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1 **Enantioseparation of binaphthol and its mono derivatives by cyclodextrin-modified**  
2 **capillary zone electrophoresis<sup>1</sup>**

3  
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9  
10 ***Abstract***

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

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


38

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

41

## 42 **1. Introduction**


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

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

88

## 89 **2. Experimental**

### 90 **2.1. Instrumentation**

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

95 was used for the pK<sub>a</sub> determinations and one 40.2 cm long (30 cm to the detector), 50 μm i.d.,  
96 was used for the enantioseparations. The capillaries were thermostated at 20.0 ± 0.1 °C. The  
97 samples were pressure-injected by 0.5 psi (34 mbar) for 3 s at the inlet side of the capillary  
98 (anode). Electrophoretic runs were performed with a 10 kV potential. The UV detection was  
99 cathodic (λ=214 nm). The System 32 Karat software (Version 4.0, Beckman Coulter) piloted  
100 the electrophoretic system and was used for controlling the data. The pH of the running  
101 solutions was measured before each experiment by using a Model IQ240 pH meter (IQ  
102 Scientific Instruments Inc., San Diego, CA, USA). A Branson 2510 sonication apparatus  
103 (Branson, Danbury, USA) was used for degassing all solutions.  
104 The data examination of the experimental design was performed with JMP software, version  
105 5.1 (SAS institute, Cary, NC, USA).

## 106 **2.2. Chemicals and reagents**

107 The racemate and R enantiomer of Binol (1,1'-binaphthalen-2,2'-diol or 1,1'-bi-2-naphthol)  
108 were provided by Sigma–Aldrich (Sigma–Aldrich France, Saint Quentin Fallavier, France).  
109 Native α-CD was purchased from Sigma–Aldrich, β-CD (98%) from Interchim (Interchim,  
110 Montluçon, France) and γ-CD (98%) from Acros Organics (Acros Organics France, Noisy-le-  
111 Grand, France). The compounds 2-hydroxypropyl-α, β and γ-CDs (HP-x-CDs) (d.s.≈0.6)  
112 were obtained from Fluka Chemie (Sigma–Aldrich–Fluka France, L'Isle d'Abeau, Chesne,  
113 France). Heptakis-2,3,6-tri-*O*-methyl-β-cyclodextrin (TM-β-CD) was purchased from Sigma–  
114 Aldrich. Sodium dihydrogenphosphate (NaH<sub>2</sub>PO<sub>4</sub>), disodium hydrogenphosphate (Na<sub>2</sub>HPO<sub>4</sub>),  
115 trisodium phosphate (Na<sub>3</sub>PO<sub>4</sub>), sodium hydroxide (+98% purity), and absolute ethanol  
116 (98.8%) were obtained from Sigma–Aldrich.

117 The Binol derivatives studied in this work were synthesized in house; the syntheses of 6-  
118 bromo-1-(2-hydroxynaphthalen-1-yl)naphthalen-2-ol (BN2) [13], 1-(2-(allyloxy)naphthalen-  
119 1-yl)naphthalen-2-ol (BN3) [14], 1-(2-(benzyloxy)naphthalen-1-yl)naphthalen-2-ol (BN4)  
120 [14] and 1-(2-metoxynaphthalen-1-yl)naphthalen-2-ol (BN5) [14] have already been reported  
121 in the literature. Their structures are shown in Table 1.

122

123 Table 1

124

125 For chiral ionic liquids, the syntheses of (R)(–)-2-hydroxy-N,N,N-trimethyl-1-phenyl-  
126 ethanaminium bis(trifluoromethylsulfonyl)imide (R-Phenylcholine NTf<sub>2</sub>) and (R)(–)-1-

127 hydroxy-N,N,N-trimethylbutan-2-ammonium bis(trifluoromethylsulfonyl)imide (R-Etcholine  
128 NTf<sub>2</sub>) were reported previously [32]. (S)(+)-tetrabutylammonium camphorsulfonate (S-tb-  
129 camphor) was prepared by neutralization of (S)(+)-camphorsulfonic acid with  
130 tetrabutylammonium hydroxide.

