



HAL
open science

Evaluation of chiral ionic liquids as additives to cyclodextrins for enantiomeric separations by capillary electrophoresis

Yannis François, Anne Varenne, Emilie Juillerat, Didier Villemin, Pierre Gareil

► **To cite this version:**

Yannis François, Anne Varenne, Emilie Juillerat, Didier Villemin, Pierre Gareil. Evaluation of chiral ionic liquids as additives to cyclodextrins for enantiomeric separations by capillary electrophoresis. *Journal of Chromatography A*, 2007, 1155 (2), pp.134-141. 10.1016/j.chroma.2006.12.076 . hal-02988932

HAL Id: hal-02988932

<https://hal.science/hal-02988932>

Submitted on 4 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 EVALUATION OF CHIRAL IONIC LIQUIDS AS ADDITIVES TO CYCLODEXTRINS FOR
2 ENANTIOMERIC SEPARATIONS BY CAPILLARY ELECTROPHORESIS

3
4 Yannis François¹, Anne Varenne¹, Emilie Juillerat¹, Didier Villemin², Pierre Gareil^{1*}

5
6
7 ¹Laboratory of Electrochemistry and Analytical Chemistry, UMR CNRS 7575, ENSCP, 11 rue
8 Pierre et Marie Curie, 75231 Paris cedex 05, France

9 ²Laboratory of Molecular and Thio-organic Chemistry, UMR CNRS 6507, ENSI Caen, 6,
10 Boulevard du Maréchal Juin, 14050 Caen cedex, France

11
12
13 Running title: Chiral ionic liquids as chiral selectors for CE

14
15 Keywords: ionic liquids, capillary electrophoresis, chiral separations, choline-based ionic liquids,
16 neutral cyclodextrins, arylpropionic acids.

17
18
19 * To whom correspondence should be addressed.

20 E-mail: pierre-gareil@enscp.fr. Tel : 33 1 55 42 63 71. Fax: 33 1 44 27 67 50

21
22

23 **Abstract**

24
25

26 A great interest has been drawn these last years towards ionic liquids in analytical chemistry,
27 especially for separation methods. Recent synthesis of chiral ILs opened the way of the
28 evaluation of new potential selectors for enantiomeric separations. This work focused on the
29 evaluation of two chiral ILs (ethyl- and phenylcholine of bis(trifluoromethylsulfonyl)imide) by
30 CE. Particular selectivities are awaited by exploiting unique ion-ion or ion-dipole interactions
31 and by tailoring the nature of the cation and the anion. To evaluate such phenomena, a study was
32 carried out with anti-inflammatory drugs 2-arylpropionic acids as model compounds. The results
33 show that these chiral ILs did not present direct enantioselectivity with regard to these model
34 analytes. The influence of chiral ILs in the electrolytes was then studied in the presence of
35 classical chiral selectors (di- or trimethyl- β -cyclodextrin). Although no general trend could be
36 established, an increase in separation selectivity and resolution was observed in some cases,
37 suggesting synergistic effects. The complementary determination of apparent inclusion constant
38 values of these IL cations in the used cyclodextrins by affinity CE provided support to the
39 understanding of the phenomena involved.

40

41 **1. Introduction**

42
43 The high proportion of chiral compounds of biological or pharmacological interest has aroused a
44 considerable need for the determination of the enantiomeric purities in the last twenty years.
45 Since the pioneering works by Zare et al. [1] and Fanali et al. [2] and as testified by the very
46 important amount of literature and a number of comprehensive reviews [3-11], capillary
47 electrophoresis (CE) has proven to be an excellent alternative to classical chromatographic
48 techniques in this field. The use in very small quantity and in free form of the chiral selector
49 makes it possible to compare the effects of various selectors and afterwards perform routine
50 analyses at lower cost.

51 A great interest is being triggered by ionic liquids (IL) as alternatives for conventional molecular
52 solvents used in organic synthesis and catalytic reactions [12]. They supplement the family of
53 “green solvents” including water and supercritical fluids. Among these, room temperature ionic
54 liquids are defined as materials containing only ionic species and having a melting point lower
55 than 298 K. They exhibit many interesting properties such as negligible vapor pressure, low
56 melting point, large liquid range, unique solvation ability and overall, the versatility of their
57 physico-chemical properties makes them really attractive. They have been proposed as solvents
58 for chemical reactions [13-15], multiphase bioprocess operations [16] and liquid-liquid
59 separations [17,18], as electrolytes for batteries and fuel cells [19], stationary phases in gas
60 chromatography [20-23] and mobile phase additives in liquid chromatography [24-26].

61 During these last years, a great attention has been paid to the relevance of these new media for
62 capillary electrophoresis (CE) [27-37] and many efforts have been directed toward the
63 understanding of the separation mechanisms involved in IL-containing background electrolytes
64 (BGE). Concerning chiral separations, two applications only have been reported so far. The first
65 one was with achiral ILs [38], 1-ethyl- and 1-butyl-3-methylimidazolium cations, associated with
66 BF_4^- or PF_6^- anions. The enantioselectivity for binaphthyl derivatives was produced by a polymeric
67 surfactant, whereas the presence of the ILs only modified the retention times and peak efficiency.
68 Nevertheless, little was elucidated about the separation mechanism. Recent synthesis of chiral ILs
69 [39,40] opened the way of the evaluation of new potential selectors for enantiomeric separations.
70 Rizvi et al. [41] realized the first chiral separation of several anionic compounds by micellar

71 electrokinetic chromatography using two new synthetic chiral ionic liquids, undecenoxycarbonyl-
72 L-pyrrolidinol bromide and undecenoxycarbonyl-L-leucinol bromide.

