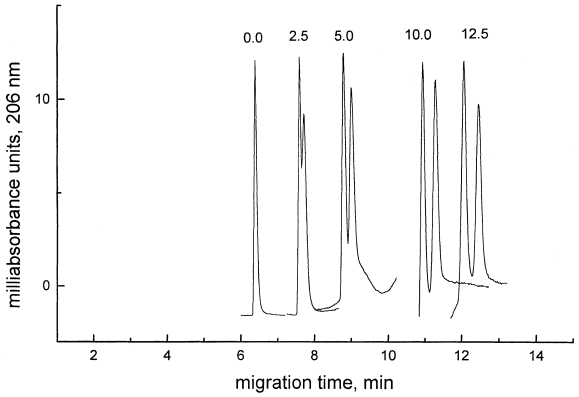

Fig. 2. Electropherograms of racemic dichlorprop with increasing concentrations (m*M*) of ethylcarbonate-β-CD in the running buffer.

migration times and resolution is clearly visible. Even with the low 7 kV applied potential and with the short length of the capillary used, complete resolution is obtained at 12.5 m*M* CD concentration. One of the electropherograms obtained with the C_6-capped-β-CD is presented in Fig. 3. Only partial enantioresolution was obtained with this derivative on the condition adopted. Both ethylcarbonate-β-CD and C_6-capped-β-CD produced large baseline drifts. This is probably due to small impurities of reagents used for synthesis, which were not completely removed during purification, although it consisted of double precipitation followed by extensive freeze-drying. In the electropherograms obtained with γ-CD, retardation due to complex formation was clearly visible, but no enantiomer separation could be observed. Results similar to those given by this last compound have been observed also with the native β-CD and with the new ethylcarbonate-γ-CD, while enantioresolution was observed with α-CD and with methyl- and hydroxypropyl-β-CD, according to reports in [24]. Fig. 4 shows the effect of increasing concentration of α-CD on the electrophoretic behaviour of the two optical isomers. The best resolution has been attained at 2.5 m*M* concentration. The plot of Fig. 5 summarises the results obtained with α-CD and ethylcarbonate-β-CD. Both the migration times and their difference for the two enantiomers depend on the concentration of chiral additive.

Measurements of migration time data at variable CD concentration were performed with all the tested CDs, and the data obtained were used for calculating the inclusion constants, which are listed in Table 1. Five of the eight CDs allowed enantiomer resolution. Under the conditions adopted, resolution of dichlorprop enantiomers was observed with the newly synthesised ethylcarbonate-β-CD, with native α-CD and with the commercially available methyl-β-CD and hydroxypropyl-β-CD, while only partial resolution was given by the C_6-capped-β-CD. The best performances, in terms of the relative difference between the constants of inclusion of the two optical antipodes, were given by the native α-CD and by the newly synthesised ethylcarbonate-β-CD. Both showed a 14% difference between the constants of

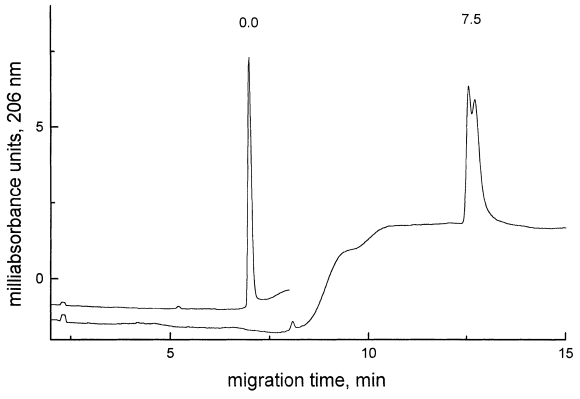

Fig. 3. Electropherogram of racemic dichlorprop obtained with 7.5 m*M* C_6-capped-β-CD added to the running buffer. The electropherogram obtained without chiral additive is superimposed for comparison.

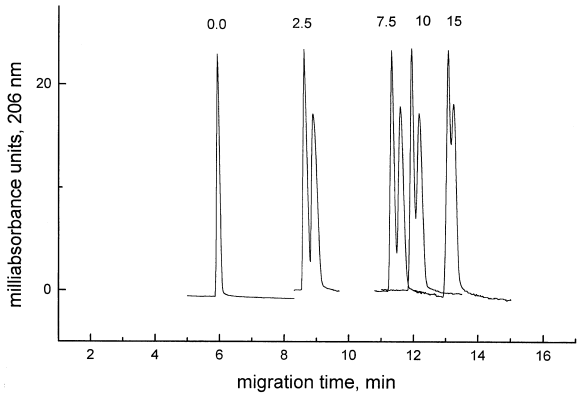

Fig. 4. Electropherograms of racemic dichlorprop with increasing concentrations (m*M*) of α-CD in the running buffer.

