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Enantioseparation of binaphthol and its monoderivatives by cyclodextrin-modified capillary zone electrophoresis: A mathematical approach

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ABSTRACT

A simple model for the separation of atropisomers of binaphthol and its monoderivatives by means of cyclodextrin-modified capillary zone electrophoresis (CD-CZE) was used to describe the migration behavior of poly charged enantiomers in a chiral separation system. This mathematical approach allowed for the determination of the optimal cyclodextrin concentrations for the enantioseparation of binaphthols by taking into account the influence of the formed complex mobilities. Moreover, using this theoretical approach, the reversal of the enantiomers' migration order as a function of cyclodextrin concentration was predicated. The apparent complexation constants between the cyclodextrins and the binaphthol and its monoderivatives could be calculated using a non-linear curve fitting method and three linear plotting methods (*x*-reciprocal, *y*-reciprocal and double reciprocal). Good agreements between the theoretical and experimental cyclodextrin concentrations were obtained.

1. Introduction

Chiral separation by means of capillary electrophoresis (CE) has been proven to be an effective technique in recent years. The major advantages of CE are simplicity, high efficiency, versatility, rapid analysis, high resolution, small sample volume and low operating costs. Enantioseparation can be achieved in CE using chiral selectors, which discriminate between enantiomers by an enantioselective complexation between the enantiomers of the analyte and the chiral selector to differentiate based on the effective electrophoretic mobility of the enantiomers. Numerous chiral selectors are currently available and can be used for enantioseparation, such as cyclodextrins (CDs), chiral crown ethers, proteins, chiral surfactants, macrocyclic antibiotics, ligand-exchange complexes and polysaccharides [1]. Among them, the cyclodextrins are the most widely used chiral selectors [2].

The mechanism of the separation is fairly well understood. Several groups have developed mathematical models as theoretical approaches to evaluate the influence of the experimental variables and to describe the migration behavior of the analytes in the chiral separation systems.

Most models assume a 1:1 complexation between the enantiomers and the chiral selector. The analyte and the chiral selector can be neutral, anionic, cationic or zwitterionic.

Wren and Rowe [3–6] developed mobility difference models, and Sänger-Van de Griend et al. [7] proposed a model based on one analyte having two or more complexation sites for the selector and able to form multiple complexes. In a series of papers, Vigh and co-workers [8–10] introduced multiple equilibrium-based mobility models, including the effects of competing binding equilibriums of the dissociated and non-dissociated analytes. They also developed the chiral charged resolving agent migration model (CHARM) [11]. Zhu et al. [12], Surapaneni et al. [13] and Lelièvre et al. [14] developed mathematical models for chiral separation systems using two chiral selectors.

The atropisomers of 1,1′-binaphthyl-2,2′-diol (or Binol) are among the most widely used ligands for the asymmetric catalytic reactions, and serve as chiral hosts in the enantiomeric separation of racemic mixtures of "guest" compounds or as chiral reagents during optical purity studies of the enantiomers in ^{1}H NMR. Furthermore, they have also been used in enantiomeric mixture tests for evaluating new or developed chiral separation systems [15]. For these reasons, the enantioseparation of Binol by CE has been the subject of several studies. In a previous paper [16], we studied the enantiomeric separation of Binol and its monoderivatives by means of CD-CZE. First, we determined the acidity constants of Binols. Second, the nature of the CDs and the optimal conditions of the separation were reported. Additionally, working in a very basic

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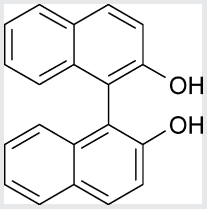
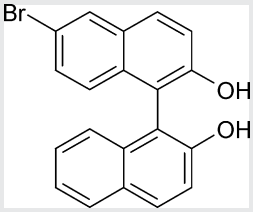
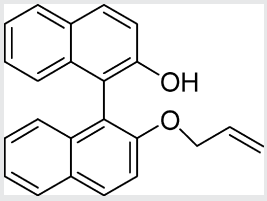
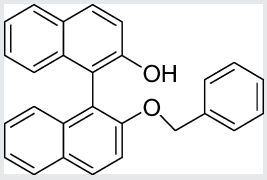
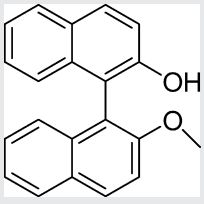
Table 1
Structures of the studied Binol and its derivatives.

Name	Abbreviation	Structure
1,1′-Binaphthalen-2,2′-diol	Binol	
6-Bromo-1-(2-hydroxynaphthalen-1-yl)naphthalen-2-ol	BN2	
1-(2-(Allyloxy)naphthalen-1-yl)naphthalen-2-ol	BN3	
1-(2-(Benzyloxy)naphthalen-1-yl)naphthalen-2-ol	BN4	
1-(2-Methoxynaphthalen-1-yl)naphthalen-2-ol	BN5	

medium (pH 11.5) was also clarified. Moreover, we observed two different types of inversion in the elution order of the two atropisomers of Binol: one as a function of the pH with γ-CD (pH range: 10–11.5) and the other as a function of the γ-CD concentration at pH 10.8.

