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# EFFECT OF THE COMBINED USE OF γ-CYCLODEXTRIN AND (R)-N,N,N-TRIMETHYL-2-AMINOBUTANOL-BIS(TRIFLUOROMETHANE-SULFON)IMIDATE CHIRAL IONIC LIQUID ON THE ENANTIOMERIC SEPARATION OF HOMOCYSTEINE BY CAPILLARY ELECTROPHORESIS

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**Abstract**

The enantioseparation of the non-protein amino acid homocysteine by CE was investigated in this article using seven neutral cyclodextrins and five chiral ionic liquids as chiral selectors. Using a previous derivatization step with FMOC and the subsequent separation under neutral conditions, homocysteine enantiomers were only separated when γ-CD or (R)-N,N,N-trimethyl-2-aminobutanol-bis(trifluoromethane-sulfon)imidate ($EtCholNTf_2$) were employed as sole chiral selectors in the separation buffer. On the one hand, γ-CD gave rise to the enantiomeric separation in 10 min with a resolution value of 1.9, whereas $EtCholNTf_2$ let to obtain a resolution value of 1.4 in more than 50 min. Then, the evaluation of the combined use of both selectors was also carried out, resulting in a considerable increase in the Rs. The best enantioseparation for homocysteine was obtained when 10 mM $EtCholNTf_2$ was added to 50 mM phosphate buffer (pH 7.0) containing 2 mM γ-CD. In an attempt to discriminate specific chiral cation effect from the salt effect, the influence of adding $LiNTf_2$ to the separation medium was also evaluated, resulting in lower resolution values for homocysteine when compared to those achieved with the addition of $EtCholNTf_2$, indicating a synergistic effect between $EtCholNTf_2$ and γ-CD. Interestingly, the enantiomer migration order changed depending on the chiral selector used. When $EtCholNTf_2$ or γ-CD were used as sole chiral selectors, D-enantiomer was the first-migrating enantiomer. However, an inversion in the migration order was observed when both selectors were employed in a dual system being the L-enantiomer the first-migrating one.

**Keywords:** Capillary electrophoresis, chiral ionic liquids, cyclodextrins, homocysteine, enantioseparation.

## 1. Introduction

Capillary electrophoresis (CE) has already demonstrated its high potential in the field of chiral separations [1-3]. In fact, this separation technique has been applied to the enantioseparation of a broad range of compounds of interest in the pharmaceutical, food or environmental fields. Among the great variety of compounds that can be used as chiral selector in CE (cyclodextrins (CDs), antibiotics, crown ethers, cyclofructants, or polysaccharides among others [1, 4] CDs continue being nowadays the most employed [2, 5-6] However, the use of all these compounds as chiral selectors have some limitations. For instance, in some cases their low solubility, high UV absorptivity, instability at high temperature, high cost and tedious synthesis could limit their use in CE [7]. All these reasons promote the search of new compounds that can be used as chiral selectors. This fact is indeed one of the most relevant challenges in the field of chiral separations by CE.

In this regard, a great interest has been paid in the last years to the evaluation of chiral ionic liquids (CILs) as new potential selectors for enantiomeric separations by CE [7-13]. Ionic liquids (ILs) are salts with melting points below 100 ºC constituted by a bulky organic cation and an organic or inorganic anion which have influence on the physicochemical properties of ILs, such as, high conductivity, low volatility, high thermal stability and miscibility in organic solvents [10]. In particular, ILs which have a chiral cation and/or anion are called CILs. CILs have been used in CE as sole chiral selectors (undecenoxycarbonyl-L-pyrrolidinol bromide, undecenoxycarbonyl-L-leucinol bromide, (R)-N,N,N-trimethyl-2-aminobutanol-bis(trifluoromethane-sulfon)imidate, (+)-N,N-dimethylephedrinium-bic(trifluoromethanesulfon)imidate, 6-O-2-hydroxypropyltrimethylammonium-β-cyclodextrin tetrafluoroborate, L-alanine tert butyl ester lactate and tetramethylammonium-lactobionate), in dual systems with other chiral selectors, ((R)-N,N,N-trimethyl-2-aminobutanol-bis(trifluoromethane-sulfon)imidate, L-

