

## AMINO ACIDS BASED CHIRAL IONIC LIQUIDS FOR ENANTIOMER SEPARATION BY CAPILLARY ELECTROPHORESIS

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### Abstract

Room temperature chiral ionic liquids (CILs) are a relatively new class of ionic compounds; they act as efficient chiral selectors and modifiers of the capacity of enantiodiscrimination in electrophoretic or chromatographic separations. The aim of this study was to synthesize and characterize according to literature new ionic liquids, tetrabutylammonium and tetramethylammonium amino acid salts (chiral anions: l-leucine, l-proline, l-histidine) and to investigate their effect on chiral separations of some pharmaceutical active ingredients racemates (ondansetron, mianserin, ofloxacin) using capillary electrophoresis. CILs alone were not able to discriminate the isomers of ondansetron, ofloxacin and mianserin, but the results proved that the enantioselectivity of hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD) increased when CILs were added in the background electrolyte (BGE) containing cyclodextrin. Several parameters (pH, CIL and cyclodextrin concentration, applied voltage and temperature) were studied and their effect on enantioselectivity was highlighted. The obtained results proved the synergistic action of CIL and cyclodextrins in the chiral recognition and enantioseparation process.

### Rezumat

Lichidele ionice chirale (CILs) sunt o clasă relativ recentă de compuși ionici; acestea pot fi utilizate fie ca selectori chirali fie ca modificali ai capacității de enantiodiscriminare în separări electroforetice sau cromatografice. Scopul acestui studiu îl reprezintă sinteza și caracterizarea, conform literaturii, a noi lichide ionice, săruri de tetrametilamoniu și tetrabutilamoniu ale aminoacizilor (anioni chirali: l-leucina, l-prolina, l-histidina) și cercetarea efectului acestora asupra separărilor chirale prin electroforeză capilară a unor substanțe farmaceutice amestecuri racemice (ondansetron, mianserină, ofloxacină). Lichidele ionice chirale, ca selector unic nu pot discrimina între izomerii ondansetronului, mianserinei sau ofloxacinei dar rezultatele obținute demonstrează că enantioselectivitatea hidroxipropil- $\beta$ -ciclodextrinei (HP $\beta$ CD) a crescut când CILs au fost adăugate în electrolitul de lucru care conține ciclodextrina. Au fost variați unii parametri experimentali (pH, concentrație de CIL și ciclodextrină, potențial aplicat și temperatură) și a fost evidențiat efectul acestora asupra enantioselectivității separărilor.

**Keywords:** chiral separations, chiral ionic liquids, capillary electrophoresis

### Introduction

The special interest in chiral separation of racemic drugs or drug metabolites is based on the data assessing differences in the pharmacological activity and pharmacokinetic profile of the enantiomers [9]. It is stated now that most biologic processes, including drug-receptor interaction and drug metabolism are chiral driven, so efficient methods for enantiomeric separation, both for analytical or preparative purpose, are now needed on a large scale. Furthermore, a strict regulatory context was established for the analysis and use of racemic drugs or pure pharmacological active enantiomers.

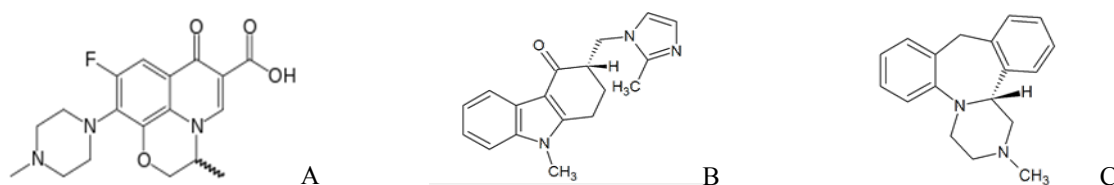
Though HPLC is now the method of choice for the analytical characterization of drug enantiomers or for enantiomeric excess determination, capillary electrophoresis becomes more and more interesting, as the amounts of analytes and chiral selectors are very small and there is a great variety of chiral selectors to be dissolved in the background electrolyte. Cyclodextrins (CDs) and their derivatives are the most important selectors, but other selectors, such as macrocyclic antibiotics, proteins, carbohydrates as glycogen or chiral ionic liquids that can be dissolved in the background electrolyte will conveniently act as chiral selectors [8, 13].