73 This work was focused on the separation performances of two chiral ILs (ethyl- and
74 phenylcholine of bis(trifluoromethylsulfonyl)imide) by CE. In a previous work, a nonaqueous
75 capillary electrophoresis (NACE) study on the electrophoretic behavior of 2-arylpropionic acids
76 (profens), which were often selected as model chiral anionic compounds [42] in the presence of
77 an achiral imidazolium-based IL evidenced peculiar ion-pairing interactions between these
78 analytes and the achiral IL [43]. In the present work, the electrophoretic behavior of the same
79 model analytes was first studied in the presence of one of both chiral choline-based ILs in
80 nonaqueous media. As these chiral ILs alone did not present any enantioselectivity with regard to
81 these model analytes under the conditions tested, the influence of the chiral ILs was then studied
82 in aqueous and hydro-organic electrolytes containing classical chiral cyclodextrin selectors (di- or
83 trimethyl- β -cyclodextrin). The figures of merit (effective enantioselectivity and resolution) of the
84 chiral separations of the six arylpropionic acids were systematically determined, depending on
85 the nature and the concentration of the chiral IL and cyclodextrin, ionic strength and hydro-
86 organic composition of the electrolyte, to investigate for possible synergistic effects between the
87 two chiral selectors. In addition to this study, apparent inclusion constant values for the used
88 chiral ILs cations and neutral cyclodextrin derivatives were determined by affinity CE to provide
89 support to the understanding of phenomena involved.

90

91 **2. Experimental**

92

93 *2.1. Chemicals and reagents*

94 Lithium bis(trifluoromethylsulfonyl)imide (LiNTf₂) (≥ 99%) was a gift from Institut Français du
95 Pétrole (Solaize, France). (R)(-) 2-hydroxy-N,N,N-trimethyl-1-phenylethanaminium (PhChol
96 NTf₂) and (R)(-) 1-hydroxy-N,N,N-trimethylbutan-2-aminium bis(trifluoromethylsulfonyl)imide
97 (EtChol NTf₂) were synthesized (see section 2.2.) in Villemin's group (Caen, France). Methanol
98 (GC grade, 99.9% purity) and sodium acetate were purchased from Prolabo (Fontenay-sous-Bois,
99 France). Formamide (> 99%) and hexadimethrin bromide (Polybrene) were supplied by Aldrich
100 (St. Louis, MO, USA). Glacial acetic acid (> 99%), Heptakis-(2,6-di-O-methyl)-β-cyclodextrin
101 (DM-β-CD) (> 90%) and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) (> 90%) were
102 obtained from Sigma (St. Louis, MO, USA). 2-arylpropionic acids (carprofen, suprofen,
103 naproxen, ketoprofen, indoprofen and ibuprofen) were donated by Rhone-Poulenc-Rorer (Vitry-
104 sur-Seine, France).

105 *2.2. Synthesis of chiral ionic liquids*

106

107 Wasserscheid *et al* have been the first to propose the use of choline derivatives as chiral ionic
108 liquid [44]. These chiral ammonium ions can be easily obtained from pure enantiomeric
109 aminoalcohol coming from the “chiral pool” as starting product.

110 The synthesis of the chiral ionic liquids were achieved in two steps: (i) permethylation of amine
111 group into ammonium group and (ii) the metathesis exchange of anion.

112 In a typical procedure of permethylation, the R (-) 2-aminobutan-1-ol (0.44 g, 5 mmol)
113 [respectively, R(-) or S(+) phenylglycin-1-ol (0.5g, 3.6 mmol)] and the iodomethane (2.13 g, 15
114 mmol) were refluxed in diethyl ether (30 ml) under argon atmosphere and was protected from the
115 light. After six days'reflux, the solvent was removed by distillation under reduced pressure. The
116 reactional mixture was solubilized in water (6 mL) and extracted three times (3 x 5 mL) with
117 CH₂Cl₂. The aqueous phase was evaporated under vacuum .

118 For the anion exchange step, the ammonium iodide (25 mmol) was dissolved in water (35 mL)
119 and an aqueous saturated solution of lithium bis(trifluoromethanesulfonyl)imide (7.2 g, 25 mmol)

120 was added. The liquid obtained was centrifuged and the ionic liquid and water were separated.
121 The ionic liquid was washed with water (3 x 10 ml) and finally vacuum-dried.

122

123 2.3. Characterization of chiral ionic liquids

124

125 The structures of the chiral ionic liquids were characterized by ^1H , ^{13}C and ^{19}F NMR
126 spectroscopy :

127 (*R*)(-) 1-hydroxy-*N,N,N*-trimethylbutan-2-aminium bis(trifluoromethanesulfonyl)imide (EtChol
128 NTf₂).

129 Colorless oil, ^1H NMR (400 MHz, MeOD) CD₃CN/TMS δ (ppm): 0.97 (t, $^3J_{\text{HH}} = 7\text{ Hz}$, 3H,
130 CH₃-CH₂), 1.93 (quint, $^3J_{\text{HH}} = 2\text{ Hz}$, 2H, CH₃-CH₂-CH), 3.24 (s, 10H, CH₃-CH₂-CH-(N-
131 (CH₃)₃)-CH₂-OH), 3.73 (dq, $^3J_{\text{HH}} = 14\text{ Hz}$, $^4J_{\text{HH}} = 4\text{ Hz}$, 1H, CH₃-CH₂-CH-(N-(CH₃)₃)-
132 CH₂-OH), 3.95 (d, $^3J_{\text{HH}} = 14\text{ Hz}$, 1H, CH₃-CH₂-CH-(N-(CH₃)₃)-CH₂-OH), 4.68 (s, 1H, CH₃-
133 CH₂-CH-(N-(CH₃)₃)-CH₂-OH); ^{13}C NMR (62.9 MHz, MeOD) CD₃CN/TMS δ (ppm): 11.93
134 (s, 1C, CH₃), 19.38 (s, 1C, CH₃-CH₂-CH), 53.55 (s, 3C, N-(CH₃)₃), 58.36 (s, 1C, CH-CH₂-
135 OH), 78.77 (s, 1C, CH₃-CH₂-CH-(N-(CH₃)₃)-CH₂-OH), 121.60 (quad, $^1J_{\text{CF}} = 1273\text{ Hz}$, 2C, N-
136 (SO₂-CF₃)₂); ^{19}F NMR (235.3 MHz, MeOD), CD₃CN/CCl₃F δ (ppm): -81.08 (s, 6F, N-(SO₂-
137 CF₃)₂)

138

139 (*R*)(-) 2-hydroxy-*N,N,N*-trimethyl-1-phenylethanaminium bis(trifluoromethanesulfonyl)imide
140 (PhChol NTf₂).

