Enantioseparation of binaphthol and its mono derivatives by cyclodextrin-modified capillary zone electrophoresis¹

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Abstract

Chiral binaphthols belong to the group of most effective ligands for asymmetrical catalysis. In this context, various binaphthols presenting original substituents have been synthesized. Their study through capillary electrophoresis is the object of this work. The literature dedicated to the separation of atropisomers by capillary electrophoresis, corresponding only to binaphthol, reveals that its enantioseparation is always delicate because of the influence of many factors and the resolutions obtained are weak. Therefore, for a structured optimization, we first successfully evaluated the acidity constants of different binaphthols by means of capillary electrophoresis. With these known physicochemical characteristics, we could successfully carry out enantiomeric separations of the different binaphthols at pH=11.5, practically in completely ionized form, in phosphate medium, and in the presence of cyclodextrin (CD), with analysis times lower than 8 minutes. The nature of CDs (α -CD, β -CD, γ -CD, hydroxypropyl- α -cyclodextrin (HP- α -CD), HP- β -CD, HP- γ -CD and trimethyl- β -CD (TM- β -CD)) and other factors in relation to enantiomeric resolution (applied voltage, nature and concentration of the electrolyte, and concentration of cyclodextrin) were optimized. These studies allowed us to determine the optimal conditions of separation (concentration and nature of CD) for each of the studied binaphthols. It is necessary to mention that, for the 1,1'-binaphthyl-2,2'-diol (Binol) at pH=11.5, the S atropisomer always migrated first, regardless of the nature and concentration of the cyclodextrin used. Moreover, an inversion in elution order of the two atropisomers as a function of pH was observed with γ -CD (pH range: 10-11.5). The R atropisomer migrated first at pH=10. At pH=10.8 the migration order of the two

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atropisomers of Binol was reversed as a function of γ -CD concentration. Finally, the addition of chiral ionic liquids (R(–)-1-hydroxy-N,N,N-trimethylbutan-2-aminium bis(trifluoromethylsulfonyl)imide and S(+)-tetrabutylammonium camphorsulfonate) was conducted. In the case of S(+)-tetrabutylammonium camphor-sulfonate, a weak antagonistic effect was observed with modeling the evolution of enantiomeric resolution by means of the experimental design, while in the case of R(–)-1-hydroxy-N,N,N-trimethylbutan-2-aminium bis(trifluoromethylsulfonyl)imide the effect was neutral.

Key words: *binaphthol derivatives, capillary electrophoresis, enantioseparation, atropisomers, cyclodextrins, chiral ionic liquid*

1. Introduction

Over the last two decades, research in the area of asymmetric synthesis has expanded. The final aim of enantioselective synthesis or catalysis is to obtain enantiomeric pure chiral compounds from achiral substrates in the presence of chiral reactants. The role of the latter is to induce the selective formation of the desired enantiomer. In reality, the situation is more complex. Indeed, both the substrate and the reactant can exist in the form of a mixture of conformational isomers, in non-negligible proportions and in different states of solvation, imposing distinct reactivities for each species present. The result is a weighted average, which generally leads to weak stereoselectivity according to the distribution and the reactivity of the implied species. A reasoned approach of stereoselectivity is based on the use of molecules that only have pure rotation elements like, for example, chiral atropisomers and, particularly, binaphthol systems [1,2]. Structurally, the binaphthols or Binols, according to the authors, are composed of two naphthyl groups carrying a hydroxyl function in position 2 and linked by a simple C-C liaison in positions 1 and 1'. As a result of the restricted gene at the level of the rotation around this C-C liaison, two conformations (S- and R-Binol) exist, hence the appellation atropisomer. Used since 1990, the atropisomers of the 1,1'-binaphthyl-2,2'-diol (or Binol) are among the most widely used ligands for asymmetric catalytic reactions. They can also be used as chiral hosts in the enantiomeric separation of racemic mixtures of “guest” compounds or even as chiral reagents during the optical purity study of enantiomers by ^1H NMR [3]. A recent paper [4] identifies all the preparation methods for Binol synthesis, as well as its uses as a reactant or ligand in various reactions.

Since 1993, the enantiomeric separation of Binol has been the subject of several studies in capillary electrophoresis (CE), the method of choice for enantioseparation. Indeed, in addition to the advantages of high efficiency and short analysis time, only very small quantities of samples and separation electrolytes are required. Under these conditions, the study of chiral compounds, which are generally extremely expensive in their pure enantiomeric form, and their use as electrolytes becomes less problematic. Finally, the potential presence of impurities within the chiral compound does not handicap their separation since they have different electrophoretic mobilities and therefore do not interfere upon separation. The majority of the possible modes in CE are applicable to the separation of enantiomers, but micellar electrokinetic chromatography (MEKC) is by far the most used. More particularly, concerning the enantioseparation of Binols and their derivatives in CE, many studies have been undertaken in capillary electrokinetic chromatography (EKC), generally by using native or derivative cyclodextrins (CD-CZE) [5-11] or even with surfactants such as sodium N-(4-n-dodecyloxybenzoyl)-L-Valinate (MEKC) [12]. These studies showed the complexity of Binol enantioseparation. Indeed these separations require the control of many parameters, on the one hand the background electrolyte (electrolyte nature, pH, ionic strength, presence or not of cosolvent) and on the other hand the chiral selector (cyclodextrin (CD) type, substitution, concentration, degree of substitution and use of charged CD or dual CD system). All studies and optimization of various factors were not always carried out systematically. The aim of this work is first to determine the acidity constants of Binol and its mono derivatives. We will also study the effect of pH on the enantioseparation of Binol in order to determine the most favorable pH of this enantioseparation. To our knowledge, such a study has never been reported. Finally, after the determination of this principal parameter, we seek to establish optimal conditions for the enantioseparation of each of the binaphthol derivatives studied using CD.