Ionic liquids (ILs) proved their analytical utility in separation when used as stationary phases (in GC) [2, 3, 26] additives or stationary phases (in HPLC) [2, 4, 26, 35] background electrolyte modifiers and supported dynamic coatings of the capillary wall capillary electrophoresis (CE) [7] or even pseudo-stationary phases micellar electrokinetic chromatography (MEKC) [14]. ILs interactions with the analytes may lead to significant changes in the analyte chromatographic and electrophoretic properties, resulting in highly selective and efficient separations [8]. When the cation, the anion or both ions possess chirality, there are premises to consider the ability of the chiral ionic liquids (CILs) to discriminate between enantiomers of various drug substances.

To the moment, experimental data proving the ability of the CILs to act as sole chiral selectors are rather few [16, 26, 31] still it is already stated a synergistic effect of chiral selectors as CDs [23-24, 27, 33] or antibiotics [25, 29, 30] and chiral ionic

liquids used together in electrophoretic enantio-separation processes [28, 32, 34].

Amino acid ionic liquids (AAILs) are ionic liquids that contain in their structure an ion derived from a natural chiral source, L-amino acids, and their zwitterionic structure allows the use both as anions or cations to obtain CILs. Several papers published in the last 15 years refer to the use of AAILs in various enantioseparations: polymers of ionic liquids derived from amino acids were used in MEKC [9, 14] and capillary electrochromatography (CEC) as polyelectrolyte multilayer coating (PEM) [6]; amino acid esters salts acted as sole chiral selectors in CE [16] or as a second selector in a dual system with vancomycin [29] or  $\beta$ -cyclodextrin [28]; tetrabutyl- [1] and tetramethylammonium derivatives [5] of AAIL obtained by ionic exchange synthesis; they can be a second chiral selector in the CE separations [27, 30, 32]. AAIL can also be used as chiral selectors in ligand exchange capillary electrophoresis (LECE) [10-12, 20].



**Figure 1.**

Chemical structures of Ofloxacin (A), Ondansetron (B) and Mianserin (C)

The purpose of our study was to investigate the effect of CILs derived from amino acids on CE enantioseparations, both as chiral selector, or as background electrolyte modifier when CDs (i.e. hydroxypropyl- $\beta$ -cyclodextrin - HP $\beta$ CD) are used as chiral selector. Our research focused on the synthesis and characterisation of AAILs obtained from L-amino acids and tetramethylammonium hydroxide or tetrabutylammonium hydroxide.

For this study the selected racemates (fig.1) were Ondansetron, 9-Methyl-3-[(2-methylimidazol-1-yl)-methyl]-2,3-dihydro-1H-carbazol-4-one (an antiemetic drug acting as a 5HT<sub>3</sub> serotonin antagonist), ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (a fluoroquinolone antibiotic) and mianserin, 1,2,3,4,10,14b-Hexahydro-2-methyl-dibenzo[c,f]-pyrazino[1,2-a]azepine (a tetracyclic antidepressant). According to the data in literature, there are several electrophoretic methods for their enantiomers' separation. Enantiodiscrimination in capillary electrophoresis is achieved using various cyclodextrins and cyclodextrin derivatives as sole chiral selectors (dimethyl derivative of  $\beta$  cyclodextrin for ondansetron) [15], HP $\beta$ CD for ofloxacin [19] and several other cyclodextrins [17, 21]  $\beta$ -

cyclodextrin ( $\beta$ CD), H $\beta$ CD and randomly methylated  $\beta$  cyclodextrin (RAMEB) for mianserin [22].

In order to describe the effect of the AAIL on chiral separations in CE using a dual chiral selectors system HP $\beta$ CD-AAIL we have studied resolution changes when several experimental parameters (pH, selectors' concentration, applied voltage, temperature) are varied at the addition of AAIL in the background electrolyte (BGE).