In this paper, we adapted a simple model to describe the enantioseparation of Binol and its derivatives by means of CD-CZE. The reversal of the enantiomer migration order as a function of the cyclodextrin concentration was achieved using this model. Moreover, a mathematical approach was developed to determine the optimal cyclodextrin concentrations. These were compared to the experimental optimal concentrations to validate the theoretical model. Finally, the apparent complexation constants between binaphthols and the CDs were determined using four different plotting methods.

2. Experimental

2.1. Instrumentation

Capillary electrophoretic experiments were performed using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) equipped with a photodiode array detector (PDA; 190–600 nm). An uncoated fused silica capillary (Thermo Electron SA, Courtaboeuf, France), 40.2 cm long (30 cm to the detector), and with a 50 μm ID was used. The capillaries were thermostated at 20.0 ± 0.1 °C. Samples were pressure-injected at 0.5 psi (34 mbar) for 3 s at the inlet side of the capillary (anode). Electrophoretic runs were performed with 10 kV potential. The UV detection was cathodic (λ = 214 nm). The electropherograms were recorded and integrated by an IBM personal computer with 32 Karat software (Version 4.0, Beckman Coulter). The pH of the electrolyte solutions was measured using a Cyberscan pH510 pH meter (Bioblock Scientific, Illkirch, France).

The experimental data were fit with the CurveExpert program (Version 1.38, Daniel G. Hyams, Hixson, TN, USA) for the nonlinear curve fitting method and with SigmaPlot8.0 (Version 8.02, Systat Software Inc., San Jose, USA) for the linear plotting methods.

2.2. Chemicals and reagents

The racemate and the R enantiomer of Binol (1,1′-binaphthyl-2,2′-diol or 1,1′-binaphthalen-2,2′-diol) were provided by

Sigma–Aldrich (Sigma–Aldrich France, Saint-Quentin-Fallavier, France). Binol derivatives studied in this work (see Table 1) were synthesized as described previously [16]. Native α-CD was purchased from Sigma–Aldrich, β-CD (98%) from Interchim (Montluçon, France) and γ-CD (98%) from Acros Organics (Acros Organics France, Noisy-le-Grand, France). 2-Hydroxypropyl-α-, β- and γ-CDs (HP-x-CDs) (d.s. ≈ 0.6) were obtained from Fluka Chemie (Sigma–Aldrich–Fluka France, L'Isle d'Abeau, Chesne, France), and heptakis-2,3,6-tri-*O*-methyl-β-cyclodextrin (TM-β-CD) was purchased from Sigma–Aldrich. Disodium hydrogenphosphate, trisodium phosphate, sodium hydroxide (+98% purity), and absolute ethanol (98.8%). were obtained from Sigma–Aldrich.

2.3. Electrolyte and sample preparation

All running electrolytes were prepared using ultrapure water produced with a Milli-Q-System water purification apparatus (Millipore France, Montigny-le-Brotonneux, France). The solutions were sonicated just before use for 10 min with a Branson 2510 sonication apparatus (Branson, Danbury, USA). For the enantioseparations and the apparent complexation constant determinations, a phosphate electrolyte (Na_2HPO_4–Na_3PO_4) with an ionic strength of 80 mM was used with cyclodextrin concentrations ranging from 0 to 20 mM or 25 mM. All samples were prepared in ethanol at a concentration of 1×10^{-4} M.

In order to determine the enantiomeric migration order, the racemic sample was spiked with the R enantiomer. New uncoated capillaries were activated by performing the following washing process: water for 2 min, 1 M NaOH for 30 min and water for 10 min. The capillary was conditioned for 15 min with the electrolyte before running and for 2 min between each run.

3. Results and discussion

3.1. Theory

In our previous work [16], we reported a reversal of the elution order of the two atropisomers of Binol as a function of the pH with γ-CD (pH range: 10–11.5). The R enantiomer of Binol migrated first at pH 10, and the second elution occurred at pH 11.5. We proposed two hypotheses for the origin of this inversion.

The first hypothesis involved the progressive ionization of γ-CD (pK_a of γ-CD is equal to 12.08) [17]. At pH 10, γ-CD is neutral and therefore is carried by the cathodic electroosmotic flow. Consequently, the enantiomer with the higher affinity for the hydrophobic cavity of the CD migrates firstly (R enantiomer of Binol), since the two enantiomers migrate towards the anode at this pH (pK_{a1} Binol = 9.04 and pK_{a2} Binol = 10.90). However, at pH 11.5, the γ-CD is slightly ionized (charge $\ll -1/2$). Thus, under such conditions, it presents an electrophoretic mobility slightly diametrically opposed to that of the electroosmotic flow, and consequently slows down the migration of the enantiomer with the greatest affinity, i.e., the R enantiomer.