2. Experimental

2.1. Instrumentation

Capillary electrophoretic experiments were performed using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) fitted with a diode array detector from 190-600 nm. An uncoated fused silica capillary (Thermo Electron SA, Courtaboeuf, France), 31.2 cm long (21 cm to the detector), 50 μm i.d. (internal diameter),

was used for the pK_a determinations and one 40.2 cm long (30 cm to the detector), 50 μm i.d., was used for the enantioseparations. The capillaries were thermostated at $20.0 \pm 0.1^\circ\text{C}$. The samples were pressure-injected by 0.5 psi (34 mbar) for 3 s at the inlet side of the capillary (anode). Electrophoretic runs were performed with a 10 kV potential. The UV detection was cathodic ($\lambda=214$ nm). The System 32 Karat software (Version 4.0, Beckman Coulter) piloted the electrophoretic system and was used for controlling the data. The pH of the running solutions was measured before each experiment by using a Model IQ240 pH meter (IQ Scientific Instruments Inc., San Diego, CA, USA). A Branson 2510 sonication apparatus (Branson, Danbury, USA) was used for degassing all solutions. The data examination of the experimental design was performed with JMP software, version 5.1 (SAS institute, Cary, NC, USA).

2.2. Chemicals and reagents

The racemate and R enantiomer of Binol (1,1'-binaphthalen-2,2'-diol or 1,1'-bi-2-naphthol) were provided by Sigma–Aldrich (Sigma–Aldrich France, Saint Quentin Fallavier, France). Native α -CD was purchased from Sigma–Aldrich, β -CD (98%) from Interchim (Interchim, Montluçon, France) and γ -CD (98%) from Acros Organics (Acros Organics France, Noisy-le-Grand, France). The compounds 2-hydroxypropyl- α , β and γ -CDs (HP-x-CDs) (d.s. $\approx$ 0.6) were obtained from Fluka Chemie (Sigma–Aldrich–Fluka France, L’Isle d’Abeau, Chesne, France). Heptakis-2,3,6-tri-*O*-methyl- β -cyclodextrin (TM- β -CD) was purchased from Sigma–Aldrich. Sodium dihydrogenphosphate (NaH_2PO_4), disodium hydrogenphosphate (Na_2HPO_4), trisodium phosphate (Na_3PO_4), sodium hydroxide (+98% purity), and absolute ethanol (98.8%) were obtained from Sigma–Aldrich.

The Binol derivatives studied in this work were synthesized in house; the syntheses of 6-bromo-1-(2-hydroxynaphthalen-1-yl)naphthalen-2-ol (BN2) [13], 1-(2-(allyloxy)naphthalen-1-yl)naphthalen-2-ol (BN3) [14], 1-(2-(benzyloxy)naphthalen-1-yl)naphthalen-2-ol (BN4) [14] and 1-(2-metoxynaphthalen-1-yl)naphthalen-2-ol (BN5) [14] have already been reported in the literature. Their structures are shown in Table 1.

Table 1

For chiral ionic liquids, the syntheses of (R)(–)-2-hydroxy-N,N,N-trimethyl-1-phenylethanaminium bis(trifluoromethylsulfonyl)imide (R-Phenylcholine NTf₂) and (R)(–)-1-

hydroxy-N,N,N-trimethylbutan-2-ammonium bis(trifluoromethylsulfonyl)imide (R-Etcholine NTf₂) were reported previously [32]. (S)(+)-tetrabutylammonium camphorsulfonate (S-tb-camphor) was prepared by neutralization of (S)(+)-camphorsulfonic acid with tetrabutylammonium hydroxide.

2.3. Running electrolyte and sample preparation

All running electrolytes were prepared, without any prerequisite purification, using ultrapure water produced by means of a Milli-Q-System water purification apparatus (Millipore France, Montigny-le-Brotonneux, France). The solutions were sonicated just before use for 10 min. For the pK_a determinations, the running electrolytes contained phosphate salts (according to the target pH value (6<pH<12)) with an ionic strength equal to 40 mM. For the enantioseparations, a phosphate electrolyte (Na₂HPO₄-Na₃PO₄) with an ionic strength equal to 80 mM, and cyclodextrin concentrations ranging from 0 mM to 20 or 25 mM were used. All samples were prepared in ethanol at a concentration equal to 10⁻⁴ M. New uncoated capillaries were activated by performing the following washing process: water for 2 min, NaOH (1 M) 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. Determination of the acidity constant

Although Binol is a well-known compound and has been used for many years, it is necessary to mention that, to date, the determination of its acidity constants has never, to our knowledge, been reported in the literature. Nevertheless, the knowledge of these dissociation constants is a key parameter, especially in transport phenomena and more particularly for electrophoretic mobility. For these reasons, it is essential to first determine these constants for Binol and its derivatives before undertaking the enantioseparation.