## Materials and Methods

### Reagents

The racemic mixtures used for this study were: ondansetron hydrochloride (Cipla, India), mianserin hydrochloride (Sigma-Aldrich, Germany) and ofloxacin (Beijin Pharmaceutical Works, China).

The amino acids used in the synthesis of ionic liquids were: l-leucine (Merck KGaA, Germany) l-histidine (Fluka, Switzerland), l-proline (Merck KGaA, Germany). Tetrabutylammonium hydroxide (TBAOH x 30 H<sub>2</sub>O) and tetramethylammonium hydroxide (TMAOH x 5 H<sub>2</sub>O) were purchased from Sigma-Aldrich, Germany. Acetonitrile was purchased from Scharlau (Spain), ethanol from Lach-Ner (Czech Republic) and Na<sub>2</sub>SO<sub>4</sub> anhydrous puriss from Riedel-de-Haën (Germany).

2-Hydroxypropyl- $\beta$ -cyclodextrin (average mass 1380) was purchased from Aldrich (Germany). The other chemicals used for the CE were: citric acid monohydrate (Merck KGaA, Germany), HCl (Merck KGaA, Germany), NaOH (Merck KGaA, Germany) and thiourea (Sigma-Aldrich, Germany).

All the solutions were prepared using ultrapure water obtained with an apparatus EASYpure RoDi (Barnstead, USA).

#### Instrumentation

The capillary electrophoresis equipment used was an Agilent Technologies G1600A, with diode array detector; the software is ChemStation ver. B.0 2.0. An uncoated silica capillary (Agilent Technologies) with 48 cm total length and an effective length of 40 cm,  $\phi = 50 \mu\text{m}$  was used.

Detection wavelengths were set at 216.4 nm for ondansetron and mianserin, 226.4 nm for ofloxacin and 279 nm for mianserin.

New capillaries were flushed with NaOH 1M for one hour and then H<sub>2</sub>O for one hour. Between analyses the capillary was flushed with water for 10 minutes, NaOH 0.1 M for 5 minutes, water for 5 minutes and then with the background electrolyte (BGE) for 10 minutes.

The applied voltage was set at 10, 15 and 20 kV in normal polarity. The temperature of the capillary was set between 16 and 25°C.

#### Sample preparation and background electrolyte

The background electrolyte for all the analysis was 50 mM citric acid/citrate, pH = 3 in which HP $\beta$ CD and tetrabutylammonium derivatives of amino acids (AATBA) and tetramethylammonium derivatives of amino acids (AATMA) were dissolved up to 5, 10 and 15 mM each.

The tested samples were 1mg/mL aqueous solutions. The electroosmotic flow (EOF) marker chosen was thiourea in concentration 0.5 mg/mL. All the solutions

were filtered through a syringe with regenerated cellulose filter with 0.45  $\mu\text{m}$  pore size (ROTH, Germany) and then sonicated prior to use to ensure proper degassing and then stored at 4°C.

#### AAILs synthesis

The AAILs were prepared according to a procedure described in the literature [1]. An aqueous solution containing 13 mmol of tetrabutylammonium or tetramethylammonium hydroxide (40%) was added to an aqueous suspension of the amino acid (13 mmol). The resulting reaction mixture was heated at 60°C for 2 hours on a heating plate with magnetic stirring (Yellow Line). The resulting water was removed in an oven at 60°C. The residue was dissolved in CH<sub>3</sub>CN (50 mL) for AATBA salts or EtOH (50 mL) for AATMA salts and filtered to remove unreacted amino acid. The filtrate was dried over Na<sub>2</sub>SO<sub>4</sub> *siccum*, filtered and the solvent was removed again in the drying oven to finally obtain the desired product. Thus, tetramethylammonium l-leucinate, l-LeuTMA (yellow liquid), tetramethylammonium l-histidinate, l-HisTMA (bright orange liquid), tetramethylammonium l-prolinate, l-ProTMA (brown-yellow liquid), tetrabutylammonium l-leucinate, l-LeuTBA, (yellow liquid), tetrabutylammonium l-histidinate, l-HisTBA (orange liquid), tetrabutylammonium l-prolinate, l-ProTBA (yellow liquid) were prepared. During storage CILs can change their colour. However, it had been stated that changes in their colour would not change their properties [1, 5].