141 Colorless oil, ^1H NMR (400 MHz, MeOD) CD₃CN/TMS δ (ppm): 2.79 (s, 1H, OH), 3.19 (s, 9H,
142 Ph-CH-(N-(CH₃)₃)-CH₂-OH), 4.22 (d, $^3J_{\text{HH}} = 13\text{ Hz}$, 1H, Ph-CH-(N-(CH₃)₃)-CH₂-OH), 4.45
143 (dd, $^3J_{\text{HH}} = 13\text{ Hz}$, $^3J_{\text{HH}} = 7\text{ Hz}$, 1H, Ph-CH-(N-(CH₃)₃)-CH₂-OH), 4.61 (dd, $^3J_{\text{HH}} = 7\text{ Hz}$,
144 $^3J_{\text{HH}} = 4\text{ Hz}$, Ph-CH-(N-(CH₃)₃)-CH₂-OH), 7.49 to 7.56 (m, 3C, 1H para and 2H ortho), 7.62
145 to 7.65 (m, 2H, 2H meta); ^{13}C NMR (62.9 MHz, MeOD) CD₃CN/TMS δ (ppm): 53.90 (s, 3C,
146 Ph-CH-(N-(CH₃)₃)-CH₂-OH). 62.04 (s, 1C, Ph-CH-(N-(CH₃)₃)-CH₂-OH), 80.35 (s, 1C, Ph-
147 CH-(N-(CH₃)₃)-CH₂-OH), 121.64 (q, $^1J_{\text{CF}} = 1273\text{ Hz}$, 2C, N-(SO₂-CF₃)₂), 130.88 (s, 3C, 1C
148 para , 2C meta), 132.35 (s, 2C, 2C ortho), 132.91 (s, 1C, C); ^{19}F NMR (235.3 MHz, MeOD),
149 CD₃CN/CCl₃F δ (ppm): -81.06 (s, 6F, N-(SO₂-CF₃)₂).

150

151 2.4. Capillary electrophoresis and procedures

152 All experiments were performed with a HP^{3D}CE (Agilent Technologies, Waldbronn,
153 Germany) capillary electrophoresis system. This apparatus automatically realized all the steps of
154 the measurement protocols, including capillary conditioning, sample introduction, voltage
155 application and diode array detection, and allows to run unattended method sequences. A CE
156 Chemstation (Agilent Technologies, Waldbronn, Germany) was used for instrument control, data
157 acquisition and data handling. Polymicro bare fused-silica capillaries of 50 μm i.d. were obtained
158 from Photonlines (Marly-le-Roi, France). They were used in 35 cm total length (26.5 cm to
159 detection). Background electrolytes (BGE) were made up with acetic acid/sodium acetate at two
160 different concentrations (5 and 60 mM) to a w_{pH} of 5.0. The methanol-water mixtures were
161 prepared by volumic mixing in 0, 10 and 25 % methanol proportions. Analytes were detected by
162 UV absorbance at 200, 230, 240, 254 and 300 nm, according to cases. Formamide (0.001 % (v/v)
163 in the BGE) was used as neutral marker to determine the electroosmotic mobility. The sample
164 solutions were prepared by dissolving each analyte at a concentration of ca 0.5 mM in methanol.
165 Samples were introduced hydrodynamically by successively applying a 30 mbar pressure for 3 s
166 (approximately, 4 nL) to the neutral marker, BGE and sample vials. New capillaries were
167 conditioned by successive flushes with 1 M and 0.1 M NaOH and then with water under a
168 pressure of 935 mbar for 10 min each. The temperature in the capillary cartridge was set at 25 °C.
169 The acquisition rate was 10 points / s. Capillaries were rinsed with water and dried by air when
170 not in use.

171
172 *2.5. Capillary coating*
173 Capillaries were dynamically coated with polybrene as described in the literature [45-47].
174 Briefly, a new fused-silica capillary was first flushed with 1 M NaOH for 20 min and rinsed with
175 water. Next, the capillary was flushed with a polybrene solution at 3 g/100 mL in water for 15
176 min. Finally, the capillary was rinsed with water for 5 min and conditioned with BGE for 5 min,
177 all these steps being performed under a pressure of 935 mbar. Recoating of the capillary with the
178 cationic polymer was accomplished by using a similar method.

179
180
181 *2.6. Complexation constant determination*

182 The apparent formation constant K for the inclusion complexes between chiral PhChol cations
183 and neutral CDs of interest, was determined by mobility shift affinity capillary electrophoresis
184 (ACE) according to a method similar to that developed for a series of imidazolium based ILs
185 cations [48].

186
187 Briefly, PhChol NTf₂ was dissolved at a concentration of 2 mM and electrophoresed in BGEs
188 (ionic strength: 5 mM) containing increasing concentrations of DM-β-CD or TM-β-CD (0 to 100
189 mM). Each injection with a given electrolyte was repeated twice. Effective mobilities (μ_{ep}) of
190 PhChol cation were calculated from migration time measurement at peak apex. The obtained
191 values were corrected to compensate for change in electrolyte viscosity due to increasing CD
192 concentrations. The corrected values μ_{ep,cor} were fitted to non-linear and linear forms (linearized
193 isotherm, x-reciprocal, y-reciprocal, double reciprocal) of the 1:1 stoichiometry complexation
194 isotherm [49,50] to determine the K value.

195

196 2.7. Calculation of the performance parameters for the chiral separations

197 The effective electrophoretic selectivity [51], α_{eff}, was calculated according to Eq. 1:

$$198 \alpha_{eff} = \frac{\mu_{ep1}}{\mu_{ep2}} \quad (1)$$

199 where μ_{ep1}, μ_{ep2} are the effective mobilities for enantiomers 1 and 2.