alanine tert butyl ester lactate, L-alanine tert butyl ester bis(trifluoromethanesulfon)imidate, 1-ethyl-3-methylimidazolium L-lactate, tetramethylammonium L-Arginine, among others) and as chiral ligands in ligand-exchange capillary electrophoresis (including 1-butyl-3-methylimidazolium L-alanine, 1-ethylpyridinium L-Lysine, 1-ethyl-3-methylimidazolium L-tartrate, etc) [13]. Most of the works that have reported the use of CILs as chiral selectors in CE were devoted to the study of their synergistic effect with other selectors, mainly CDs, using drugs [14-22] or protein amino acids as model compounds [23]. With the exception of a work in which an improvement in the chiral resolution was not obtained by adding the CILs to the separation media [24] all the other works demonstrated that combination of CILs can be a useful tool to increase the enantiomeric resolution and selectivity obtained with other chiral selectors, in particular, CDs, polysaccharides or macrocyclic antibiotics. However, until now, dual systems combining a CD and a CIL have scarcely been used to achieve the enantioseparation of non-protein amino acids. In fact, only a work reported the use of a dual system based on β-CD and D-alanine tert butyl ester bis(trifluromethane)sulfonamide (D-Ala$C_4NTf_2$) to carry out the enantiomeric separation of the non-protein amino acid pipecolic acid [25]. Non-protein amino acids, are a class of compounds which are not found in protein chains, but play important roles in metabolic pathways as intermediates [26]. A high number of these non-protein amino acids are chiral molecules.

Homocysteine (Hcy) is a sulfur containing non-protein amino acid implied in the metabolism of methionine and whose metabolism is related with other important metabolites like S-adenosylmethionine, folic acid and B vitamins [27, 28]. This non-protein amino acid is also considered a biomarker in cardiovascular and neurodegenerative diseases since high levels of Hcy in serum and plasma are associated

97 with coronary heart disease [29], Alzheimer`s [30] and Parkinson`s diseases [27].
98 Although Hcy is a chiral amino acid, only few works reported its enantiomeric separation
99 by HPLC [31-34] and just one work reported its enantiomeric separation by CE using a
100 high concentration of γ-CD with a resolution value of 1.26 [35].
101 The aim of this work was to study the enantiomeric separation of the non-protein amino
102 acid homocysteine with different neutral CDs and CILs as sole chiral selectors in CE, and
103 to investigate the effect of the combined use of both types of chiral selectors on the
104 enantioseparation of this model compound.
105

106 **2. Materials and methods**

107 **2.1. Reagents and samples**

108 All chemicals and reagents used were of analytical grade. Boric acid, sodium hydroxide
109 and pentane were purchased from Sigma-Aldrich (Madrid, Spain). Disodium hydrogen
110 phosphate was provided by Panreac Química S.A. (Barcelona, Spain). Acetonitrile and
111 hydrochloric acid were obtained from Scharlau (Barcelona, Spain). The chiral selector β-
112 CD, Heptakis(2,3,6-tri-O-methyl)-β-CD, (2-Hydroxi)propyl-β-CD (DS ~ 3), and γ-CD
113 were purchased from Fluka (Buchs, Switzerland). α-CD, methyl-β-CD and Heptakis(2,6-
114 di-O-methyl)-β-CD were from Sigma-Aldrich (Madrid, Spain). Water used to prepare
115 solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA).

116 DL-homocysteine (DL-Hcy), L-homocysteine (L-Hcy), DL-homocystine, DL-
117 homocysteine thiolactone hydrochloride, and the derivatization reagent 9-
118 fluorenylmethoxycarbonyl chloride (FMOC-Cl) were provided by Sigma-Aldrich
119 (Madrid, Spain). Two of the five CILs employed in this work, namely 1-ethyl-3-
120 methylimidazolium L-lactate ([EMIm][L-Lactate]) and 2-hydroxiethyl-
121 trimethylammonium L-Lactate were obtained from Sigma-Aldrich (Madrid, Spain)

whereas the other three were synthesized following different procedures previously reported in the literature. Thus, L-alanine tert butyl ester L-lactate (L-Ala$C_4$L-Lactate) was synthesized by our research group according to the procedure described by Bwambok et al. [36], whereas L-alanine tert butyl ester bis(trifluromethane)sulfonamide (L-Ala$C_4NTf_2$) and (R)-N,N,N-trimethyl-2-aminobutanol-bis(trifluoromethane-sulfon)imidate (EtCholN$Tf_2$) were synthesized by the Center for Applied Chemistry and Biotechnology (CQAB) from the University of Alcalá following previously optimized procedures [36, 37]. **Figure 1** shows the chemical structures of the five CILs employed in this work.