## Results and Discussion

#### Optical rotation

The experiments were performed with a Perkin Elmer Polarimeter 341. The optical rotation of 0.3 - 0.6% aqueous solutions was measured and compared with the amino acids at 20°C.

**Table I**  
Optical rotations of AAIL

Molecule/anion	Amino acid		AATBA		AATMA	
	c (%)	$[\alpha]_{\text{D}}^{20}$	c (%)	$[\alpha]_{\text{D}}^{20}$	c (%)	$[\alpha]_{\text{D}}^{20}$
l-Leu	0.59%	-8.4	0.34%	-3.26	0.33%	0.05
l-His	0.54%	-38.40	0.64%	-5.53	0.22%	-4.83
l-Pro	0.60%	-84.26	0.64%	-25.22	0.67%	-35.19

As it can be seen from Table I for every ionic liquid the optical rotation changes compared to the starting amino acids. The highest shift can be observed for the tetraalkylammonium derivatives from proline.

#### NMR spectra

The NMR spectra were performed with a Varian Gemini 300 BB at a frequency of 300 MHz. The samples were dissolved in deuterated water (D<sub>2</sub>O) using the residual peak of water (4.79 ppm) as reference. The results are shown in Tables II and III.

**Table II**  
Chemical shifts of the protons from  $\alpha$ -carbon for amino acids and amino acid anions in the AAILs

Chemical shift (ppm)	Pro	Leu	His	ProTMA	LeuTMA	HisTMA	ProTBA	LeuTBA	HisTBA
$\delta \text{H}^{\alpha}$ (ppm)	4.080	3.699	3.912	3.627	3.352	3.412	3.755	3.498	3.554
$\Delta \delta \text{H}^{\alpha}$ (ppm)	-	-	-	0.452	0.346	0.500	0.324	0.201	0.359

**Table III**

Chemical shifts of the protons from  $\alpha$ -carbon for tetraalkylammonium hydroxides and tetraalkylammonium cations in the AAILs

Chemical shift (ppm)	TMAOH	TBAOH	ProTMA	LeuTMA	HisTMA	ProTBA	LeuTBA	HisTBA
$\delta H^\alpha$ (ppm)	3.116	3.137	3.126	3.135	3.065	3.153	3.159	3.106
$\Delta \delta H^\alpha$ (ppm)	-	-	-0.01	-0.019	-0.051	-0.015	-0.021	-0.031

The size of the cation  $N(C_4H_9)_4^+$  compared to  $N(CH_3)_4^+$  results in an ecranation (smaller chemical shifts  $\delta <$ ) for the  $\alpha$  proton from all of the tetra-butylammonium derivatives. When an equimolecular mixture of amino acid and tetraalkylammonium hydroxide is added,  $\delta H^\alpha$  from amino acid chain is shifted up field. The change of the signal from  $\alpha$  position was caused by the exchange of the carboxylic proton from the amino acid, resulting in an ionic liquid. The NMR spectra proved that quaternary salts of the amino acids were obtained. The signals for  $\alpha$  protons are different than the signals from the amino acid of origin; the deprotonation for the carboxylic moiety occurs.

#### IR spectra

A JASCO FT/IR-4200 spectrophotometer with an accessory ATR PRO450-S with optic diamond was used for the analysis. It was worked on attenuated total reflectometry (ATR) in the domain 400 - 4000  $cm^{-1}$ , at a resolution of 4  $cm^{-1}$ .