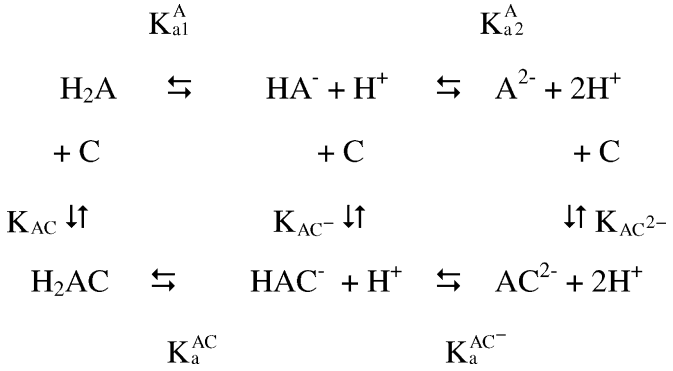
The second hypothesis was based upon the conclusions of the works of Vigh and co-workers [8–10], who modeled the enantiomeric separation of chiral weak acids and bases in CE. They showed that based on

- the electrophoretic mobilities of the dissociated analytes/CD complexes (μ^-_{RCD} and μ^-_{SCD}),
- the complex formation constants of the analytes in the dissociated and non-dissociated forms with the CD (K^-_{RCD}, K^-_{SCD} and K_{HRCD}, K_{HSCD}),
- there are three kinds of separation. One works as a function of the pH, one based on the CD concentration, and one is systematically neutral. The three kinds are:
  - desionoselective separation or Type I ($\mu^-_{RCD} = \mu^-_{SCD}$, $K^-_{RCD} = K^-_{SCD}$ and $K_{HRCD} \neq K_{HSCD}$),
  - ionoselective separation or Type II ($\mu^-_{RCD} \neq \mu^-_{SCD}$, $K^-_{RCD} \neq K^-_{SCD}$ and $K_{HRCD} = K_{HSCD}$),
  - duoselective separation or Type III ($\mu^-_{RCD} \neq \mu^-_{SCD}$, $K^-_{RCD} \neq K^-_{SCD}$ and $K_{HRCD} = K_{HSCD}$).

The last two allow for, if so desired, the reversal of the migration order of the enantiomers as a function of the electrolyte composition (pH, CD concentration).

Therefore, the origin of the reversal migration order of the Binol enantiomers as a function of the pH in presence of γ-CD is more likely to act according to the behavior of Type II or III than in the progressive ionization of γ-CD. In fact, the last option is unlikely even at pH 11.5, because the absolute value of its electrophoretic mobility is likely small in comparison to the electroosmotic flow.

However, the case of binaphthol and BN2 proves to be more complicated because they are diacids. Thus, all of the equilibriums reported below that are based on acid–base equilibrium must be taken into account.



where K^A_{a1} and K^A_{a2} are the first and the second acidity constants of the diacid H_2A (or A); K^{AC}_a and K^{AC-}_a are the acidity constants of the formed complexes H_2AC and HAC^-, respectively; and K_{AC}, K_{AC^-} and $K_{AC^{2-}}$ are the complexation formation constants of H_2A in its non-ionized, ionized and doubly ionized forms, respectively.

Not all of the data necessary to model the electrophoretic behavior of the enantiomers as a function of the pH and the cyclodextrin concentration was accessible experimentally. Thus, we decided to simplify the model by using an apparent equilibrium and apparent constants, as proposed by Lelièvre and Gareil [18], in the case of the monoacids, as an alternative approach to that of Vigh and co-workers [8–10].

If A′ and AC′ represent the analyte and the complex, respectively, in their ionized and non-ionized forms, then [A′] is the total concentration of the analyte and its related species, and [AC′] is the total concentration of the complex and its related species, so that

$$[A'] = [A] + [A^-] + [A^{2-}], \qquad [AC'] = [H_2AC] + [HAC^-] + [AC^{2-}] \tag{1}$$

Then, as in the case of monoacids [18], the whole system can be described by an apparent equilibrium and an apparent equilibrium constant K':

$$A' + C \rightleftarrows AC', \qquad K = \frac{[AC']}{[A'][C]} \tag{2}$$

with

$$K' = K_{AC}\frac{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}{[H^+]^2 + [H^+]K_{a1}^A + K_{a1}^A K_{a2}^A}$$
$$= K_{AC^-}\frac{K_{a1}^A}{K_a^{AC}}\frac{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}{[H^+]^2 + [H]^+K_a^A + K_{a1}^A K_{a2}^A}$$
$$= K_{AC^-}\frac{K_{a1}^A K_{a2}^A}{K_a^{AC}K_a^{AC^-}}\frac{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}{[H^+]^2 + [H^+]K_{a1}^A + K_{a1}^A K_{a2}^A} \quad (3)$$