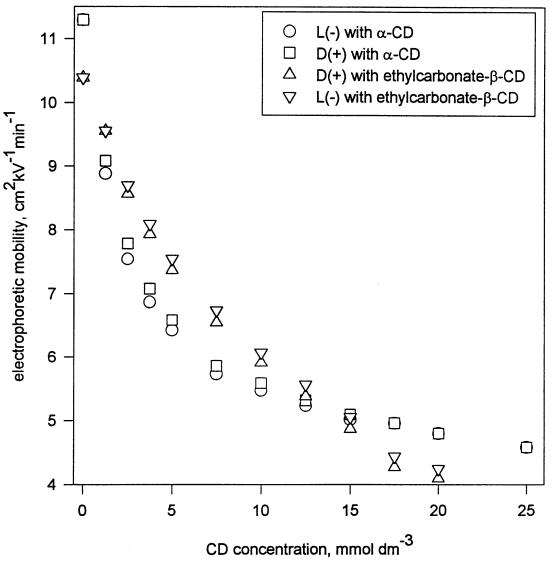

Fig. 5. Migration times of D-(+)- and L-(−)-dichlorprop as a function of cyclodextrin concentration in the running buffer.

the two enantiomers. The negative value of the α-CD relative difference is due to the fact that the L-(−)-enantiomer was eluted first with this additive, while all the remaining CDs caused the D-(+)-enantiomer to migrate faster. The inclusion constant values of the three native CDs are inversely proportional to their dimensions, those of γ-CD being approximately one order of magnitude smaller than those of α-CD, this last being the only native CD capable of enantioresolution. Molecular modeling can help in interpreting such experimental findings. In Fig. 6, the molecular models of the inclusion complexes of dichlorprop with the three native CDs are reported. The guest molecule fits rather tightly into the α-CD cavity, while the distance between the host and guest molecules increases remarkably as the dimension of the CD ring increase. The closer the two molecules are, the larger the energy of interaction between them is expected to be, as the inclusion constants values demonstrate. On the other hand, limited space in the CD cavity implies limited freedom for the included molecule of assuming the position which minimises the thermodynamic energy of this molecular system. In this case, the inclusion of the enantiomer capable of best matching the chiral centres of the host molecule, will be energetically favoured, thus allowing effective enantioresolution. On the contrary, larger CDs permit each enantiomer to find a position which minimises the energy of the system, thus reducing the stereospecific aspects of complexation.

The derivatized β-CDs show inclusion constants lower than that of the native β-CD, but they can produce enantiomer separation despite their lower complexation ability. The molecular models represented in Figs. 7 and 8 show that the CDs' substituents cause some hindrance for the guest molecule, thus reducing the energy of interaction, but also its degrees of freedom, thus creating the conditions for enantiomer resolution to occur. The dimensions of the γ-CD cavity are probably so big, with respect to those of dichlorprop, that not even derivatisation can create conditions favourable for enantioresolution.

In CD-assisted CZE, peak separation is known to depend on the concentration of the chiral selector and on the relative difference in the inclusion constants of the two enantiomers, independently of their absolute value [27]. In fact, native β-CD,

Table 1
Inclusion constants

Cyclodextrin	D-(+) constant, k_D	L-(−) constant, k_L	$[(k_L - k_D)/k_D)\cdot 100$	Optimum [CD] (mM dm^{-3})
α-Cyclodextrin	411	352	−14	2.6
β-Cyclodextrin	257	257	0	−
γ-Cyclodextrin	40	40	0	−
C_6-Capped-β-CD	64	69	8	15.0
Ethylcarbonate-β-CD	74	84	14	12.6
Ethylcarbonate-γ-CD	74	74	0	−
Methyl-β-CD	53	59	11	17.9
Hydroxypropyl-β-CD	82	91	11	11.6