200

201 The chiral resolution, R_s, between two enantiomers, 1 and 2, was calculated according to:

$$202 R_s = 1.177 \frac{t_2 - t_1}{\delta_1 + \delta_2} \quad (3)$$

203 where t₁, t₂ are the migration times and δ₁, δ₂ are the temporal peak widths at half height.

204

205

206 3. Results and discussion

207 In a previous work, interactions between an achiral IL (1-butyl-3-methylimidazolium
208 bis(trifluoromethylsulfonyl)imide) and a series of 2-arylpropionic acids were studied in non
209 aqueous capillary electrophoresis (NACE) [43]. The results indicated a quadratic effect of the

210 concentration of the achiral IL in the BGE on profen electrophoretic mobilities due to
211 antagonistic interactions between anionic analytes and imidazolium cations either adsorbed to the
212 capillary wall or free in the BGE electrolyte. With a view to evaluate a new family of chiral
213 selectors, the same conditions have been investigated with two chiral choline-based ILs (ethyl-
214 and phenylcholine bis(trifluoromethylsulfonyl)imide). No enantioselectivity has been shown in
215 these conditions for this family of compounds. This work was then directed to the study of the
216 association of a chiral IL to the best chiral selectors, reported previously for the
217 enantiorecognition of profens, DM- β -CD and TM- β -CD [52,53], to search for possible
218 synergistic effects. The use of CDs nevertheless is poorly compatible with that of nonaqueous
219 BGEs, to preserve adequate CD solubilization and partial formation of inclusion complexes. This
220 study was therefore realized in water and 90:10 and 75:25 (v/v) water-MeOH mixtures. The
221 choice of MeOH as molecular solvent in hydro-organic mixtures was based on its favorable
222 anion-solvating properties and ion-pairing and its ability to dissolve the tested CD.

223 The aim of this work was then to determine if a synergistic effect may exist between the chiral IL
224 cation and the CD, and possibly to elucidate the interaction system bringing into play the three
225 different entities: analyte, chiral IL and β -CD derivative (Figure 2). The main parameters
226 expected to impact this complex system were the nature and concentration of the IL, the nature
227 and concentration of the CD, the concentration of the buffer and the hydro-organic composition
228 of the BGE. The influence of adding LiNTf₂ to the separation electrolyte in place of the chiral ILs
229 was tested under the same conditions to discriminate specific chiral cation effect from a mere salt
230 effect. Also, the study was conducted either with bare fused silica capillaries or polybrene-coated
231 capillaries, to assess the influence of IL cation adsorbed to the capillary wall.

232 Owing to the number of parameters to be studied, only three model profens (naproxen, carprofen
233 and suprofen (Figure 1)) were investigated for the part of the experiments realized with bare
234 silica capillaries. For the experiments performed with polybrene-coated capillaries, which were
235 only realized in aqueous media, the following six profens were selected: naproxen, carprofen,
236 suprofen, ketoprofen, indoprofen and ibuprofen (Figure 1). The retained parameters for
237 discussion were effective electrophoretic chiral selectivity, α_{eff} , (thermodynamic parameter,
238 independent of electroosmotic flow variation) and chiral resolution, R_s , (global parameter). It is
239 to note that no enantioselectivity was obtained for naproxen under all conditions tested and for
240 suprofen under all DM- β -CD conditions. The results obtained for carprofen and suprofen with

241 bare silica capillaries are given in Table 1, while those obtained for the five profens showing
242 enantioselectivity with polybrene-coated capillaries are presented in Table 2.

243 In a number of cases, an increase in resolution R_s and a decrease in selectivity α_{eff} were observed
244 for the experiments with chiral ILs, as compared to the experiments without salt, but no general
245 trend on the evolution of R_s and α_{eff} can be traced.

246

247 *Influence of electroosmotic flow and total salt concentration on R_s*

248

249 The two chiral choline IL derivatives, EtChol and PhChol, were used in this work at a
250 concentration of 10 mM and at two buffer salt concentrations (5 mM and 60 mM), in keeping
251 with the preliminary study realized with achiral imidazolium-based IL cation by NACE [38].
252 Indeed, the chiral IL addition in solution caused a change of system properties such as a possible
253 variation of the electrolyte viscosity, a marked increase in the total salt concentration, especially
254 when the buffer salt concentration is 5 mM, and a modification of the capillary wall. These three
255 parameters could mask a specific effect of the chiral IL on the enantiomeric separation.

256 The viscosity of each solution was measured using CE instrumentation by the method described
257 in the literature [54]. The results showed no difference upon adding an IL or LiNTf₂ salt to a
258 solution already containing a CD. So, there was no viscosity effect due to the IL addition on
259 enantiomeric separation.

260 As the addition of the chiral IL was changing the total salt concentration of the solution, the same
261 experiments were realized with LiNTf₂ salt in place of chiral IL to discriminate between a mere
262 salt effect and a specific effect due to the chiral nature of IL cations.

263 In effect, in a lot of cases, Table 1 shows an increase in R_s upon chiral IL addition, but also upon
264 LiNTf₂ addition. Salt addition caused a decrease in electroosmotic mobility (μ_{eo}) and under these
265 counter-electroosmotic flow condition an increase in R_s values [55]. As expected, a more
266 important μ_{eo} variation and hence R_s increase was observed at the lower starting level of buffer
267 salt concentration (5 mM), for which the relative variation in concentration was higher (Figure 3).

268 It was also noted that, with bare silica capillaries, in the majority of cases the addition of a chiral
269 IL caused a more important decrease in μ_{eo} than LiNTf₂ did. This decrease was likely due to the
270 adsorption of the IL cation to the capillary wall, as already mentioned by Stalcup [27,28]. To
271 further discriminate between IL cation wall adsorption and salt effect, the same experiments were

272 resumed with polybrene-coated capillaries which are anticipated to eliminate the IL cation
273 interaction with capillary wall. Table 2 shows that in a majority of cases, an increase in R_s for the
274 experiments with chiral IL and LiNTf₂ was still observed as compared to CD-alone experiments.
275 In all these cases, a decrease in μ_{eo} was also observed, due to the increase in salt concentration.
276 These experiments with positively charged capillaries highlighted the significance of salt effects
277 on the chiral resolution of the five model profens.