During the past several years, capillary electrophoresis has been demonstrated to be a convenient and precise method for the determination of acidity constants (K_a) in aqueous [15,16] and non-aqueous mediums [17,18]. Several experimental approaches, linear or non-linear, are applicable. In order to determine the acidity constants we only need to follow the evolution of the effective electrophoretic mobility (μ_{eff}) of the target sample as a function of the pH of the running electrolyte, the ionic strength of which is kept constant. The

experimental K_a value is then determined from the slope of the graph obtained; the inflection point of the graph $\mu_{\text{eff}} = f(\text{pH})$ is equal to the $\text{p}K_a$. The $\text{p}K_a$ is then deduced thermodynamically by using the equation (1):

$$\text{p}K_a^{\text{thermo}} = \text{p}K_a^{\text{exp}} - \frac{0.5085\sqrt{I}}{1 + 0.328 \times r_i \times \sqrt{I}} \quad (1)$$

where I is the ionic strength of the running electrolyte and r_i is the hydrated radius of the species studied. A value of 0.5 nm is generally recommended [19].

Despite the fact that Binol and its derivatives have high $\text{p}K_a$ values because they are very weak acids, the use of CE allowed us to determine their $\text{p}K_a$ values without difficulties, as the pH value of the running electrolytes can reach about 12. The results obtained for the different Binols studied are grouped in Table 2.

Table 2

As was expected, Binol and its derivatives have high acidity constants: Binols with two hydroxyl functions have values of values 9/9.4 for the first acidity and 10.9 for the second, and Binols with only one hydroxyl function have a value of approximately 10.2, Table 2. Before starting the optimization of the enantioseparation of binaphthol and its four derivatives in the presence of cyclodextrins as chiral selectors, in the case of Binol, we studied the evaluation of the enantioseparation as a function of the pH by using a native cyclodextrin as the chiral selector.

3.2. Study of the enantioseparation of Binol

The enantioseparation depends directly on the affinity of the two enantiomers to the cyclodextrin (CD) used. Therefore, the use of different cyclodextrins (native or derivative, neutral or charged) strongly affects the results. For the enantioseparation of Binol racemate and its derivatives, generally denoted as BN in the remainder of this text, the study was limited to the case of neutral cyclodextrin. To our knowledge, the enantioseparation of Binol and its derivatives has never been carried out in a genuinely satisfactory way (electrophoretic enantioselectivity $\alpha_{\text{exp}} > 1$), with the exception of the use of trimethyl- β -CD (TM- β -CD, $\alpha_{\text{exp}} = 1.22$ [5]), hydroxypropyl- α -cyclodextrin (HP- α -CD) and TM- β -CD, (HP- α -CD, $R_s = 1.2$ and TM- β -CD, $R_s = 1.2$ [6]). With other neutral CDs, either no enantioseparation was

systematically observed (in the case of α -CD and HP- γ -CD) or, according to the authors, no enantioseparation was obtained at the pH used [5,6]. On the contrary, specific enantioselectivity was noted (in the case of β -CD, γ -CD and HP- β -CD at pH 10.5) [10]. Although enantioselectivities are weaker than those reported above, K. Kano et al. [5] and H. Nishi [6] did not observe a chiral resolution of the Binol racemate at pH 10.5, while at this same pH, O. Zerbinati et al. obtained certain enantioselectivity [10]. All of the CDs studied are naturally exempt from all charge, so it proved imperative to charge the analytes in order to confer a charge to the BN/CD complexes, and thus ensure different velocities of the experimental electrophoretic migration for the S and R enantiomers. Taking into account the obtained pK_a values of Binol and its derivatives (the range of the pK_a corresponding to the first acidity is between 9 and 10.2, see Table 2), a significant negative charge for the Binol racemate can only be obtained at $pH \geq 10.5$. A weak acid presents a charge equal to -1 when the pH of the electrolyte used is higher or equal to $pK_a + 2$. Consequently, we decided to examine a range of pH between 9.5 and 12. However, it should be noted that the experiments conducted at $pH=12$ showed to be delicate due to problems encountered concerning the repeatability as well as the stability of the electrophoretic current. Therefore, we were forced to limit the range of the studied pH between 9.5 and 11.5. Using the Binol racemate as a sample test, we studied the influence of the ionic strength and the pH of the electrolyte on the enantioseparation.