If all of equilibriums are fast, then μ_A, the mobility of H_2A (or A), is a linear combination of its mobility in the free solution (μ_A) and its mobility when it is totally complexed (μ_C):

$$\mu_A = \frac{[A']}{[A'] + [AC']}\mu_f + \frac{[AC']}{[A'] + [AC']}\mu_C$$
$$\mu_f = \frac{[H^+]^2}{[H^+]^2 + [H^+]K_{a1}^A + K_{a1}^A K_{a2}^A}\mu_A + \frac{[H^+]K_{a1}^A}{[H^+]^2 + [H^+]K_{a1}^A + K_{a1}^A K_{a2}^A}\mu_{A^-} + \frac{K_{a1}^A K_{a2}^A}{[H^+]^2 + [H^+]K_{a1}^A + K_{a1}^A K_{a2}^A}\mu_{A^{2-}} \quad (4)$$
$$\mu_C = \frac{[H^+]^2}{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}\mu_{AC} + \frac{[H^+]K_a^{AC}}{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}\mu_{AC^-} + \frac{K_a^{AC}K_a^{AC^-}}{[H^+]^2 + [H^+]K_a^{AC} + K_a^{AC}K_a^{AC^-}}\mu_{AC^{2-}}$$

where μ_{A^-}, $\mu_{A^{2-}}$, μ_{AC}, μ_{AC^-} and $\mu_{AC^{2-}}$ are the absolute mobilities of HA^-, A^{2-}, H_2AC, HAC^- and AC^{2-}, respectively.

The first term in the equation that defines μ_f is systematically null, because the acid compounds present a null electrophoretic mobility in ion suppression.

Therefore, the mobility of H_2A (or A) can be expressed as a function of the apparent equilibrium constant and the ligand concentration, which is the same equation in the case of a monoacid, but with a different constant:

$$\mu_A = \frac{1}{1 + K'[C]}\mu_f + \frac{K'[C]}{1 + K'[C]}\mu_C \quad (5)$$

If A is a racemic mixture, then the mobility of each enantiomer (S or R) can be described using:

$$\mu_S = \frac{\mu_f + \mu_{SC}K'_S[C]}{1 + K'_S[C]}, \qquad \mu_R = \frac{\mu_f + \mu_{RC}K'_R[C]}{1 + K'_R[C]} \quad (6)$$

where μ_S and μ_R are the mobility of the S and R enantiomers, respectively; μ_f is the mobility of the free enantiomer; μ_{SC} and μ_{RC} are the mobilities of the complexed S and R enantiomers, respectively; K'_S and K'_R are the apparent complexation constants between the S and R enantiomers, respectively, and the ligand; and [*C*] is the ligand concentration.

As a result, similar equations are obtained for both the monoacid and diacid, but with different apparent complexation constants. Moreover, the mobilities of the complexed S and R enantiomers are slightly different from each other due to the difference in their acidity constants, as can be observed from Eq. (4).

For the determination of the apparent complexation constant, Eq. (6) can be used. Additionally, linear plotting (Eqs. (7)–(9)) can be obtained from these equations, which have been used in CE for the determination of binding constants. In fact, these linear plotting equations are analogous to those that have been employed for spectroscopy, chromatography and calorimetry techniques. These three linear plotting equations are known as double reciprocals (Eq. (7)) with K' = intercept/slope, *y*-reciprocal (Eq. (8)) with K' = slope/intercept, and *x*-reciprocal (Eq. (9)) with K' = −slope:

$$\frac{1}{\mu_i - \mu_f} = \frac{1}{(\mu_C - \mu_f)K'}\frac{1}{[C]} + \frac{1}{\mu_C - \mu_f} \quad (7)$$

$$\frac{[C]}{\mu_i - \mu_f} = \frac{1}{\mu_C - \mu_f}[C] + \frac{1}{(\mu_C - \mu_f)K'} \quad (8)$$

$$\frac{\mu_i - \mu_f}{[C]} = -K'(\mu_i - \mu_f) + K'(\mu_C - \mu_f) \quad (9)$$

Armstrong et al. [19] compared these different equations for the estimation of the binding constants by CE for both achiral and chiral analytes. Bellini et al. [20] determined the apparent association constants of four model drugs with β-CD by CE and compared the results obtained by these three linear plotting methods. The great advantage of the non-linear curve fitting method (Eq. (6)) is the determination of μ_f and μ_C by fitting the experimental data to the equations, so we can compare μ_f and its experimentally determined value when no chiral selector is present. Moreover, we do not need to measure μ_C experimentally in order to determine the binding constant. In fact, μ_C often cannot be accurately measured or even is impossible to be determined due to experimental limitations such as ligand solubility [21] or capillary wall binding by the ligand [22]. The non-linear curve fitting methods avoid these problems and disadvantages. Similarly, the linear methods (Eqs. (7)–(9)) are advantageous for systems where the mobility of the analyte–ligand complex is not known and cannot be adequately measured with a marker or at saturating concentrations of the ligand.