278

279 *Influence of chiral IL on α_{eff}*

280

281 Finally, effective electrophoretic selectivity, α_{eff} , designed to be independent of electroosmotic
282 mobility, was the only parameter able to indicate a possible synergistic effect between the two
283 selectors. In some cases, when the initial buffer salt concentration was 5 mM, an increase in α_{eff}
284 was observed upon adding 10 mM LiNTf₂ salt. This behavior can only be understood in
285 considering that the apparent inclusion constants for profens into the CDs, which control α_{eff} , can
286 be depending on electrolyte ionic strength. Apart from this, an increase in α_{eff} , with a difference
287 of more than 3%, in the presence of a chiral IL additive as compared to the experiments with the
288 same concentration of LiNTf₂ was noted in five cases with bare silica capillaries (Table 1) and in
289 four cases with the polybrene-coated capillaries (Table 2). Such a relative difference was
290 considered as the limit of significance based on a mean 3% error for experimental electrophoretic
291 values of chiral compounds (Table 1 and Table 2). Among these nine cases, eight were obtained
292 with 5 mM buffer salt concentration and all five cases identified in the experiments reported in
293 Table 1 were obtained with aqueous and hydroorganic media. It is to note that the experiments
294 with polybrene-coated capillaries were performed with both 5 mM (results shown in Table 2) and
295 60 mM (results not shown) buffer salt concentrations, but no case of synergy was observed at the
296 higher concentration. In spite of the lack of general trend, this behavior suggests that the
297 synergistic effect observed between the two selectors may be due to specific ion-pairing
298 interaction between the analyte and the chiral IL cation.

299 The presence of the phenyl group in the chiral choline cation did not appear to be of importance
300 in the observation of apparent synergistic effects, whereas most cases were observed with TM- β -
301 CD. For a better understanding of the interactions brought into play and to assess a possible
302 competition between the analyte and the IL cation for inclusion complex formation with the CD,

303 a study on possible inclusion complexation between chiral IL cation and β -CD derivatives was
304 undertaken. Concerning EtChol NTf₂, a recent study realized by our group on inclusion constant
305 determination between quite a large number of neutral CDs and alkyl(methyl)methylimidazolium
306 cations [48], revealed that the inclusion of IL cation almost exclusively depends on the alkyl
307 chain length. For 1-ethyl-3-methylimidazolium cation, no inclusion was measured with any
308 tested CD. On analogy, it seems reasonable to conclude that there is no inclusion between EtChol
309 cation and the two β -CD derivatives of the present study. The previously used mobility shift
310 affinity CE method was adapted to determine the apparent inclusion constant for PhChol cation
311 and DM- and TM- β -CD in a acetic acid/sodium acetate buffer at pH 5.0 (ionic strength, 30 mM) .
312 The results obtained in this work showed that there was no inclusion of PhChol cation into TM-
313 β -CD cavity but that this cation formed a complex with DM- β -CD having an apparent constant of
314 144 ± 3 at 25°C. This difference in behavior could be explained by the more important steric
315 hindrance of TM- β -CD as compared to DM- β -CD.

316 Eventually, the study of inclusion phenomena between chiral IL cations and used CDs showed
317 that there was an influence of the CD nature on the competition between the analyte and the IL
318 cation with the CD. Nevertheless, the two thirds of apparent synergistic cases were observed with
319 TM- β -CD with respect to DM- β -CD for EtChol as well as PhChol ILs, which does not allow to
320 further clarify which factor is the most influent.

321

322

323 **4. Conclusion**

324
325 This work focused on the evaluation of two chiral ILs (ethyl- and phenylcholine of
326 bis(trifluoromethylsulfonyl)imide) by CE. No direct enantioselectivity was observed for these
327 two chiral IL cations with respect to a series of arylpropionic acids, selected as model
328 compounds, in various nonaqueous BGE conditions. BGEs containing both a chiral IL cation and
329 a classical chiral selector (di- or trimethyl- β -cyclodextrin) in water and water-MeOH mixtures
330 were subsequently investigated to look for a compromise between the selective formation of
331 inclusion complexes, favored in aqueous electrolyte, and of ion-pairs, favored in nonaqueous
332 media. In most cases, an increase in resolution was observed upon adding one of the chiral IL,
333 but this variation was most often due to a decrease in electroosmotic flow, resulting from the
334 increase in salt concentration and a possible wall adsorption. In nine cases, however,
335 simultaneous increase in α_{eff} and R_s was observed as compared to a simple salt effect, which
336 suggests a synergistic effect of the two selectors. Apparent inclusion constant for EtChol and
337 PhChol cations and the used cyclodextrins were evaluated, demonstrating an influence of the CD
338 nature on the competition between the analyte and the IL cation with respect to CD
339 complexation. Nevertheless, the presence of the phenyl group in the IL cation appeared to be of
340 less importance in promoting these synergistic effects than that of methanol and of a low salt
341 concentration in the BGE, which suggests that specific ion-pairing interactions may be involved.

342

343

344

345 **Acknowledgements**

346

347 The authors thank Julie du Mazaubrun and Estelle Davesne for their collaboration in this work,
348 Jean-Marc Busnel and Thomas Le Saux for very fruitful discussions.