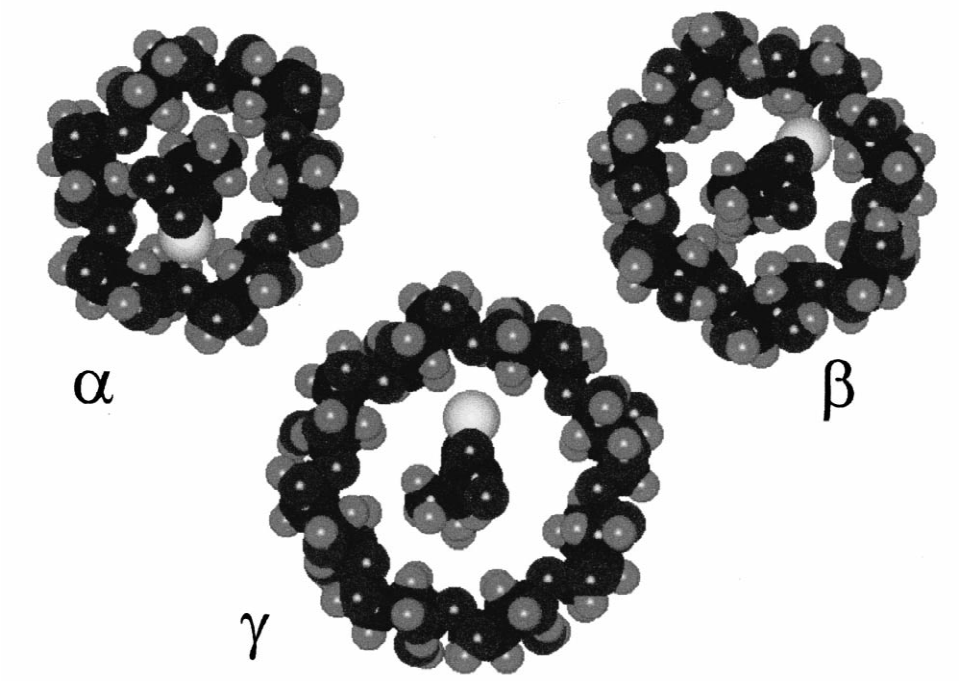

Fig. 6. Molecular models representing the inclusion complexes of dichlorprop with α-, β- and γ- CD. Secondary hydroxyl sides of CDs are facing the reader.

although is a stronger complexing agent than its derivatives, does not separate dichlorprop enantiomers, while hydroxypropyl-, methyl- and ethylcarbonate-CD derivatives produced enantioresolution. On the other hand, neither native γ-CD nor its ethylcarbonate derivative were capable of producing enantiomer separation, although both have inclusion constants of the same order of magnitude as those of the β-CD derivatives.

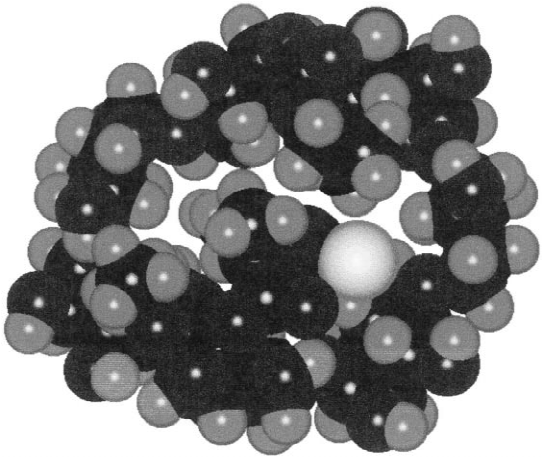

Fig. 7. Chemical structure of the ethylcarbonate-β-CD–dichlorprop complex. Secondary hydroxyl side of CD is facing the reader.

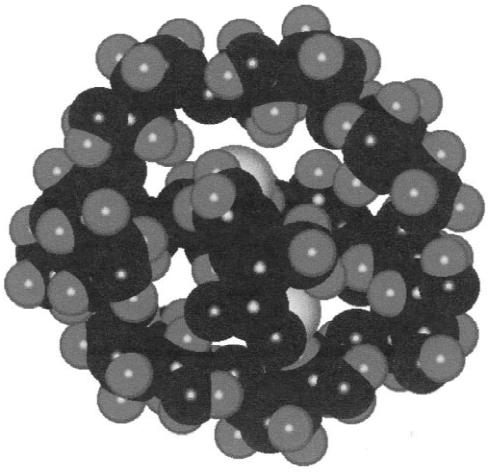

Fig. 8. C_6-Capped-β-CD complex with dichlorprop. Secondary hydroxyl side of CD is facing the reader.