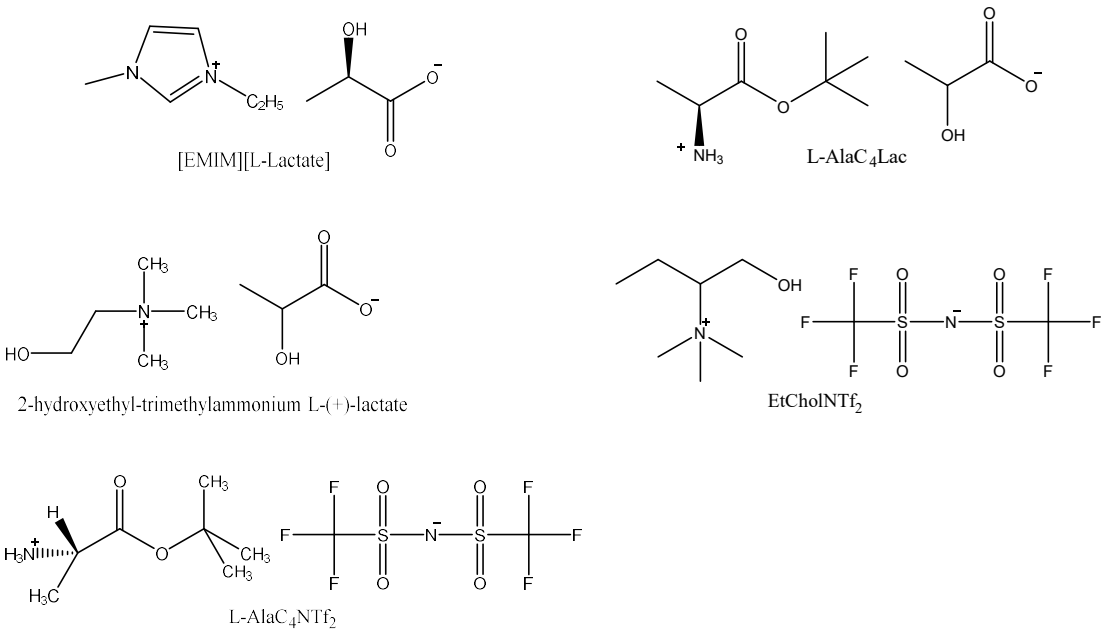



**Figure 1.** Chemical structures of CILs employed in this work.

## 2.2. CE conditions

CE analyses were carried out using an Agilent 7100 CE system (Agilent Technologies, Waldbronn, Germany). Detection was performed with a DAD working at 210 nm with a

bandwidth of 4 nm. The instrument was controlled by the ChemStation software (B. 04. 03 SP1) from Agilent Technologies. Separation capillary was an uncoated fused-silica capillary of 50 µm ID (362.8 µm OD) with a total length of 58.5 cm (50 cm effective length) provided by Polymicro Technologies (Phoenix, AZ, USA). Injections were made applying a pressure of 50 mbar for 4 s and the electrophoretic separation was achieved using a voltage of 20 kV and a working temperature of 20 ºC.

Before its first use, the capillary was conditioned (applying 1 bar) with 1 M sodium hydroxide for 30 min, followed by 5 min with Milli-Q water and with buffer solution for 60 min. At the beginning of each day the capillary was pre-washed (applying 1 bar) with 0.1 M sodium hydroxide during 10 min, Milli-Q water for 5 min, buffer solution for 15 min and BGE during 10 min. Between runs, the capillary was conditioned with 0.1 M sodium hydroxide (2 min), Milli-Q water (1 min) and BGE (3min).

### 2.3. Preparation of solutions and samples

Borate buffer solution (200 mM, pH 9.0) needed for the derivatization step was prepared dissolving the appropriate amount of boric acid in Milli-Q water. The buffer solution was prepared dissolving the amount needed of disodium hydrogen phosphate to achieve a concentration of 50 mM and adjusting the pH with hydrochloric acid before completing the volume with water. The BGE was obtained by dissolving the proper amount of chiral selectors in the buffer solution. The stock standard solution of Hcy was prepared by dissolving the appropriate amount of the amino acid in borate buffer and stored at 4 ºC until its derivatization with FMOC.