### 131 ***2.3. Running electrolyte and sample preparation***

132 All running electrolytes were prepared, without any prerequisite purification, using ultrapure  
133 water produced by means of a Milli-Q-System water purification apparatus (Millipore France,  
134 Montigny-le-Brotonneux, France). The solutions were sonicated just before use for 10 min.  
135 For the pK<sub>a</sub> determinations, the running electrolytes contained phosphate salts (according to  
136 the target pH value (6 < pH < 12)) with an ionic strength equal to 40 mM. For the  
137 enantioseparations, a phosphate electrolyte (Na<sub>2</sub>HPO<sub>4</sub>-Na<sub>3</sub>PO<sub>4</sub>) with an ionic strength equal to  
138 80 mM, and cyclodextrin concentrations ranging from 0 mM to 20 or 25 mM were used. All  
139 samples were prepared in ethanol at a concentration equal to 10<sup>-4</sup> M. New uncoated capillaries  
140 were activated by performing the following washing process: water for 2 min, NaOH (1 M)  
141 for 30 min and water for 10 min. The capillary was conditioned for 15 min with the  
142 electrolyte before running and for 2 min between each run.

## 143 **3. Results and discussion**

### 144 ***3.1. Determination of the acidity constant***

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

151 During the past several years, capillary electrophoresis has been demonstrated to be a  
152 convenient and precise method for the determination of acidity constants (K<sub>a</sub>) in aqueous  
153 [15,16] and non-aqueous mediums [17,18]. Several experimental approaches, linear or non-  
154 linear, are applicable. In order to determine the acidity constants we only need to follow the  
155 evolution of the effective electrophoretic mobility ( $\mu_{\text{eff}}$ ) of the target sample as a function of  
156 the pH of the running electrolyte, the ionic strength of which is kept constant. The

157 experimental  $K_a$  value is then determined from the slope of the graph obtained; the inflection  
158 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  
159 thermodynamically by using the equation (1):

$$160 \quad \text{p}K_a^{\text{thermo}} = \text{p}K_a^{\text{exp}} - \frac{0.5085\sqrt{I}}{1 + 0.328 \times r_i \times \sqrt{I}} \quad (1)$$

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

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

167

168 Table 2

169 As was expected, Binol and its derivatives have high acidity constants: Binols with two  
170 hydroxyl functions have values of values 9/9.4 for the first acidity and 10.9 for the second,  
171 and Binols with only one hydroxyl function have a value of approximately 10.2, Table 2.

172 Before starting the optimization of the enantioseparation of binaphthol and its four derivatives  
173 in the presence of cyclodextrins as chiral selectors, in the case of Binol, we studied the  
174 evaluation of the enantioseparation as a function of the pH by using a native cyclodextrin as  
175 the chiral selector.

### 176 ***3.2. Study of the enantioseparation of Binol***

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

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

205

### 206 3.2.1. Ionic strength optimization

207 For the pH range studied, the charge of the two enantiomers of Binol was maximal at pH  
208 11.5, with a charge slightly higher than -1.5. The influence of ionic strength on the  
209 enantioseparation was therefore studied at this pH. Indeed, the electroosmotic flow is cathodic  
210 and the greatest resolution with neutral CDs should be achieved under these conditions. In  
211 fact, at weak ionic strength, the electroosmotic flow is very significant and consequently the  
212 electrophoretic peaks corresponding to the two enantiomers of Binol were hardly  
213 differentiated from that of the electroosmotic flow due to their electrophoretic migration  
214 direction, which is diametrically opposed to that of the electroosmotic flow. Despite the  
215 possibility of operating under very intense electric fields, consequently obtaining very high  
216 efficiencies, i.e. the joule effect was perfectly dissipated, we did not obtain a satisfactory  
217 resolution regardless of the size of the cyclodextrin used ( $\alpha$ -CD,  $\beta$ -CD or  $\gamma$ -CD).  
218 Progressively reducing the electroosmotic flow by increasing the ionic strength of the