These four plotting methods were used to estimate the apparent complexation constants of binaphthols and cyclodextrins at pH 11.5. The advantage of working at this pH for the enantioseparation of Binols was previously reported [16].

The mobilities of the analytes were systematically corrected for the electrolytes' viscosity changes due to the varied CD concentration. Thus, the electrophoretic mobilities were multiplied by the ratio between the currents obtained with and without CD in the solution [23].

The pK_a of cyclodextrins is about (12.08–12.3) [24], thus the cyclodextrin can begin to be dissociated at pH 11.5 and could exist in ionized form. However, the ionization percentage is almost poor ≈15%, and as a first approximation, we hypothesized that this percentage does not influence the determination of the apparent complexation constant or complex mobility. This hypothesis was validated by the comparison between the calculated mobility of free Binol in the case of the various studied CDs using Eq. (6) and the experimental values measured without CD (see Table 2). In fact, a good agreement is observed between the experimental and calculated values, and therefore, at pH 11.5, the dissociation of CD was effectively considered to be negligible and not influencing the calculation.

As a result, the proposed model could be used for estimating the apparent complexation constants between binaphthols and cyclodextrins at pH 11.5.

3.2. Apparent complexation constants and complex mobilities

As was illustrated above with a mathematical approach, in the case of binaphthol, the mobilities of the S- and R-complexed enantiomers are different from each other. Fig. 1a reports the theoretical evolution of the electrophoretic mobilities as a function of the γ-CD concentration for the R and S enantiomers of Binol. Table 3 contains the apparent complexation constants between these two

Table 2
Comparison between the experimental and calculated values of free Binol mobility in the case of various experimentations realized during the enantioseparation of Binol by means of various cyclodextrins at pH 11.5.

	$\mu_{free,exp}$ ($\times 10^{-8}$ m^2 V^{-1} s^{-1})	Binol (S, R)	$\mu_{free,cal}$ ($\times 10^{-8}$ m^2 V^{-1} s^{-1})
α-CD	−2.00	S	−2.00
		R	−2.01
β-CD	−2.00	S	−2.02
		R	−2.02
γ-CD	−2.01	S	−2.01
		R	−2.02
HP-α-CD	−2.03	S	−2.02
		R	−2.02
HP-β-CD	−2.09	S	−2.10
		R	−2.11
HP-γ-CD	−2.13	S	−2.12
		R	−2.13
TM-β-CD	−2.12	S	−2.13
		R	−2.13

$\mu_{free,exp}$: experimental value and $\mu_{free,cal}$: calculated value using (Eq. (6)).