349

350

351 **References**

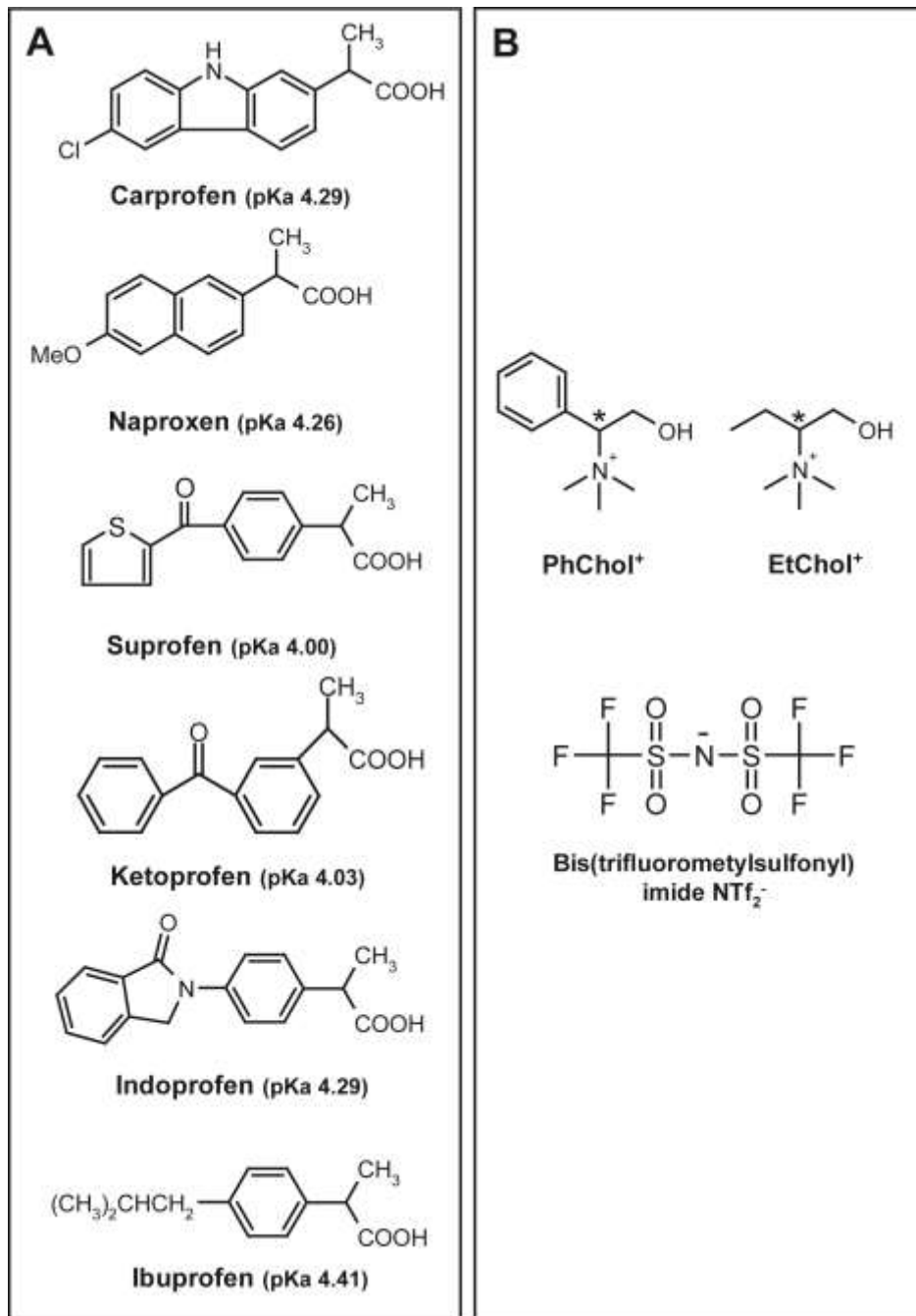
352

- 353 [1] E. Gassmann, J.E. Kuo, R.W. Zare, *Science* 230 (1985) 813
- 354 [2] S. Fanali, *J. Chromatogr.* 494 (1989) 441
- 355 [3] B. Chankvetadze, *Capillary Electrophoresis in Chiral Separation*, Wiley and Sons, Chichester, 1997.
- 356 [4] M.I. Jimidar, W. Van Ael, P. Van Nyen, M. Peeters, D. Redlich, M. De Smet, *Electrophoresis* 25 (2004) 2772.
- 357 [5] B. Chankvetadze, G. Blaschke, *J. Chromatogr. A* 906 (2001) 309.
- 358 [6] H. Nishi, S. Terabe, *J. Chromatogr. A* 875 (2000) 1.
- 359 [7] G. Vigh, A.D. Sokolowski, *Electrophoresis* 18 (1997) 2305.
- 360 [8] G. Gübitz, M.G. Schmid, *Electrophoresis* 25 (2004) 3981.
- 361 [9] M. Lämmerhofer, *J. Chromatogr. A* 1068 (2005) 3
- 362 [10] M. Lämmerhofer, *J. Chromatogr. A* 1068 (2005) 31
- 363 [11] A. Van Eeckhaut, Y. Michotte, *Electrophoresis* 27 (2006), 2880.
- 364 [12] P. Wasserscheidt, T. Weldon, *Ionic Liquids in Synthesis*, Wiley-VCH, New-York, 2003.
- 365 [13] J. Dupont, R.F. de Souza, P.A.Z. Suarez, *Chem. Rev.* 102 (2002) 3667.
- 366 [14] P. Wasserscheidt, W. Keim, *Angewandte Chem. Int. Ed.* 39 (2000) 3772.
- 367 [15] M.J. Earle, K.R. Seddon, *Pure Appl. Chem.* 72 (2000) 1391.
- 368 [16] S.G. Cull, J.D. Holbrey, V. Vargas-Mora, K.R. Seddon, G.J. Lye, *Biotechnol. Bioeng.* 69 (2000) 227.
- 369 [17] J.G. Huddleston, H.D. Willauer, R.P. Swatloski, A.E. Visser, R.D. Rogers, *Chem. Comm.* (1998) 1765.
- 370 [18] A.G. Fadeev, M.M. Meagher, *Chem. Comm.* (2001) 295.
- 371 [19] A.E. Visser, R.P. Swatloski, R.D. Rogers, *Green Chem.* 2 (2000) 1.
- 372 [20] F. Pachole, H.T. Butler, C.F. Poole, *Anal. Chem.* 54 (1982) 1938.
- 373 [21] D.W. Armstrong, J.L. Andersen, J. Ding, T. Welton, *J. Am. Chem. Soc.* 124 (2002) 14247.
- 374 [22] A. Berthod, L. He, D.W. Armstrong, *Chromatographia* 53 (2001) 63.
- 375 [23] A. Heintz, D.W. Kulikov, S.P. Verevkin, *J. Chem. Eng. Data* 47 (2002) 894.
- 376 [24] M.J. Ruiz-Angel, S. Carda-Broch, A. Berthod, *J. Chromatogr. A* 1119 (2006) 202.
- 377 [25] M.P. Marszall, T. Baczek, R. Kaliszan, *J. Sep. Sci.* 29 (2006) 1138.
- 378 [26] X. Xiao, L. Zhao, X. Liu, S. Jiang, *Anal. Chim. Acta* 519 (2004) 207.
- 379 [27] E.G. Yanes, S.R. Gratz, A.M. Stalcup, *The Analyst* 125 (2000) 1919.
- 380 [28] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robinson, A.M. Stalcup, *Anal. Chem.* 73 (2001) 3838.
- 381 [29] M. Vaheer, M. Koel, M. Kaljurand, *Chromatographia* 53 (2001) S-302.
- 382 [30] M. Vaheer, M. Koel, M. Kaljurand, *Electrophoresis* 23 (2002) 426.
- 383 [31] M. Vaheer, M. Koel, M. Kaljurand, *J. Chromatogr. A* 979 (2002) 27.
- 384 [32] R. Kuldvee, M. Vaheer, M. Koel, M. Kaljurand, *Electrophoresis* 24 (2003) 1627.
- 385 [33] M. Vaheer, M. Koel, *J. Chromatogr. A* 990 (2003) 225.
- 386 [34] K. Tian, S. Qi, Y. Cheng, X. Chen, Z. Hu, *J. Chromatogr. A* 1078 (2005) 181.
- 387 [35] S. Qi, Y. Li, Y. Deng, Y. Cheng, X. Chen, Z. Hu, *J. Chromatogr. A* 1109 (2006) 300.