219 electrolyte, at a constant pH equal to 11.5, we noticed a simultaneous increase in the  
220 enantioresolution. Thus, we obtained a resolution superior to 1.5 for an ionic strength equal to  
221 80 mM, while, in such conditions, the applied voltage could not exceed  
222 10 kV. Consequently, for the remainder of the study, the ionic strength was maintained at  
223 80 mM and the applied voltage was fixed at 10 kV.

224

### 225 3.2.2. Choice of the pH of the medium

226 The electrophoretic enantioselectivity ( $\alpha_{\text{exp}}$ : the ratio of the electrophoretic mobilities of the  
227 two enantiomers) proved to be slightly greater with  $\gamma$ -CD ( $\alpha_{\text{exp}}=1.05$ ) than with the other two  
228 native CDs ( $\alpha_{\text{exp}}=1.04$  with  $\alpha$ -CD as well as with  $\beta$ -CD). Therefore, we studied the influence  
229 of the pH on the enantioseparation of the Binol racemate with  $\gamma$ -CD. The ionic strength and  
230 applied voltage were fixed at 80 mM and 10 kV, respectively. In fact, the study of the  
231 evolution of the resolution was simultaneously conducted as a function of the pH and  $\gamma$ -CD  
232 concentration, in the pH range between 9.5 and 11.5 and  $\gamma$ -CD concentrations between 0 and  
233 20 mM, in order to determine the best experimental conditions.

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

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

239

240 Figure 1

241

242 Such an observation could originate in the progressive ionization of  $\gamma$ -CD as the pH is  
243 increased. In fact, in the pH range between 10.2 and 10.5, we observed the coelution of the  
244 two enantiomers. If we refer to the literature, we find that the  $\text{pK}_{\text{a}}$  of  $\gamma$ -CD is equal to 12.08  
245 [20].

246 At pH 10,  $\gamma$ -CD is neutral and therefore is carried by the electroosmotic flow, which is  
247 cathodic. Consequently, the enantiomer with more affinity towards the hydrophobic torus of  
248 CD migrates first, since the two non-complexed enantiomers migrate towards the anode at  
249 this pH ( $\text{pK}_{\text{a}1}$  Binol=9.04 and  $\text{pK}_{\text{a}2}$  Binol=10.90, see Table 2). Thus, in light of the  
250 electropherograms reported in Figure 1, we conclude that the R enantiomer of Binol has the

251 greatest affinity for  $\gamma$ -CD. On other hand, at pH 11.5,  $\gamma$ -CD is partially ionized (charge  $\ll -$   
252  $1/2$ ) and thus, under such conditions, it presents an electrophoretic mobility that is  
253 diametrically opposed to that of the electroosmotic flow, and, consequently, it slows down the  
254 migration of the enantiomer that has the greatest affinity with it, that is to say the R  
255 enantiomer.

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

261

262 Figure 2

263

264 Furthermore, it should be noted that when the pH of the electrolyte is lower than 10, the  
265 resolution drops rapidly ( $R_s$  is about 0.6 when the pH of the electrolyte reaches 9.5). Indeed,  
266 the two enantiomers of Binol present small charges, while  $\gamma$ -CD is neutral. Thus, their  
267 migration becomes differentiated from that of the electroosmotic flow and, consequently, the  
268 difference in their migration velocity is enough only to be displayed as the beginning of the  
269 separation. Secondly, it appears that the optimal concentration of  $\gamma$ -CD is approximately the  
270 same at the two pH values considered. In fact, at pH 10, the optimal concentration of  $\gamma$ -CD is  
271 equal to 10 mM, where the enantiomeric resolution reaches its maximum value, while, at pH  
272 11.5, this concentration is equal to 6 mM.