All solutions were filtered before its use through 0.45 µm pore size disposable nylon filters from Scharlau (Barcelona, Spain).

## 2.5. Derivatization procedure

Homocysteine was derivatized following the procedure previously described in the literature [38, 39]. Taking into account that an excess of FMOC of at least three times was necessary to obtain a complete derivatization of homocysteine, a solution of 30 mM in ACN was freshly prepared each day. Then, 200 µL of this solution were mixed with 200 µL of homocysteine standard solution (10 mM). The reaction was kept at room temperature for 2 min. The excess of FMOC-Cl was extracted with 0.5 mL pentane and the resulting solution was diluted ten times with Milli-Q water before injection in the CE system.

## 2.6. Data treatment

Migration times and values of resolution (Rs), calculated from the migration times of enantiomers and their peak widths at half height, were obtained using the Chemstation software from Agilent Technologies. The effective electrophoretic selectivity [14, 40] ($\alpha_{eff}$) was calculated according to the following equation:

$$\alpha_{eff} = \mu_{ep1}/\mu_{ep2}$$

were $\mu_{ep1}$ and $\mu_{ep2}$ are the effective mobilities of enantiomers 1 and 2, respectively.

Origin 8.0 software was used to carry out the composition of graphs with different electropherograms.

# 3. Results and discussion

## 3.1. Enantiomeric separation of homocysteine with neutral CDs as sole chiral selectors

In order to achieve the enantioselective separation of Hcy using CDs, a set of different neutral CDs (α-CD, β-CD, γ-CD, methyl-β-CD, Heptakis(2,3,6-tri-O-methyl)-β-CD, (2-Hydroxi)propyl-β-CD, Heptakis(2,6-di-O-methyl)-β-CD) was selected to evaluate their discrimination power at pH 7.0 in which FMOC-Hcy is negatively charged (pKa = 3.77). In these experiments, all CDs were tested using a concentration of 10 mM in 50 mM phosphate buffer (pH 7.0) using a voltage of 20 kV and a temperature of 20 ºC. Among all the CDs employed, only the use of γ-CD gave rise to the chiral separation of Hcy. The separation of the Hcy enantiomers was achieved in 10 min with a resolution value of 1.9. The influence of the concentration of γ-CD on the enantioselective separation of Hcy was evaluated in the range from 1 to 15 mM due to the fact that the concentration affects the affinity of the enantiomers for the chiral selector. As it can be seen in **Figure 2** and from the data shown in **Table 1**, the resolution gradually improved when the γ-CD concentration increased up to 10 mM. A higher concentration of γ-CD led to a slight decrease in the resolution value. Regarding to the enantiomer migration order for Hcy, it was established injecting a solution of DL-Hcy spiked with L-Hcy, so that it was possible to assign the D-Hcy as the first migrating enantiomer and L-Hcy as the second one.

The separation conditions optimized in this work with γ-CD enabled to obtain the best chiral separation of Hcy in the shortest migration time when comparing these results with that previously reported in the literature for the enantiomeric separation of Hcy [35].

**Table 1.** Migration times, electrophoretic selectivity and resolution for Homocysteine enantiomers using different chiral selectors.

| Chiral selector | [CS](mM) | $t_1$ (min) | $t_2$ (min) | $R_s$ | α (eff) | Enantiomer Migration order |
|---|---|---|---|---|---|---|
| γ-CD | 1 | 9.955 | 10.013 | 0.5 | 1.01 | D - L |
| | 2 | 9.87 | 10.008 | 1.0 | 1.01 | |
| | 5 | 9.764 | 9.956 | 1.7 | 1.02 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 9.813 | 10.008 | 1.9 | 1.02 | |
| | 15 | 9.892 | 10.068 | 1.8 | 1.02 | |
| **EtCholNTf2** | 1 | 22.031 | - | - | - | |
| | 5 | 27.636 | - | - | - | |
| | 10 | 34.611 | 34.935 | 0.6 | 1.01 | D - L |
| | 20 | 39.098 | 39.618 | 0.8 | 1.01 | |
| | 40 | 51.745 | 53.682 | 1.4 | 1.04 | |
| | 60 | 54.393 | 55.900 | 1.6 | 1.03 | |
| **Dual system 1mM γ-CD + EtCholNTf2** | 1 | 10.066 | 10.151 | 1.0 | 1.01 | L - D |
| | 5 | 10.525 | 10.938 | 4.7 | 1.04 | |
| | 10 | 12.767 | 13.52 | 4.8 | 1.06 | |
| | 20 | 15.839 | 16.516 | 3.1 | 1.04 | |
| **Dual system 2 mM γ-CD + EtCholNTf2** | 1 | 9.741 | 10.264 | 4.0 | 1.05 | L - D |
| | 5 | 10.235 | 10.900 | 7.7 | 1.06 | |
| | 10 | 11.890 | 12.737 | 8.0 | 1.07 | |
| | 20 | 12.716 | 13.456 | 5.9 | 1.06 | |
| **Dual system 2 mM γ-CD + $LiNTf_2$** | 1 | 10.591 | 11.014 | 4.1 | 1.04 | L - D |
| | 5 | 10.968 | 11.719 | 7.1 | 1.07 | |
| | 10 | 11.386 | 12.105 | 6.4 | 1.06 | |
| | 20 | 12.038 | 12.561 | 3.8 | 1.04 | |