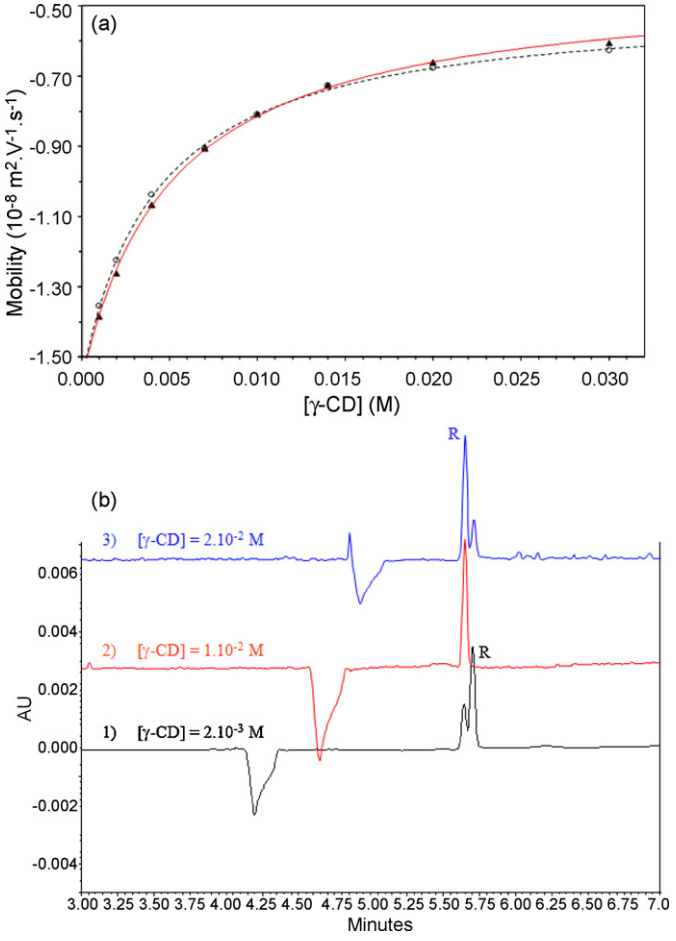


Fig. 1. (a) Variations of the electrophoretic mobility of Binol enantiomers as a function of γ-CD concentration. Curves identification: (▲) experimental points and continuous line for R-Binol; (○) experimental points and dashed line for S-Binol. (b) Enantioseparation of Binol enantiomers with different concentrations of γ-CD. *Experimental conditions*: uncoated silica capillary (30 (40.2) cm × 50 μm ID); hydrodynamic injection: 3 s at 0.5 psi; T = 20 °C; λ = 214 nm; voltage: 10 kV; electrolyte: Na_2HPO_4/Na_3PO_4; pH 10.8; ionic strength: 8×10^{-2} M; [γ-CD]: variable; sample: Binol racemate spiked with the R enantiomer.

Table 3
The apparent complexation constants, and the complex mobilities μ_{SC}, μ_{RC} between the S, R enantiomers of Binol and γ-CD at pH 10.8.

K'_S (M^{-1})	235
μ_{SC} ($\times 10^{-8}$ m^2 V^{-1} s^{-1})	−0.49
R^2	0.9996
K'_R (M^{-1})	194
μ_{RC} (10^{-8} m^2 V^{-1} s^{-1})	−0.43
R^2	0.9996

enantiomers and γ-CD and the complex mobilities deduced from the theoretical approach and the correlation coefficients.

It should be noted that these correlation coefficients are good. Through examination of Table 3, we find that the apparent complexation constants between the two enantiomers and γ-CD are different (235 M^{-1} for the S enantiomer against only 194 M^{-1} in the case of the R enantiomer), and the electrophoretic mobilities of the formed complexes are also slightly different. In addition, the absolute values of the electrophoretic mobilities of the S-Binol/γ-CD complex are higher than that of the R-Binol/γ-CD complex. In fact, the electrophoretic mobilities of these two complexes, as they are deduced from the theoretical treatment, are equal to -0.49×10^{-8} and -0.43×10^{-8} m^2 V^{-1} s^{-1}, respectively. This difference in the apparent complexation constants for the Binol enantiomers as well as the difference in the mobilities of the Binol/γ-CD complexes ($K'_S > K'_R$ and $\mu_{SC} > \mu_{RC}$) rationalize the inversion observed experimentally and shown in Fig. 1b.

The apparent complexation constants calculated between the two Binol enantiomers and γ-CD ($K'_S = 235$ M^{-1} and $K'_R = 194$ M^{-1}) are similar to those reported by Zerbinati and Trotta [25] for the same enantiomers and the same cyclodextrin (272 M^{-1} for the S enantiomer and 246 M^{-1} for the R enantiomer). In fact, the constants that we deduced have the same magnitude reported by these authors, and the interactions of the S enantiomer with the hydrophobic cavity of γ-CD are more favorable in the two cases. On the other hand, the K'_S/K'_R ratio, and thus the thermodynamic selectivity, is higher in our case than in the study of Zerbinati and Trotta (1.22 versus 1.11). However, the approach used by these authors is critical since they deduced their different values by applying the model of Wren and Rowe [3–6], meaning without taking into account the incomplete dissociation of binaphthol.

Table 4 shows some examples of the complex mobility of various binaphthol (S,R)/CD couples calculated using Eq. (6) and for which no inversion in the enantiomer migration order was observed as a function of the cyclodextrin concentration.

Such an observation could be attributed to an insufficient difference of the electrophoretic mobilities of the S- and R-formed complexes together with the magnitudes of the apparent com-

Table 4
Calculated mobilities of the formed complexes in the case of various BN/CD couples at pH 11.5 using Eq. (6).

Binols	CD	$\mu_{c,cal}$ ($\times 10^{-8}$ m^2 V^{-1} s^{-1})
Binol (S)	α-CD	−0.37
Binol (R)		−0.39
BN2 (1)	HP-γ-CD	−0.45
BN2 (2)		−0.44
BN3 (1)	γ-CD	−0.38
BN3 (2)		−0.34
BN4 (1)	HP-α-CD	−0.46
BN4 (2)		−0.43
BN5 (1)	β-CD	−0.34
BN5 (2)		−0.32

(1) and (2) corresponding to the first and second peaks of BN racemates.

Table 5
Apparent complexation constants of different binaphthols and cyclodextrins calculated at pH 11.5 by the four plotting methods: (X) *x*-reciprocal, (Y) *y*-reciprocal, (XY) double reciprocal and (XYZ) non-linear (K' in M^{-1}).