As reported in [27,30] the optimum concentration of chiral additive, that is, the concentration at which maximum resolution occurs, depends on the value of the two complexation constants according to the following relationship:

$$C_{opt} = 1/(K_1 K_2)^{1/2} \quad (6)$$

where C_{opt} is the concentration of additive which gives the maximum selectivity, K_1 and K_2 are the formation constants. By substituting into this expression the values of the calculated constants, the optimum concentration for each CD capable of giving enantioseparation was obtained, and the results are reported in Table 1. For α-CD, the concentration for obtaining maximum difference in the enantiomer mobilities was 2.6 m*M*, while that for ethylcarbonate-β-CD was 12.7 m*M*. The optimum concentration of C_6-capped-β-CD should be 15.0 m*M*, but the solubility of this compound in the run buffer was only 12.5 m*M* which was lower than the optimum.

The difference in the mobility of the enantiomers can be calculated as a function of the CD concentration by means of the following relationship [27,30]:

$$\Delta\mu = \frac{[\mathrm{CD}](\mu_f - \mu_c)(K_2 - K_1)}{1 + [\mathrm{CD}](K_1 + K_2) + K_1 K_2 [\mathrm{CD}]^2} \quad (7)$$

where $\Delta\mu$ is the calculated difference in mobility, μ_f and μ_c are the mobilities of the free and of the complexed analytes, K_1 and K_2 are the inclusion constants and [CD] is the CD concentration. The results obtained for α-CD and for ethylcarbonate-β-CD are plotted in Fig. 9. The maxima of the two functions are located at the CD concentrations already calculated by means of Eq. (1). The electropherograms in Figs. 2 and 4 show that the CD concentrations for maximum difference in mobility are those calculated.

The two peaks were resolved better by ethylcarbonate-β-CD than by α-CD. The electrophoretic resolution depends on the ratio between the difference in the mobility of the analytes and the sum of the width of the peaks. This ratio was more favourable with ethylcarbonate-β-CD than with α-CD, thus resulting in better separation performances with the former.

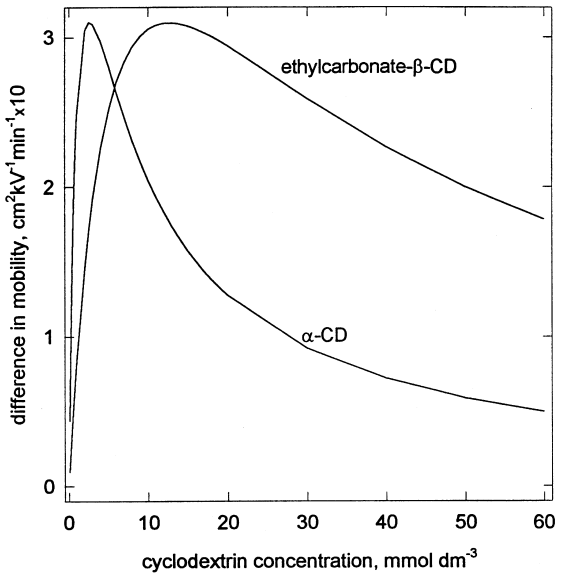

Fig. 9. Difference in the mobility of the two enantiomers of dichlorprop calculated as a function of chiral running buffer additive.

4. Conclusions

The chiral selector properties of eight CDs were investigated. The inclusion constant values were calculated by means of migration time data measured a variable CD concentration. A new ethylcarbonate derivative of β-CD was found to resolve the two optical antipodes of dichlorprop better than native α-CD or the commercially available hydroxypropyl-β-CD and methyl-β-CD. Although the complexation constants of the β-CD derivatives were found to be smaller than that of the native β-CD, efficient chiral resolution was possible due to the large relative difference between the complexation constants of the two enantiomers. An interpretation of these facts based on the examination of molecular models of the inclusion complexes has been proposed. C_6-Capped-β-CD and native β-CD gave unsatisfactory or no resolution of the racemate, thus evidencing the specific character of the interaction between analyte and resolving agent needed to achieve optical resolution. The knowledge of the values of the complexation constants allowed precise calculation of the dependence of the difference of the enantiomer mobilities on the concentration of the chiral additive.

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