3.2.1. Ionic strength optimization

For the pH range studied, the charge of the two enantiomers of Binol was maximal at pH 11.5, with a charge slightly higher than -1.5. The influence of ionic strength on the enantioseparation was therefore studied at this pH. Indeed, the electroosmotic flow is cathodic and the greatest resolution with neutral CDs should be achieved under these conditions. In fact, at weak ionic strength, the electroosmotic flow is very significant and consequently the electrophoretic peaks corresponding to the two enantiomers of Binol were hardly differentiated from that of the electroosmotic flow due to their electrophoretic migration direction, which is diametrically opposed to that of the electroosmotic flow. Despite the possibility of operating under very intense electric fields, consequently obtaining very high efficiencies, i.e. the joule effect was perfectly dissipated, we did not obtain a satisfactory resolution regardless of the size of the cyclodextrin used (α -CD, β -CD or γ -CD). Progressively reducing the electroosmotic flow by increasing the ionic strength of the

electrolyte, at a constant pH equal to 11.5, we noticed a simultaneous increase in the enantioresolution. Thus, we obtained a resolution superior to 1.5 for an ionic strength equal to 80 mM, while, in such conditions, the applied voltage could not exceed 10 kV. Consequently, for the remainder of the study, the ionic strength was maintained at 80 mM and the applied voltage was fixed at 10 kV.

3.2.2. Choice of the pH of the medium

The electrophoretic enantioselectivity (α_{exp} : the ratio of the electrophoretic mobilities of the two enantiomers) proved to be slightly greater with γ -CD ($\alpha_{\text{exp}}=1.05$) than with the other two native CDs ($\alpha_{\text{exp}}=1.04$ with α -CD as well as with β -CD). Therefore, we studied the influence of the pH on the enantioseparation of the Binol racemate with γ -CD. The ionic strength and applied voltage were fixed at 80 mM and 10 kV, respectively. In fact, the study of the evolution of the resolution was simultaneously conducted as a function of the pH and γ -CD concentration, in the pH range between 9.5 and 11.5 and γ -CD concentrations between 0 and 20 mM, in order to determine the best experimental conditions.

Figure 1 reports the electropherograms obtained at the end of the analysis of Binol racemate spiked with pure R enantiomer at pH 10 (the upper line) and pH 11.5.

A comparison of the two electropherograms showed that the migration order of the two enantiomers of Binol was inversed at pH 11.5 compared with that obtained at pH 10. The R enantiomer of Binol migrated initially at pH 10 and eluted a second time at pH 11.5.

Figure 1

Such an observation could originate in the progressive ionization of γ -CD as the pH is increased. In fact, in the pH range between 10.2 and 10.5, we observed the coelution of the two enantiomers. If we refer to the literature, we find that the pK_{a} of γ -CD is equal to 12.08 [20].

At pH 10, γ -CD is neutral and therefore is carried by the electroosmotic flow, which is cathodic. Consequently, the enantiomer with more affinity towards the hydrophobic torus of CD migrates first, since the two non-complexed enantiomers migrate towards the anode at this pH (pK_{a1} Binol=9.04 and pK_{a2} Binol=10.90, see Table 2). Thus, in light of the electropherograms reported in Figure 1, we conclude that the R enantiomer of Binol has the

greatest affinity for γ -CD. On other hand, at pH 11.5, γ -CD is partially ionized (charge $\ll -1/2$) and thus, under such conditions, it presents an electrophoretic mobility that is diametrically opposed to that of the electroosmotic flow, and, consequently, it slows down the migration of the enantiomer that has the greatest affinity with it, that is to say the R enantiomer.

Figure 2 summarizes the evolution of the resolution of the Binol racemate as a function of the pH in the range explored. As this graph highlights, the resolution value at pH 11.5 ($R_s=2.4$) is greater than at pH 10 ($R_s=0.9$), that is to say, before the inversion of the enantiomer migration order. Such an observation was made not only in the case of γ -CD as the chiral selector, but was systematically noticed in this study regardless of the cyclodextrin used.

Figure 2

Furthermore, it should be noted that when the pH of the electrolyte is lower than 10, the resolution drops rapidly (R_s is about 0.6 when the pH of the electrolyte reaches 9.5). Indeed, the two enantiomers of Binol present small charges, while γ -CD is neutral. Thus, their migration becomes differentiated from that of the electroosmotic flow and, consequently, the difference in their migration velocity is enough only to be displayed as the beginning of the separation. Secondly, it appears that the optimal concentration of γ -CD is approximately the same at the two pH values considered. In fact, at pH 10, the optimal concentration of γ -CD is equal to 10 mM, where the enantiomeric resolution reaches its maximum value, while, at pH 11.5, this concentration is equal to 6 mM.

We tried to find if such an inversion could be observed as a function of pH with the other two native cyclodextrins (α -CD and β -CD), as well as with different neutral cyclodextrins, either hydroxypropylated (HP- α -CD, HP- β -CD and HP- γ -CD) or trimethylated (TM- β -CD). In fact, such an inversion in the migration order of the Binol enantiomers was observed with β -CD as well as with two out of three hydroxypropylated cyclodextrins (HP- β -CD and HP- γ -CD). On the other hand, no inversion was observed in the case of α -CD, HP- α -CD or TM- β -CD. However, we noticed that in the case of α -CD, a coelution could be observed as a function of the pH, unlike in the case of HP- α -CD and TM- β -CD.