388 [36] M.P. Marszall, M.J. Markuszewski, R. Kaliszan, *J. Pharm. Biomed. Anal.* 41 (2006) 329.
389 [37] M.E. Yue, Y.P. Shi, *J. Sep. Sci.* 29 (2006) 272.
390 [38] S.M Mwongela, A. Numan, N.L. Gill, R.A. Agbaria, I.M. Warner, *Anal. Chem.* 75 (2003) 6089.
391 [39] J. Ding, D. W. Armstrong, *Chirality* 17 (2005) 281.
392 [40] C. Baudequin, D. Brégeon, J. Levillain, F. Guillen, J-C. Plaquenvent, A-C. Gaumont, *Tetrahedron: Asymmetry*
393 16, (2005) 3921.
394 [41] S.A.A. Rizvi, S.A. Shamsi, *Anal. Chem.* 78 (2006) 7061.
395 [42] B.K. Patel, M. Hanna-Brown, M.R. Hadley, A.J. Hutt, *Electrophoresis* 25 (2004) 2625.
396 [43] Y. Francois, A. Varenne, E. Juillerat, A-C. Servais, P. Chiap, P. Gareil, *J. Chromatogr. A* 1138 (2007) 268.
397 [44] P. Wasserscheid, A. Bosman, C. Bolm, *Chem. Commun.* (2002) 200.
398 [45] Y.J. Yao, S.F.Y. Li, *J. Chromatogr. A* 680 (1994) 431.
399 [46] E. Cordova, J. Gao, G.M. Whitesides, *Anal. Chem.* 69 (1997) 1337.
400 [47] A. Macia, F. Borrull, M. Calull, C. Aguilar, *Electrophoresis* 25 (2004) 3441.
401 [48] Y. Francois, A. Varenne, J. Sirieix, P. Gareil, *J. Sep. Sci.* in press.
402 [49] K.A. Connors, *Binding Constants. The Measurements of Molecular Complex Stability.* John Wiley & Sons,
403 New York, 1987
404 [50] K.L. Rundlett, D.W. Armstrong, *J. Chromatogr. A* 721 (1996) 173.
405 [51] F. Lelièvre, P. Gareil, A. Jardey, *Anal. Chem.* 69 (1997) 385.
406 [52] S. Fanali, Z. Aturki, *J. Chromatogr. A* 694 (1995) 297.
407 [53] F. Lelièvre, P. Gareil, *J. Chromatogr. A* 735 (1996) 311.
408 [54] Y. Francois, K. Zhang, A. Varenne, P. Gareil, *Anal. Chim. Acta* 562 (2006) 164.
409 [55] C. Schwer, E. Kenndler, *Chromatographia* 33 (1992) 331
410
411
412
413
414
415
416
417