273 We tried to find if such an inversion could be observed as a function of pH with the other two  
274 native cyclodextrins ( $\alpha$ -CD and  $\beta$ -CD), as well as with different neutral cyclodextrins, either  
275 hydroxypropylated (HP- $\alpha$ -CD, HP- $\beta$ -CD and HP- $\gamma$ -CD) or trimethylated (TM- $\beta$ -CD). In fact,  
276 such an inversion in the migration order of the Binol enantiomers was observed with  
277  $\beta$ -CD as well as with two out of three hydroxypropylated cyclodextrins (HP- $\beta$ -CD and HP- $\gamma$ -  
278 CD). On the other hand, no inversion was observed in the case of  $\alpha$ -CD, HP- $\alpha$ -CD or TM- $\beta$ -  
279 CD. However, we noticed that in the case of  $\alpha$ -CD, a coelution could be observed as a  
280 function of the pH, unlike in the case of HP- $\alpha$ -CD and TM- $\beta$ -CD.

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

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

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

302 Thereby the origin of the reversal migration order of Binol enantiomers as a function of pH in  
303 the presence of  $\gamma$ -CD is more likely be found in the behavior of Type II or III, concerning the  
304 enantioseparation, than in the progressive ionization of  $\gamma$ -CD. In fact, the latter method  
305 remains modest even at pH 11.5.

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

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

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

324

### 325 *3.2.3. Optimization of cyclodextrin concentration as a function of its nature*

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

334

335 Table 3


336

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

340 It appears that, at pH 11.5 and contrary to what has been observed in the literature at pH 9  
341 [24], 10.5 [5,6] and 11 [25], we can obtain a resolution of the Binol racemate with all of the  
342 cyclodextrins studied. In fact, the enantioseparation of the Binol racemate has only been  
343 reported with TM- $\beta$ -CD, either at pH 10.5 [5,6] or at pH 11 [5,25], with HP- $\alpha$ -CD at pH 10.5  
344 [6], with HP- $\beta$ -CD at pH 10.5 [10], with  $\gamma$ -CD either at pH 9 [24] or at pH 10.5 [10], and with  
345  $\beta$ -CD at pH 9 [24] and 10.5 [10].

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

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

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

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

### 368 **3.3. Enantioseparation of Binol derivatives**

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

377

378 Table 4

379

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

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

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

### 398 ***3.4. Reversal of enantiomer migration order as a function of $\gamma$ -CD concentration***

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

409

410 Figure 3

411

412 This phenomenon of inversion in enantiomer migration order was widely interpreted by B.  
413 Chankvetadze [26]. The author presented and discussed the different parameters responsible  
414 for the origin of such an inversion, such as the pH, the inversion of the direction of  
415 electroosmotic flow or even the change of the chiral selector nature. However, there are very  
416 few examples of inversion reported in the literature where the experimental parameter of the  
417 inversion origin is the cyclodextrin concentration directly [27-29]. For example, T. Schmitt  
418 and H. Engelhardt [27] reported the case of dansylated phenylalanine racemate with HP- $\beta$ -  
419 CD, where the migration order of the two enantiomers is reversed at low and high  
420 concentrations of HP- $\beta$ -CD. The authors explain this reversal migration order by the  
421 modifications of the electrophoretic mobilities as a function of CD concentration: at low  
422 concentrations, the electrophoretic mobilities reflect the differential interactions with HP- $\beta$ -  
423 CD, while at high concentrations the migration order is directly controlled by the  
424 electrophoretic mobility of the diastereomeric formed complexes.

425 The case described in this paper follows principally the same rationalization. Thus, we can  
426 postulate that at low  $\gamma$ -CD concentrations, the two Binol enantiomers are separated according  
427 to their affinity with  $\gamma$ -CD, while at high  $\gamma$ -CD concentrations the principle of the resolution  
428 of Binol enantiomers is due to the electrophoretic mobilities of diastereomeric complexes  
429 formed between each of the Binol enantiomers and  $\gamma$ -CD. However, such a hypothesis could  
430 be validated by the evaluation of the complexation constants and the complex mobilities  
431 according to B. Chankvetadze [30].