205 $t_1$: time of the first-migrating enantiomer

206 $t_2$: time of the second-migrating enantiomer

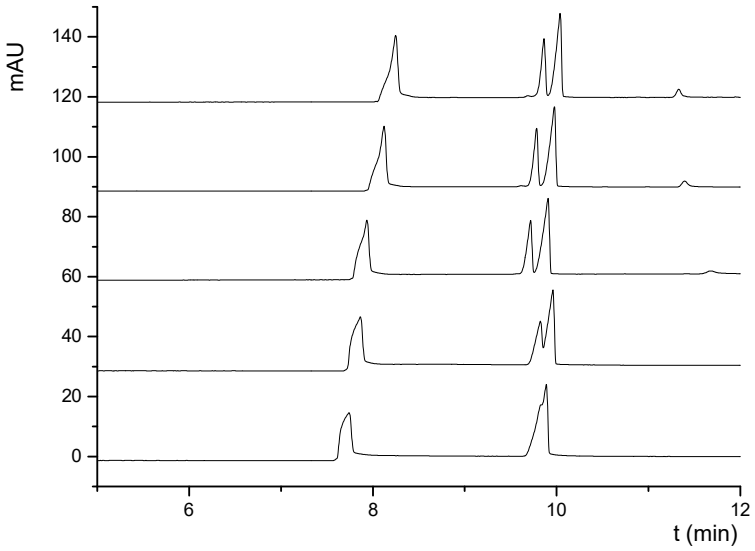
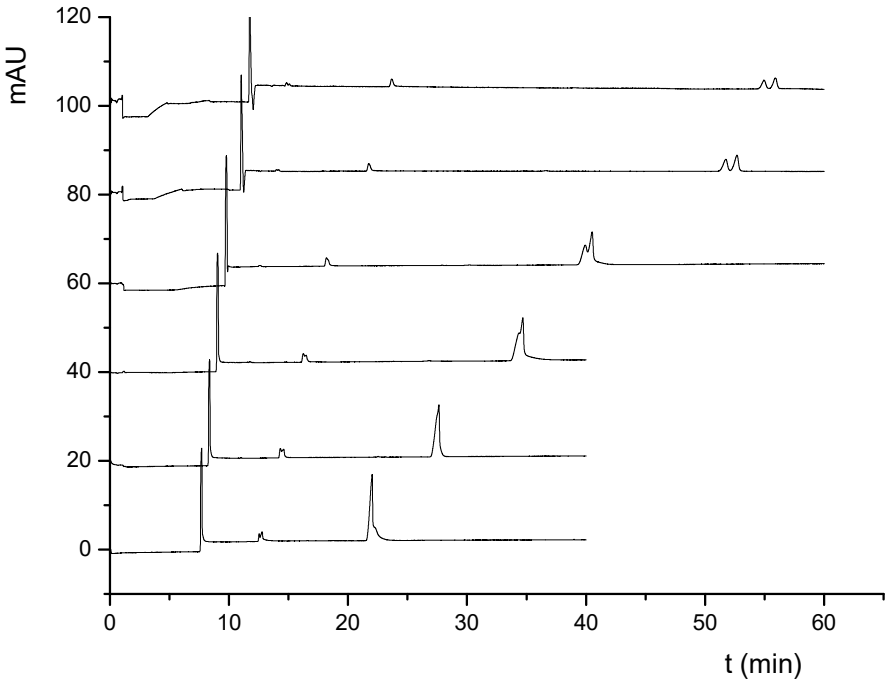