The migration order observed at a pH greater or equal to 10.6 corresponds to the migration order observed by O. Zerbinati and F. Trotta [10] at pH 10.5, during a partial resolution of the

Binol racemate in the presence of γ -CD. In that study, the authors determined the association constants of the two Binol enantiomers with γ -CD and acceded in that way to the thermodynamic enantioselectivity for the Binol/ γ -CD couple. The treatment used is based on non-charged-CDs. In fact, such a hypothesis seems reasonable, as, at this pH, the charge of γ -CD is very weak when taking into account its pK_a (12.08). Such an observation is also applied in the present study, and so the origin of the migration order reversal of the Binol enantiomers as a function of pH must found elsewhere, approximating the charge of γ -CD to zero. In reality, in a very interesting study, G. Vigh et al. [21-23] modeled the enantiomeric separation of chiral weak acids and bases in CE. They showed that according to the magnitude orders of (1) the electrophoretic mobilities of (dissociated analytes / CD) complexes (μ_{RCD^-} and μ_{SCD^-}), and (2) the complex formation constants of the analytes in their dissociated and non-dissociated forms with CD (on the one hand K_{RCD^-} , K_{SCD^-} and on the other hand K_{HRCD} , K_{HSCD}), three kinds of separation could be envisaged as a function of the pH and CD concentration, while the latter is systematically neutral. The three kinds of separation 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 $K_{HRCD} = K_{HSCD}$), and duoselective separation or Type III ($\mu_{RCD^-} \neq \mu_{SCD^-}$, $K_{RCD^-} \neq K_{SCD^-}$ and $K_{HRCD} \neq K_{HSCD}$).

The two latter types allow for, if so desired, the reversal of the migration order of the enantiomers as a function of the electrolyte composition (pH, CD concentration). Thereby the origin of the reversal migration order of Binol enantiomers as a function of pH in the presence of γ -CD is more likely be found in the behavior of Type II or III, concerning the enantioseparation, than in the progressive ionization of γ -CD. In fact, the latter method remains modest even at pH 11.5.

A fortiori in the present case, the reversal of the migration order of the enantiomers as a function of the pH probably results from the behavior reflected either in an ionoselective separation (Type II) or a duoselective separation (Type III). In fact, if we observe the pK_a values of the previously studied cyclodextrins, they prove to be even greater than that of γ -CD. They increase from γ -CD ($pK_a=12.08$) to α -CD ($pK_a=12.33$). The pK_a of β -CD is intermediary and is equal to 12.20 [20]. On the other hand, the most acidic sites must be the most reactive and we can therefore suppose that the pK_a values of the derivative cyclodextrins are appreciably higher than the corresponding native CDs. Consequently, there is a likelihood that, in the considered pH range, the charge of the cyclodextrin is very weak, even null concerning HP- β -CD and HP- γ -CD, and that the second hypothesis for the explication of the

reversal of the migration order of the enantiomers as a function of pH is more plausible than the first one. Therefore, we consider that the possible charge of CD is negligible in the pH range considered.

Finally it should be noted that when no inversion of the migration order was observed as a function of the pH (HP- α -CD and TM- β -CD), we systematically remarked that the enantioselective resolution of Binol racemate increased regularly with the pH of the electrolyte and that it was much higher at pH 11.5 than at other any pH. Consequently, this pH value (11.5) was used for the remainder of the study.

3.2.3. Optimization of cyclodextrin concentration as a function of its nature

The optimal conditions were thus defined in the case of the (R,S Binol/ γ -CD) couple. We then determined the optimal concentrations of the other six neutral CDs studied, namely the two natives (α -CD and β -CD), the hydroxypropylated (HP- α -CD, HP- β -CD and HP- γ -CD) and the trimethylated (TM- β -CD). The pH and the ionic strength were fixed at 11.5 and 80 mM, respectively. Table 3 reports (1) the optimal concentration of cyclodextrin (where the electrophoretic enantioselectivity, also consigned in this table, is maximal), (2) the literature data concerning the enantioseparation of the Binol racemate when the same CD is used as the chiral selector, and (3) the pH for each of the CD used in this study.

Table 3

In fact the optimal concentrations of CDs were experimentally deduced from $\Delta\mu^{\text{eff}} = f([\text{CD}])$ curves ($\Delta\mu^{\text{eff}} = \mu_2 - \mu_1$ the difference in electrophoretic mobilities of the two atropisomers, μ_1 and μ_2).

It appears that, at pH 11.5 and contrary to what has been observed in the literature at pH 9 [24], 10.5 [5,6] and 11 [25], we can obtain a resolution of the Binol racemate with all of the cyclodextrins studied. In fact, the enantioseparation of the Binol racemate has only been reported with TM- β -CD, either at pH 10.5 [5,6] or at pH 11 [5,25], with HP- α -CD at pH 10.5 [6], with HP- β -CD at pH 10.5 [10], with γ -CD either at pH 9 [24] or at pH 10.5 [10], and with β -CD at pH 9 [24] and 10.5 [10].

Such an observation clearly demonstrates the definite advantages of working in a more basic medium and thus at pH 11.5.