	Binol		BN2 (S/R)		BN3 (S/R)		BN4		BN5	
	S	R					1	2	1	2
α-CD										
K' (X)	27	18	33		34		32	23	22	15
K' (Y)	27	20	33		34		31	23	22	15
K' (XY)	29	20	30		34		34	25	22	15
K' (XYZ)	25	23	32		25		23	15	22	20
	Binol		BN2		BN3		BN4		BN5	
	S	R	1	2	1	2	1	2	1	2
β-CD										
K' (X)	125	83	454	289	116	41	154	105	95	32
K' (Y)	129	85	447	299	114	41	155	106	92	33
K' (XY)	113	73	445	274	123	39	158	105	106	32
K' (XYZ)	150	106	450	319	90	48	145	108	75	41
	Binol		BN2		BN3		BN4		BN5	
	S	R	1	2	1	2	1	2	1	2
γ-CD										
K' (X)	118	75	639	564	171	145	145	97	63	39
K' (Y)	118	76	639	559	173	142	160	107	65	41
K' (XY)	121	75	652	581	178	154	134	89	60	36
K' (XYZ)	117	81	628	554	167	135	170	122	74	49
	Binol		BN2 (S/R)		BN3		BN4		BN5	
	S	R			1	2	1	2	1	2
HP-α-CD										
K' (X)	60	29	21		91	53	95	72	56	23
K' (Y)	60	29	21		91	53	93	69	55	23
K' (XY)	62	29	24		93	56	106	83	57	25
K' (XYZ)	56	25	17		86	57	79	49	51	21
	Binol		BN2 (S/R)		BN3		BN4		BN5	
	S	R			1	2	1	2	1	2
HP-β-CD										
K' (X)	264	159	344		195	125	443	268	219	113
K' (Y)	268	160	335		183	118	457	270	208	109
K' (XY)	263	160	340		204	135	439	266	234	128
K' (XYZ)	279	164	362		197	117	460	272	212	109
	Binol		BN2		BN3		BN4		BN5	
	S	R	1	2	1	2	1	2	1	2
HP-γ-CD										
K' (X)	91	52	607	476	109	90	122	71	34	14
K' (Y)	95	53	644	470	115	94	125	73	35	15
K' (XY)	93	51	598	473	103	84	124	72	34	14
K' (XYZ)	96	57	655	483	121	96	137	81	36	15
	Binol		BN2		BN3		BN4		BN5	
	S	R	1	2	1	2	1	2	1	2
TM-β-CD										
K' (X)	125	51	38	24	59	42	155	71	95	49
K' (Y)	128	51	40	24	60	42	158	70	94	48
K' (XY)	120	50	34	24	54	43	156	77	90	48
K' (XYZ)	135	55	51	17	65	38	163	66	92	44

plexation constants between the two enantiomers and the CD. In fact, the reported values of $\Delta\mu_C$ in Table 4 are at most equal to 0.04×10^{-8} m^2 V^{-1} s^{-1}, which must be closer to the calculated $\Delta\mu_C$ value in the case of Binol and γ-CD (0.06×10^{-8} m^2 V^{-1} s^{-1}). These results remain insufficient for such an inversion to be observed in the range of the experimentally explored concentration.

Table 5 shows the apparent complexation constant calculated for the various studied binaphthol/CD couples by the four plotting methods (*x*-reciprocal, *y*-reciprocal, double reciprocal and non-linear).

A good linearization with all of the three plotting methods was observed with an average correlation coefficient of 0.997. Strong correlation coefficients were also observed for the non-linear method (average of 0.9991). As a result, a satisfactory agreement between the four methods was observed for all considered BN/CD couples.

In comparing the affinity between the different cyclodextrins and the binaphthols, with regard to the studied CDs, HP-β-CD has the highest affinity for the binaphthols, while α-CD has the lowest one. Moreover, we found that the derivative CDs have relatively stronger affinities for the binaphthols than the corresponding native ones, except for γ-CD, which globally developed stronger interactions than its homologue HP-γ-CD derivative.

Finally, in the case of the Binol with the γ-CD, the effect of increasing the electrolyte pH by 0.7 units led to a decrease in the affinity of the two enantiomers for the hydrophobic cavity of the cyclodextrin, as we could expect, since the ionization degree is reinforced for the two partners.