432

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

### 438 ***3.5 Simultaneous adding of chiral ionic liquids***

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

443 methylsulfonyl) imide (R-Etcholine NTf<sub>2</sub>). Firstly, we envisaged the successive addition of  
444 these chiral ionic liquids for the enantioseparation of binaphthol. Unfortunately,  
445 R-Phenylcholine NTf<sub>2</sub> has a chromophore, which generates a strong absorbance at the  
446 detection wavelength, and consequently it was impossible to carry out such experimentation.  
447 On the other hand, R-Etcholine NTf<sub>2</sub> is transparent and well adapted for the test. In order to  
448 evaluate the potential of R-Etcholine NTf<sub>2</sub> in combination with  $\gamma$ -CD at pH 11.5 for the  
449 enantioseparation of binaphthol, we resorted to the experimental design methodology. The  
450 aim of this study was to highlight the potential interactions between the operation parameters,  
451 i.e.  $\gamma$ -CD concentration and R-Etcholine NTf<sub>2</sub> concentration. In order to model the response  
452 surface (electrophoretic enantioselectivity  $\alpha_{\text{exp}}$ ) in the explored experimental domain ( $\gamma$ -CD  
453 concentration between 0 and  $3 \cdot 10^{-2}$  M and R-Etcholine NTf<sub>2</sub> concentration between 0 and  
454  $2 \cdot 10^{-2}$  M) we resorted to a central composite design with uniform precision (CCD-UP). This  
455 design consists of 13 runs and 2 factors with 2 levels. The factors and their levels are reported  
456 in Table 5, where  $\alpha$  is equal to 1.41 in adequacy with the theory ( $\alpha = (2^n)^{1/4}$  with n=2) [33].

457

458 Table 5

459

460 The evolution of the electrophoretic enantioselectivity ( $\alpha_{\text{exp}}$ ) of the two atropisomers of  
461 binaphthol as a function of  $\gamma$ -CD and R-Etcholine NTf<sub>2</sub> concentration is presented in Figure  
462 4a.

463

464 Figure 4

465

466 As has been highlighted previously, if there is an effect during the addition of R-Etcholine  
467 NTf<sub>2</sub> in the presence of  $\gamma$ -CD, then it is weak. In order to objectively analyze this potential  
468 effect, we substituted lithium NTf<sub>2</sub> in the second series of runs for R-Etcholine NTf<sub>2</sub> and  
469 studied the response surface ( $\alpha_{\text{exp}}$ ) in an identical experimental domain using CCD-UP. The  
470 response surface (electrophoretic enantioselectivity  $\alpha_{\text{exp}}$ ) values after the end of the new series  
471 of 13 runs is shown in Figure 4b. A comparison between Figures 4a and 4b clearly shows that  
472 there is neither a synergistic nor an antagonistic effect from adding R-Etcholine NTf<sub>2</sub> to the  
473 implemented electrophoretic system during the enantioseparation of atropisomers.

474 At this stage in the study, we tried to find an ionic liquid without chromophore among the

475 chiral ionic liquids already reported in the literature. If this material exists, its synthesis will