Secondly, it appears that, with TM- β -CD, the enantioselectivity is maximal ($\alpha_{\text{exp}}=1.27$ at optimal concentration of TM- β -CD equal to 10^{-2} M). Otherwise, the three hydroxypropylated cyclodextrins led approximately to the same enantioselectivity (α_{exp} about 1.13) with a slight advantage for HP- α -CD ($\alpha_{\text{exp}}=1.14$). However, the optimal concentrations of the three hydroxypropylated cyclodextrins are not identical, and the highest concentration is for HP- α -CD ($C_{\text{opt}}=1.5 \cdot 10^{-2}$ M) against only $8 \cdot 10^{-3}$ M for HP- γ -CD and only $4.4 \cdot 10^{-3}$ M for HP- β -CD, which proved to be the most interesting among the three hydroxypropylated CDs when taking into account the concept of consummated quantity.

Finally, the three native cyclodextrins led to a weaker enantioselectivity than the other CDs studied. Once again, with a first approximation, we can say that they led more or less to the same enantioselectivity, but for concentrations of CD varying from single to triple. For γ -CD, which presents the lowest optimal concentration of CD among the three native CDs ($C_{\text{opt}}=6 \cdot 10^{-3}$ M), a slightly greater enantioselectivity is obtained ($\alpha_{\text{exp}}=1.05$ against $\alpha_{\text{exp}}=1.04$ with α -CD as well as with β -CD).

In the case of γ -CD, an inversion in the migration order of the two enantiomers of Binol was observed at pH 10.8 as a function of cyclodextrin concentration; this case is reported below. However, before discussing this important case and taking into account all of these positive results, we evaluated the capacity of the seven CDs, implemented previously, towards the resolution of the racemic mixtures of various Binol derivatives (structures given in Table 1) at pH 11.5 with an ionic strength of 80 mM.

3.3. Enantioseparation of Binol derivatives

For the four Binol derivatives, we alternatively used each of the seven CDs studied as chiral selectors. For each (racemate of Binol derivative/CD) couple we studied the evolution of the electrophoretic enantioselectivity (α_{exp}) as a function of the CD concentration. The range of the explored concentration was between 0 and $2.5 \cdot 10^{-2}$ M taking into account the previously obtained results for the Binol racemate. Such a study allowed us to determine the various optimal concentrations of cyclodextrins for each of twenty-eight (Binol derivative/CD) couples. C_{opt} depended on the CD structures, where the enantioselectivity was maximal and thus the resolution was maximal. The results obtained are given in Table 4.

Table 4

Examination of Table 4 confirms the first conclusions of the experiments conducted with Binol. α -CD proved to be the least favorable CD among the seven cyclodextrins studied. In fact, it did not allow the enantioseparation of two out of four Binol derivative racemates, that is to say BN2 and BN3.

On the other hand, the cyclodextrins that are constituted by seven or eight glucose units (β -CD and γ -CD natives or derivatives) provided better results than their α homologue, which is constituted by six glucose units. In fact, taking into consideration all the CDs studied with seven glucose units (native β -CD and its derivatives HP- β -CD and TM- β -CD), only one out of four Binol derivative racemates, namely BN2, could not be separated with this CD family. On the contrary, taking into consideration the studied cyclodextrins with eight glucose units (native γ -CD and its derivative HP- γ -CD), all of the Binol derivative racemates could be separated using this CD family as the chiral selector.

Finally, in general the cyclodextrin derivatives led to higher enantioseparations than that observed with their homologous native CD. However, we note one exception to this rule: HP- β -CD proved to be less satisfactory than β -CD towards the resolution of the BN2 racemate, since it did not lead to any enantioseparation, while we observed an enantioselectivity ($\alpha_{\text{exp}}=1.05$) with β -CD. Moreover, except for the case of BN5, in general TM- β -CD led to the highest enantioselectivity observed during this study.

3.4. Reversal of enantiomer migration order as a function of γ -CD concentration

At pH 10.8, when the γ -CD concentration progressively increased from 2.10^{-3} M to 6.10^{-3} M, the electrophoretic peak corresponding to the S enantiomer of Binol (enantiomer migrates firstly) gradually approached the peak of the R enantiomer. We then observed the coelution of the two enantiomers at γ -CD concentrations ranging from 7.10^{-3} M to 15.10^{-3} M. The concentration range of the observed coelution appears to be relatively important. Finally when the γ -CD concentration became higher than $1.5.10^{-2}$ M, we once again observed a resolution of the Binol racemate, but in this case the R enantiomer migrated first. Figure 3 shows three electropherograms concerning the analysis of the Binol racemate effectuated at pH 10.8. In the presence of a variable concentration of γ -CD, these three electropherograms show enantioseparations in each of the three above-mentioned zones of γ -CD concentration.