**Figure 2.** Electropherograms corresponding to the enantiomeric separation of Hcy using different concentrations of γ-CD. Experimental conditions: 50 mM phosphate buffer (pH 7.0); uncoated fused-silica capillary, 58.5 cm (50 cm to the detector window) x 50 µm ID; UV detection at 210 nm; applied voltage, 20 kV; temperature 20 ºC; injection by pressure, 50 mbar for 4s.

### 3.2. Enantiomeric separation of homocysteine with CILs as sole chiral selectors

As mentioned in the introduction, the potential of CILs as selectors in chiral CE has been demonstrated in different works [13]. For this reason, the discrimination power of different CILs to achieve the enantioselective separation of Hcy was also investigated. A total of five different CILs were tested. Two of them were commercially available (namely [EMIm][L-lactate] and 2-hydroxiethyl-trimethylammonium L-Lactate), other two were amino acid based ionic liquids (L-Ala$C_4$N$Tf_2$ and L-Ala$C_4$Lac) which synthesis is easy, low cost and the chirality is stable [15], and the last one was EtCholN$Tf_2$ that was chosen due to the good results obtained when it was employed both as sole chiral selector for the enantioseparation of different compounds including alcohols, amines, acids and protein amino acids [41] and in combination with CDs forming a dual separation system

for the enantioseparation of drugs [14], different binaphthols [24] and a synthetic key intermediate of benzopyran derivatives [42]. The evaluation of the discrimitation power of the five CILs was performed by a screening test in which each CIL was at a concentration of 20 mM in 50 mM phosphate buffer (pH 7.0) and applying a voltage of 20 kV at 20 ºC. The results obtained in these experiments showed that only the use of $EtCholNTf_2$ gave rise to a partial separation of DL-Hcy (Rs = 0.8) (data not shown). Then, the influence of the concentration of this chiral ionic liquid on the Hcy enantioresolution was investigated. The concentration range studied was from 1 to 60 mM, and the electropherograms obtained at each concentration are shown in **Figure 3**. As it can be observed, at low concentrations (1 and 5 mM) the enantioseparation was not achieved. When concentrations of 10 and 20 mM were employed, the separation was not satisfactory (Rs values of 0.6 and 0.8, respectively). However, an increase in the concentration resulted in a significant increase in resolution (Rs values of 1.4 and 1.5 for 40 and 60 mM, respectively) although the analysis time was too high (> 50 min) and peak broadening took place. Using $EtCholNTf_2$ as chiral selector, the enantiomeric migration order for Hcy was the same as that obtained using γ-CD, i.e. D-enantiomer migrates faster than the L-enantiomer.




242

243 **Figure 3.** Effect of $EtCholNTf_2$ concentration on the separation of Hcy enantiomers.
244 Other conditions same as in Fig 2.

245

246 An interesting fact than can be seen in **Figure 3** is that as the concentration of $EtCholNTf_2$
247 increased, the peak corresponding to the electroosmotic flow migrated always around the
248 same analysis time, therefore its electrophoretic mobility was practically constant in all
249 the experiments. This seems to indicate the no adsorption (or a low adsorption) of the
250 cationic moiety of the CIL on the capillary wall since otherwise a decrease in the
251 electroosmotic mobility should be observed [14].