476 not be based on the choline derivative already employed. With this reasoning, we next  
477 evaluated the effect of the simultaneous addition of S(+)-tetrabutylammonium  
478 camphorsulfonate (S-tb-camphor) to the experimental mixture.  
479 Resorting again to the previously employed experimental design (CCD-UP), we were able to  
480 plot the response surface ( $\alpha_{\text{exp}}$ ) after 13 runs, Figure 4c.  
481 As Figure 4c clearly demonstrates, the addition of S-tb-camphor is not neutral to the  
482 resolution of the Binol racemate under the operation conditions established previously,  
483 contrary to what we observed with the addition of R-Etcholine NTf<sub>2</sub>. In fact, Figure 4c  
484 highlights a weak antagonistic effect for S-tb-camphor. Thus the degree of the  
485 enantioselectivity, obtained by using  $\gamma$ -CD at pH 11.5, is progressively decreased as the S-tb-  
486 camphor concentration is increased, regardless of the  $\gamma$ -CD concentration.  
487 Finally, it must be noted that no enantioseparation of the Binol racemate was observed with  
488 R-Etcholine NTf<sub>2</sub> as the only chiral selector. Unfortunately, a similar situation was also  
489 observed with the other chiral ionic liquids tested. In fact, no separation was found when only  
490 S-tb-camphor was used as the electrophoretic medium in the range of explored concentrations  
491 (0 - 3.10<sup>-2</sup> M).

#### 492 **4. Conclusion**

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

496 The result of this study is that the enantioselectivity of Binol atropisomers and its mono  
497 substituted derivatives increases systematically with pH, regardless of the Binol/cyclodextrin  
498 couple being considered. Consequently, it was interesting to carry out such separations at pH  
499 11.5, where no difficulty was met in realizing the enantioseparations.

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

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

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

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

525 Finally, the addition of the chiral ionic liquid (R-Etcholine NTf<sub>2</sub> or S-tb-camphor) did not  
526 perceptibly reinforce the enantioselectivity of the system considered.

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

530

531

## 532 5. References

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576 Figure Captions

577

578 Figure 1: Enantioseparation of Binol racemate in presence of  $\gamma$ -CD.

579 *Experimental conditions: uncoated silica capillary (40.2 (30) cm  $\times$  50  $\mu$ m ID); hydrodynamic*  
580 *injection: 3s at 0.5 psi; T=20°C;  $\lambda$ =214 nm; voltage: 10kV; electrolyte: Na<sub>2</sub>HPO<sub>4</sub>/ Na<sub>3</sub>PO<sub>4</sub>,*  
581 *ionic strength: 8.10<sup>-2</sup> M; [ $\gamma$ -CD]=4.10<sup>-3</sup>M; sample: Binol racemate spiked with the R*  
582 *enantiomer; (A) at pH=10 and (B) at pH=11.5.*

583

584

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

586 *Experimental conditions: uncoated silica capillary (40.2 (30) cm  $\times$  50  $\mu$ m ID); hydrodynamic*  
587 *injection: 3s at 0.5 psi; T=20°C;  $\lambda$ =214 nm; voltage: 10kV; electrolyte: phosphate with ionic*  
588 *strength=8.10<sup>-2</sup> M; [ $\gamma$ -CD]=4.10<sup>-3</sup>M; sample: Binol racemate spiked with the R enantiomer.*

589

590

591 Figure 3: Electropherograms recorded during the enantioseparation of Binol with variable  
592 concentrations of  $\gamma$ -CD.

593 *Experimental conditions: uncoated silica capillary (40.2 (30) cm  $\times$  50  $\mu$ m ID); hydrodynamic*  
594 *injection: 3s at 0.5 psi; T=20°C;  $\lambda$ =214 nm; voltage: 10kV; electrolyte: Na<sub>2</sub>HPO<sub>4</sub>/ Na<sub>3</sub>PO<sub>4</sub>;*  
595 *pH=10.8; ionic strength: 8.10<sup>-2</sup> M; [ $\gamma$ -CD]: variable; sample: Binol racemate spiked with the*  
596 *R enantiomer.*

597

598

599 Figure 4: Response surface plots for the electrophoretic enantioselectivity of Binol racemate  
600 ( $\alpha_{\text{exp}}$ ) as a function of  $\gamma$ -CD and ionic liquid concentrations a) R-Etcholine NTf<sub>2</sub>, b) LiNTf<sub>2</sub>  
601 and c) S-tb-camphor.

602

603