3.3. Determination of ($\Delta\mu_{S,R\,max}$) and the optimal concentration of cyclodextrin

The mobility difference between a pair of S and R enantiomers ($\Delta\mu_{S,R}$) can be described from Eq. (6) in its most general form as

$$\Delta\mu_{S,R} = \frac{\mu_f + \mu_{RC}K'_R[C]}{1 + K'_R[C]} - \frac{\mu_f + \mu_{SC}K'_S[C]}{1 + K'_S[C]} \quad (10)$$

so ($\Delta\mu_{S,R}$) depends not only on the apparent complexation constants of both of the S and R enantiomers with the chiral selector, but also on the electrophoretic mobility of each complex formed between the S and R enantiomers with the chiral selector and on the concentration of the chiral selector. Therefore, the optimal chiral selector concentration, which leads to a maximum electrophoretic

Table 6
Comparison between the theoretical optimal concentration of the cyclodextrin $[C_{opt}]_{cal}$ calculated from (Eq. (12)) and the experimental optimal concentration of the cyclodextrin determined experimentally $[C_{opt}]_{exp}$ at pH = 11.5 for various binaphthols and cyclodextrins (concentration in mM).

	Binol	BN2	BN3	BN4	BN5
α-CD					
$[C_{opt}]_{cal}$	56.2	–	–	25	26.3
$[C_{opt}]_{exp}$	20	–	–	20	20
β-CD					
$[C_{opt}]_{cal}$	3.9	1.4	6.7	5.2	12.6
$[C_{opt}]_{exp}$	10	1.2	8	4	10
γ-CD					
$[C_{opt}]_{cal}$	5	1.5	4.4	4.5	7.3
$[C_{opt}]_{exp}$	6	2	7.5	4	7
HP-α-CD					
$[C_{opt}]_{cal}$	14.8	–	9.3	14.6	17.4
$[C_{opt}]_{exp}$	15	–	10	10	15
HP-β-CD					
$[C_{opt}]_{cal}$	4.2	–	5.2	2.8	5.2
$[C_{opt}]_{exp}$	4.4	–	10	3.3	6
HP-γ-CD					
$[C_{opt}]_{cal}$	9.8	1.6	7.5	7.3	10.8
$[C_{opt}]_{exp}$	8	2	7.5	7.5	8
TM-β-CD					
$[C_{opt}]_{cal}$	9.9	30	13.6	8.3	9.7
$[C_{opt}]_{exp}$	10	25	15	7.5	10

mobility ($\Delta\mu_{S,R\,max}$) difference, can be achieved when

$$\frac{\partial \Delta\mu_{S,R}}{\partial C} = 0 \quad (11)$$

Wren and Rowe [3,4] simplified the determination of $[C_{opt}]$ by assuming that $\mu_{SC} = \mu_{RC} = \mu_C$, and then $[C^{opt}] = 1/\sqrt{K_S K_R}$. In the case of the Binols and as it was illustrated above, $\mu_{SC} \neq \mu_{RC}$, so Wren–Rowe's model is not valid; therefore, a direct resolution of Eq. (11) must be used, which leads to a classic polynomial equation in the form of

$$a[C]^2 + b[C] + c \quad \text{with } [C^{opt}] = \frac{-b + \sqrt{\Delta}}{2a} \quad (12)$$

where $\Delta = b^2 - 4ac$, $a = K'_S K'_R[K'_S(\mu_{RC} - \mu_f) - K'_R(\mu_{SC} - \mu_f)$, $b = 2K'_S K'_R(\mu_{RC} - \mu_{SC})$ and $c = K'_R(\mu_{RC} - \mu_f) - K'_S(\mu_{SC} - \mu_f)$.

Table 6 shows the comparison between the optimal concentration of the cyclodextrin $[C_{opt}]_{cal}$ calculated with Eq. (12) and the optimal concentration of the cyclodextrin $[C_{opt}]_{exp}$ determined experimentally by plotting ($\Delta\mu_{S,R\,exp}$) as a function of the cyclodextrin concentration (0–20 or 25 mM).

We found a good agreement between the two concentrations. Moreover, by eliminating the $[C_{opt}]_{cal}$ value corresponding to Bin1 and α-CD outside of the studied concentration range, we obtained a 94% correlation between $[C_{opt}]_{cal}$ and $[C_{opt}]_{exp}$.

As a result, the proposed model is perfectly adapted to describe the electrophoretic behavior of the enantioseparation of binaphthols by CD-CZE and to support the formed hypothesis that the weak electrophoretic mobilities of the various studied cyclodextrins could be negligible.

4. Conclusion

With regard to these results, we conclude that the proposed theoretical model is well-suited to describe the electrophoretic behavior of the binaphthols in CD-CZE under the studied conditions (pH range from 10.8 to 11.5) and, consequently, to model the enantioseparation of binaphthols by means of CD-CZE.

The optimal cyclodextrin concentrations for the enantioseparation of binaphthols determined by this model are in strong agreement with the values deduced from experimental data. Moreover, the electrophoretic mobilities of the complexes formed between the binaphthols and the various studied cyclodextrins played a significant role in the enantioseparation. This model can be applied to

- determine the optimal concentration of the chiral selector for the weak monoacid and diacid analytes, and
- the reversal of the enantiomer migration order as a function of the chiral selector concentration, using the apparent complexation constants and the complex mobilities.

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