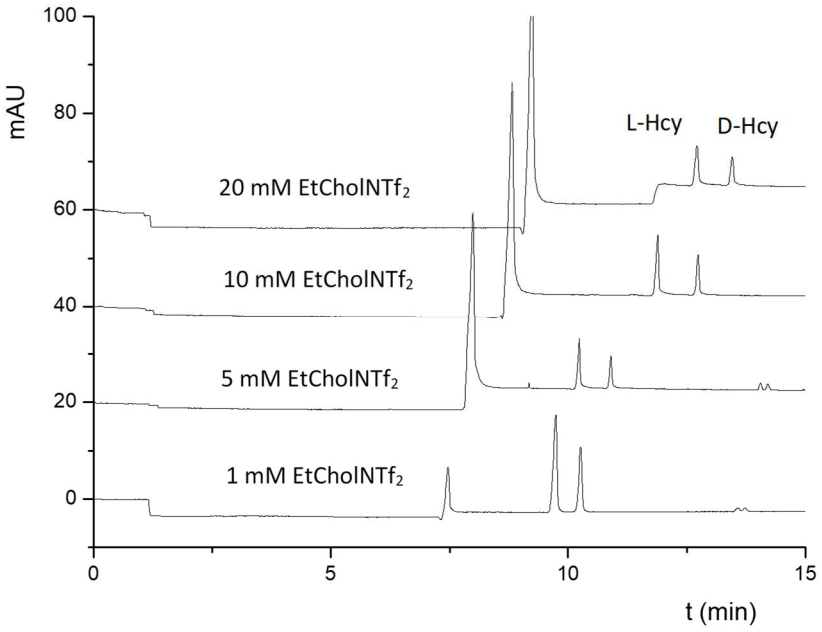
252

253 **3.3. Effect of the combined use of γ-CD and $EtCholNTf_2$ on the enantiomeric**
254 **separation of homocysteine**

255 Until now, most of the applications of CILs in chiral CE have employed dual recognition
256 systems based on the combination of a CIL with other chiral selector (mainly CDs) [13].

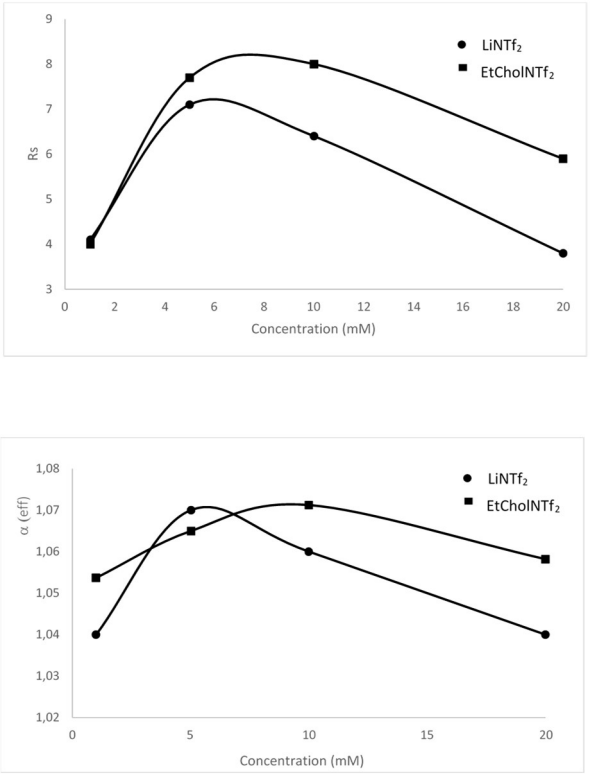
257 Taking into account that among the different selectors evaluated in this research work,
258 only the use of γ-CD and EtCholNTf$_2$ were able to provide the enantioseparation of Hcy,
259 the dual system formed by the combination of both selectors was evaluated in order to
260 determine if a synergistic effect may exist between them to improve the enantiomeric
261 separation of Hcy.
262 With this aim, low concentrations of γ-CD (1 and 2 mM) were chosen since both enabled
263 to achieve a partial resolution of Hcy (Rs values of 0.5 and 1.0, respectively). With these
264 concentrations of γ-CD, different concentrations of EtCholNTf$_2$ (from 1 to 60 mM) were
265 investigated to evaluate the role of the CIL in a possible synergistic effect. As it can be
266 seen from the data shown in **Table 1**, for both combinations of γ-CD plus EtCholNTf$_2$,
267 the resolution of Hcy increased with the concentration of EtCholNTf$_2$ till reaching a
268 maximum value when 10 mM EtCholNTf$_2$ was employed. In fact, Rs values of 4.7 and
269 8.0 were obtained using 1 or 2 mM γ-CD plus 10 mM EtCholNTf$_2$, respectively. Higher
270 concentrations of EtCholNTf$_2$ gave rise to a decrease in the Rs probably due to a
271 saturation in the complexation between the chiral selector and the analyte. The results
272 obtained in these experiments demonstrated a significant improvement of the
273 enantiomeric separation of Hcy (Rs increased 8 and 14.5 times) when a dual system was
274 employed compared to the use of the chiral selector alone at the same concentration than
275 that employed in the dual system. **Figure 4** depicts the electropherograms obtained for
276 the enantiomeric separation of Hcy using 2 mM of γ-CD and different concentrations of
277 EtCholNTf2. It can be seen that the best results were achieved when adding 10 mM CIL.
278 Interestingly, a reversal in the enantiomeric migration order was observed for Hcy using
279 the dual system. In fact, D-Hcy was the first-migrating enantiomer when using γ-CD or
280 EtCholNTf$_2$ as sole chiral selectors whereas when the dual system was employed, the
281 opposite migration order was observed being the D-enantiomer the second-migrating one.