Figure 3

This phenomenon of inversion in enantiomer migration order was widely interpreted by B. Chankvetadze [26]. The author presented and discussed the different parameters responsible for the origin of such an inversion, such as the pH, the inversion of the direction of electroosmotic flow or even the change of the chiral selector nature. However, there are very few examples of inversion reported in the literature where the experimental parameter of the inversion origin is the cyclodextrin concentration directly [27-29]. For example, T. Schmitt and H. Engelhardt [27] reported the case of dansylated phenylalanine racemate with HP- β -CD, where the migration order of the two enantiomers is reversed at low and high concentrations of HP- β -CD. The authors explain this reversal migration order by the modifications of the electrophoretic mobilities as a function of CD concentration: at low concentrations, the electrophoretic mobilities reflect the differential interactions with HP- β -CD, while at high concentrations the migration order is directly controlled by the electrophoretic mobility of the diastereomeric formed complexes. The case described in this paper follows principally the same rationalization. Thus, we can postulate that at low γ -CD concentrations, the two Binol enantiomers are separated according to their affinity with γ -CD, while at high γ -CD concentrations the principle of the resolution of Binol enantiomers is due to the electrophoretic mobilities of diastereomeric complexes formed between each of the Binol enantiomers and γ -CD. However, such a hypothesis could be validated by the evaluation of the complexation constants and the complex mobilities according to B. Chankvetadze [30].

Finally in very recent works, the utilization of chiral ionic liquids is reported for the first time either as a single chiral selector [31] or by combination with cyclodextrins [32] for the enantioseparation of alcohols, amine, acid, and amino acids, and 2-arylpropionic acids. We decided to evaluate the effect of adding a chiral ionic liquid in the case of atropisomer enantioseparation, a problem that has not yet been reported in the literature.

3.5 Simultaneous adding of chiral ionic liquids

P. Gareil et al. [32] showed weak synergistic effects concerning the enantioseparation of racemic mixtures of 2-arylpropionic acids by simultaneously using cyclodextrins and (R)(-)-2-hydroxy-N,N,N-trimethyl-1-phenylethanaminium bis(trifluoromethylsulfonyl)imide (R-Phenylcholine NTf₂) or (R)(-)-1-hydroxy-N,N,N-trimethylbutan-2-aminium bis (trifluoro-

methysulfonyl) imide (R-Etcholine NTf₂). Firstly, we envisaged the successive addition of these chiral ionic liquids for the enantioseparation of binaphthol. Unfortunately, R-Phenylcholine NTf₂ has a chromophore, which generates a strong absorbance at the detection wavelength, and consequently it was impossible to carry out such experimentation. On the other hand, R-Etcholine NTf₂ is transparent and well adapted for the test. In order to evaluate the potential of R-Etcholine NTf₂ in combination with γ -CD at pH 11.5 for the enantioseparation of binaphthol, we resorted to the experimental design methodology. The aim of this study was to highlight the potential interactions between the operation parameters, i.e. γ -CD concentration and R-Etcholine NTf₂ concentration. In order to model the response surface (electrophoretic enantioselectivity α_{exp}) in the explored experimental domain (γ -CD concentration between 0 and 3.10⁻² M and R-Etcholine NTf₂ concentration between 0 and 2.10⁻² M) we resorted to a central composite design with uniform precision (CCD-UP). This design consists of 13 runs and 2 factors with 2 levels. The factors and their levels are reported in Table 5, where α is equal to 1.41 in adequacy with the theory ($\alpha = (2^n)^{1/4}$ with n=2) [33].

Table 5

The evolution of the electrophoretic enantioselectivity (α_{exp}) of the two atropisomers of binaphthol as a function of γ -CD and R-Etcholine NTf₂ concentration is presented in Figure 4a.

Figure 4

As has been highlighted previously, if there is an effect during the addition of R-Etcholine NTf₂ in the presence of γ -CD, then it is weak. In order to objectively analyze this potential effect, we substituted lithium NTf₂ in the second series of runs for R-Etcholine NTf₂ and studied the response surface (α_{exp}) in an identical experimental domain using CCD-UP. The response surface (electrophoretic enantioselectivity α_{exp}) values after the end of the new series of 13 runs is shown in Figure 4b. A comparison between Figures 4a and 4b clearly shows that there is neither a synergistic nor an antagonistic effect from adding R-Etcholine NTf₂ to the implemented electrophoretic system during the enantioseparation of atropisomers.

At this stage in the study, we tried to find an ionic liquid without chromophore among the chiral ionic liquids already reported in the literature. If this material exists, its synthesis will

not be based on the choline derivative already employed. With this reasoning, we next evaluated the effect of the simultaneous addition of S(+)-tetrabutylammonium camphorsulfonate (S-tb-camphor) to the experimental mixture. Resorting again to the previously employed experimental design (CCD-UP), we were able to plot the response surface (α_{exp}) after 13 runs, Figure 4c. As Figure 4c clearly demonstrates, the addition of S-tb-camphor is not neutral to the resolution of the Binol racemate under the operation conditions established previously, contrary to what we observed with the addition of R-Etholine NTf₂. In fact, Figure 4c highlights a weak antagonistic effect for S-tb-camphor. Thus the degree of the enantioselectivity, obtained by using γ -CD at pH 11.5, is progressively decreased as the S-tb-camphor concentration is increased, regardless of the γ -CD concentration. Finally, it must be noted that no enantioseparation of the Binol racemate was observed with R-Etholine NTf₂ as the only chiral selector. Unfortunately, a similar situation was also observed with the other chiral ionic liquids tested. In fact, no separation was found when only S-tb-camphor was used as the electrophoretic medium in the range of explored concentrations (0 - 3.10⁻² M).