418 **Captions**

419



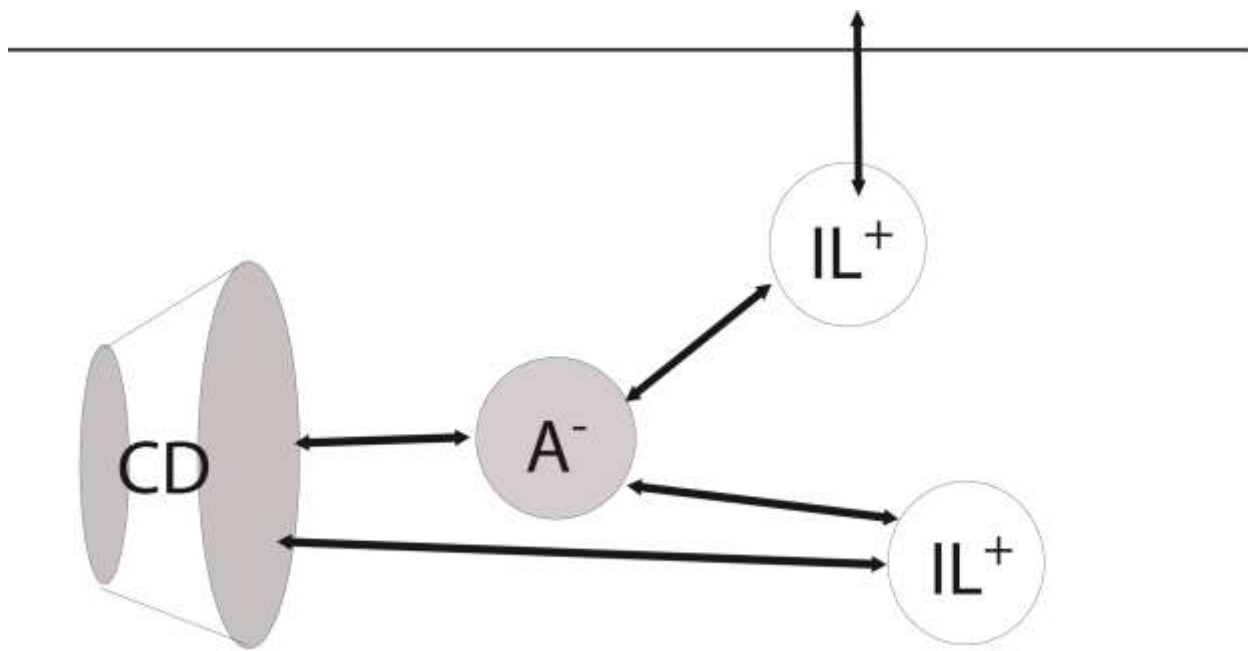
420

421 Figure 1: Structures of (A) the studied arylpropionic acids and (B) ionic liquids ethylcholine and

422 phenylcholine bis(trifluoromethanesulfonyl)imide (EtCholNTf₂, PhCholNTf₂). pK_a values at 26-

423 27 °C from [53]

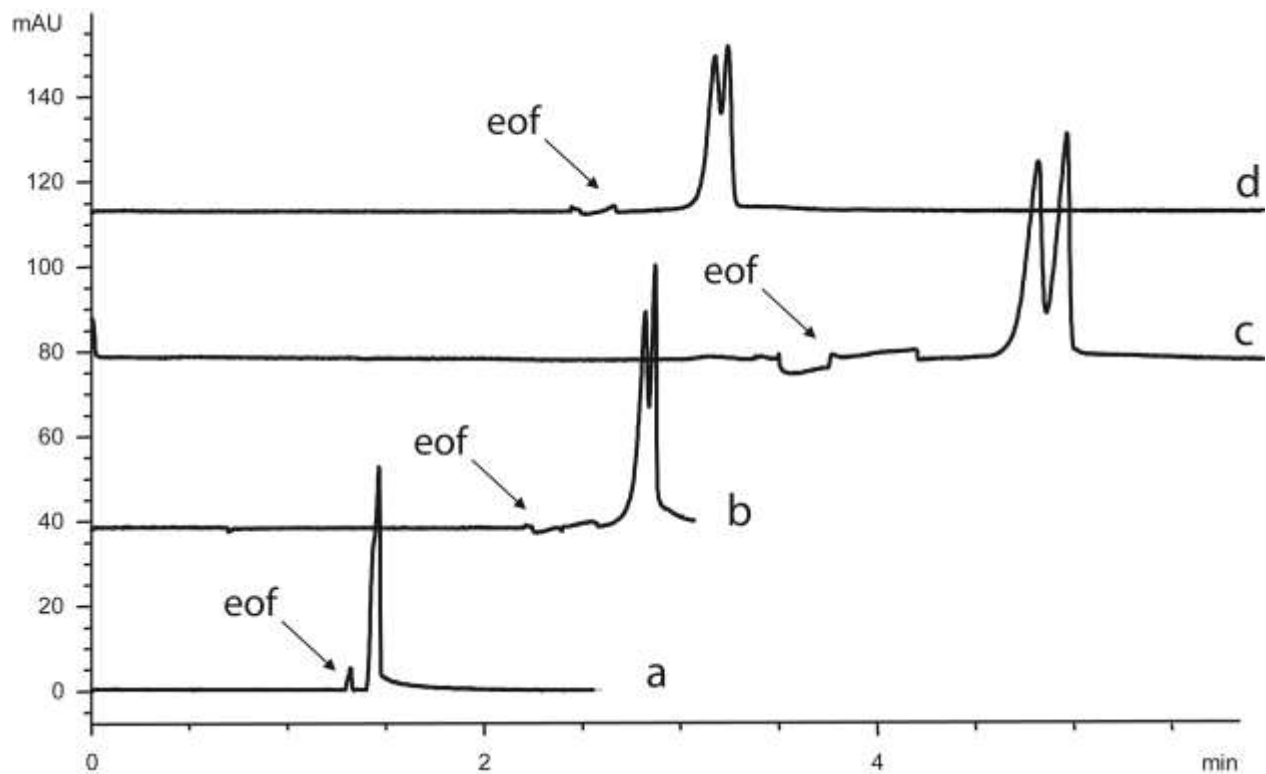
424



425
426 Figure 2: Schematic description of the interaction system between anionic profen, chiral IL
427 cation, free in the BGE or adsorbed onto the capillary wall, and β -CD derivatives.

428

429



430
 431 Figure 3: Enantioseparation of carprofen in the presence of TM- β -CD and chiral ILs.
 432 Fused silica capillary, 50 μ m i.d. 35 cm (effective length, 26.5 cm). Electrolyte: 2.63 mM acetic
 433 acid, 5.0 mM sodium acetate buffer, w_p pH 5.0 containing (a) 30 mM TM- β -CD, (b) 30 mM TM-
 434 β -CD + 10 mM EtCholNTf₂, (c) 30 mM TM- β -CD + 10 mM PhCholNTf₂ (d) 30 mM TM- β -CD
 435 + 10 mM LiNTf₂ in (90:10, v/v) H₂O-MeOH mixture. Applied voltage: 25 kV. Temperature: 25
 436 °C. UV absorbance at 230 nm. Hydrodynamic injection (30 mbar, 3 s). EOF: electroosmotic
 437 flow.

438
 439
 440
 441
 442