282 To study if the increase in the Rs value was due to a synergistic effect between both chiral
283 selectors (CD and CIL) and to discriminate specific chiral cation effect from the salt
284 effect, the influence of adding $LiNTf_2$ to the separation medium instead of the CIL was
285 investigated under the same experimental conditions. With this aim, increasing
286 concentrations of this salt (from 1 to 20 mM) were added to the separation medium when
287 a 2 mM concentration of γ-CD was employed. Along with the resolution values, the
288 effective electrophoretic selectivity (αeff), a thermodynamic parameter independent of
289 the electroosmotic flow variation, was also calculated (see **Table 1**). **Figure 5** depicts the
290 variation of Rs and αeff as a function of the concentration of both $LiNTf_2$ and
291 $EtCholNTf_2$. As it can be observed, with the addition of the salt, Rs and αeff increased
292 till 5 mM where they reached their highest values (7.07 and 1.07, respectively) and then,
293 they decreased when increasing the salt concentration. These results were in agreement
294 with those previously observed by other authors [14, 42] and were difficult to explain
295 since the salt is not chiral and the migration times for the Hcy enantiomers were very
296 similar when using the salt and when using the CIL being the variations in the
297 electroosmotic flow not significant. In the case of $EtCholNTf_2$, the highest values for Rs
298 and αeff were obtained at a 10 mM concentration and it was this CIL which originated
299 the highest values of Rs showing the existence of a synergistic effect between γ-CD and
300 $EtCholNTf_2$.




301

302 **Figure 4.** Electropherograms corresponding to the chiral separation of Hcy using a dual
303 system based on the combination of □-CD (whose concentration was fixed at 2 mM) and
304 increasing concentrations of EtCholNTf2. Other experimental conditions as in Fig 2.




305

306 **Figure 5.** Effects of the addition of EtCholNTf2 and LiNTf2 at different concentrations
307 on Rs and αeff values. Fixed concentration of 2 mM γ-CD. Other conditions as in Fig 2.

Finally, as shown in **Figure 4** the optimized separation conditions enabled to observe the degradation of Hcy in solution over the time since two additional peaks were observed in the electropherograms. In fact, Hcy can form the chiral dymer homocystine via dehydrogenation reaction or give rise to the homocysteine lactone [43]. Both degradation products were injected in the CE system to identify which of them corresponded to the peaks observed in the electropherograms. Homocystine could be clearly identified as the degradation product gradually formed from Hcy although it could not be clarified it the two peaks observed corresponded to the separation of two enantiomers or two diastereomers (homocystine has two chiral centers).

## 4. Conclusions

A set of seven neutral cyclodextrins and five chiral ionic liquids were evaluated to achieve the enantiomeric separation of homocysteine, a non-protein amino acid selected as model compound. Enantiomeric discrimination was observed when both γ-CD and EtCholNTf$_2$ were employed as sole chiral selectors in the separation buffer, but resolution values lower than 2 were obtained. Then, a BGE based on the combination of both selectors was subsequently investigated to look for a possible synergistic effect which could increase the enantiomeric separation of homocysteine. A dual system combining 2 mM γ-CD plus 10 mM EtCholNTf$_2$ enabled the enantiomeric separation of Hcy in a short analysis time (~11 min) with a high Rs value (8.0). Under these conditions, the L-enantiomer migrated faster than the D-enantiomer, what was opposite to the migration order observed when both chiral selectors were used alone. The simultaneous increase of αeff and resolution obtained in the presence of EtCholNTf$_2$, compared to the experiments with salt and γ-CD alone indicated a synergistic effect between EtCholNTf$_2$ and γ-CD towards the

enantioseparation of homocysteine. This is the first time that EtCholNTf$_2$ is applied to the enantiomeric separation of a non-protein amino acid.

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