4. Conclusion

The aim of this work was to study the evolution of the enantioselectivity of various racemates of Binol and four of its mono substituted derivatives in the presence of different cyclodextrins (seven native or derivative cyclodextrins).

The result of this study is that the enantioselectivity of Binol atropisomers and its mono substituted derivatives increases systematically with pH, regardless of the Binol/cyclodextrin couple being considered. Consequently, it was interesting to carry out such separations at pH 11.5, where no difficulty was met in realizing the enantioseparations.

On the other hand, this study highlighted an inversion in the enantiomer migration order as a function of the pH, in the case of the Binol and the γ -cyclodextrin. Thus, in this specific case, there is a pH value where no resolution could be observed. Based on experimental results, this pH value is situated between 10.3 and 10.5.

Having defined the pH, we studied the evolution of the enantioselectivity as a function of the cyclodextrin concentration. Once again, we were able to highlight a particular behavior in the case of the Binol/ γ -cyclodextrin couple by observing an inversion in the enantiomer migration order as a function of the γ -cyclodextrin concentration at pH 10.8. The S enantiomer migrated

before the R enantiomer at low cyclodextrin concentration. Consequently, at concentrations between 7.10^{-3} M and 15.10^{-3} M, no enantioselective separation could be observed in the case of Binol and γ -cyclodextrin. This double inversion in the enantiomer migration order, on the one hand as a function of the pH and on the other hand as a function of the concentration of chiral selector, is remarkable. It is an illustration of the Type II enantioseparation (ionoselective) or the Type III enantioseparation (duoselective), according to the terminology established by G. Vigh following his theoretical treatment of modeling the enantioseparation of weak chiral acids.

Globally, α -CD proved to be the least favorable CD among the seven cyclodextrins studied. On the other hand, the cyclodextrins that are constituted by seven or eight glucose units (β -CD and γ -CD natives or derivatives) provided better results than their α homologue, which is constituted by six glucose units. In fact, taking into consideration all the CDs studied with seven glucose units (native β -CD and its derivatives HP- β -CD and TM- β -CD), only one out of four Binol derivative racemates, namely BN2, could not be separated with this CD family. On the contrary, taking into consideration the cyclodextrins studied with eight glucose units (native γ -CD and its derivative HP- γ -CD), all of the Binol derivative racemates could be separated using this CD family as chiral selector.

Finally, the addition of the chiral ionic liquid (R-Etcholine NTf₂ or S-tb-camphor) did not perceptibly reinforce the enantioselectivity of the system considered.

We envisage, in the near future, studying the enantioseparation of other Binol derivatives in order to discover if the observed inversion of the enantiomer migration order can be observed in these systems as well.

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Figure Captions

Figure 1: Enantioseparation of Binol racemate in presence of γ -CD.

Experimental conditions: uncoated silica capillary (40.2 (30) cm $\times$ 50 μ m ID); hydrodynamic injection: 3s at 0.5 psi; $T=20^{\circ}\text{C}$; $\lambda=214$ nm; voltage: 10kV; electrolyte: $\text{Na}_2\text{HPO}_4/\text{Na}_3\text{PO}_4$, ionic strength: 8.10^{-2} M; $[\gamma\text{-CD}]=4.10^{-3}\text{M}$; sample: Binol racemate spiked with the R enantiomer; (A) at pH=10 and (B) at pH=11.5.

Figure 2: Resolution of Binol racemate as a function of the pH of the running electrolyte.

Experimental conditions: uncoated silica capillary (40.2 (30) cm $\times$ 50 μ m ID); hydrodynamic injection: 3s at 0.5 psi; $T=20^{\circ}\text{C}$; $\lambda=214$ nm; voltage: 10kV; electrolyte: phosphate with ionic strength= 8.10^{-2} M; $[\gamma\text{-CD}]=4.10^{-3}\text{M}$; sample: Binol racemate spiked with the R enantiomer.

Figure 3: Electropherograms recorded during the enantioseparation of Binol with variable concentrations of γ -CD.

Experimental conditions: uncoated silica capillary (40.2 (30) cm $\times$ 50 μ m ID); hydrodynamic injection: 3s at 0.5 psi; $T=20^{\circ}\text{C}$; $\lambda=214$ nm; voltage: 10kV; electrolyte: $\text{Na}_2\text{HPO}_4/\text{Na}_3\text{PO}_4$; pH=10.8; ionic strength: 8.10^{-2} M; $[\gamma\text{-CD}]$: variable; sample: Binol racemate spiked with the R enantiomer.

Figure 4: Response surface plots for the electrophoretic enantioselectivity of Binol racemate (α_{exp}) as a function of γ -CD and ionic liquid concentrations a) R-Etcholine NTf₂, b) LiNTf₂ and c) S-tb-camphor.