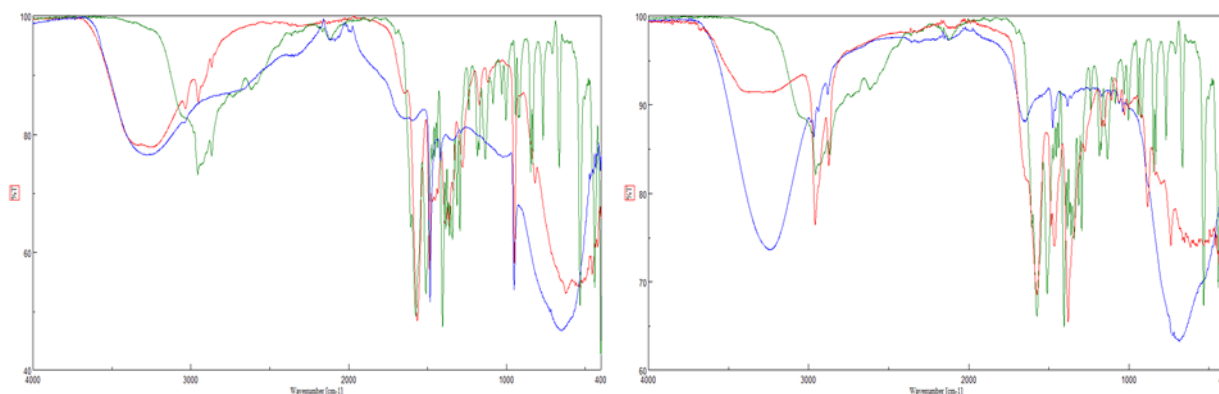
The spectra of amino acid, tetraalkylammonium hydroxides and ionic liquids were recorded. For every ionic liquid significant difference in the spectra can be observed than the spectra of the starting amino acid or of tetraalkylammonium hydroxide.

The analysis of all spectra revealed that the signals corresponding to the amino group cannot be attributed.

Because of the zwitterionic nature of amino acids the signal of  $-COOH$  group cannot be identified in the amino acid. The signals corresponding to  $-CH_3$  cannot be found in histidine and proline due to their chemical structure. The signals corresponding to  $\nu_{-CH_3}$  symmetric and  $\nu_{-CH_3}$  asymmetric can be observed around 2950 and 2870  $cm^{-1}$ . The peaks for  $\nu_{-CH_3}$  and  $\nu_{-CH_2}$  are overlapping in CILs; in AATBA the transmittances of the peaks corresponding to  $\nu_{-CH_3}$  are lower than in AATMA. The signal corresponding  $\nu_{C=O}$  from carboxylate group has a signal around 1560 and 1570  $cm^{-1}$ . The signals of the same group,  $\nu_{C=O}$  symmetric and  $\delta_{C=O}$  are overlapping with other peaks.

In the case of LeuTBA and LeuTMA the peak from 1404  $cm^{-1}$  corresponding to  $\nu_{C=O}$  from the  $-COOH$  is absent which means that the deprotonation occurred. The signal corresponding to  $\nu_{C=O}$  asymmetric from the carboxylate group can be found around 1570  $cm^{-1}$ . Although it cannot be attributed, the signal corresponding to the amino can be observed around 3200 - 3000  $cm^{-1}$ .

The intensity of the peak from 2950  $cm^{-1}$  corresponding to  $\nu_{-CH_3}$  asymmetric is higher in LeuTBA than in LeuTMA and the corresponding amino acid. Around the value of 2870  $cm^{-1}$  the vibration  $\nu_{-CH_3}$  symmetric can be found in each compound.

**Figure 2.**

IR spectra of LeuTMA-red, TMAOH-blue and Leu-green (left) and LeuTBA-red, TBAOH-blue and Leu-green (right)

#### CE analysis

Ondansetron ( $pK_a = 7.4$ ) and mianserin ( $pK_a = 8.3$ ) are basic drugs, fairly soluble in water in acidic solutions, ofloxacin ( $pK_{a1} = 5.97$ ,  $pK_{a2} = 9.28$ ) is also soluble. The racemates of ofloxacin and ondansetron are partially resolved or even fully separated (mianserin) using HP $\beta$ CD as chiral selector [19, 22].

To emphasize the effect of the AAILs on electrophoretic chiral separations (used as chiral selectors or as background electrolyte modifiers) when HP $\beta$ CD is used as chiral selector the selectivity and resolution changes against several experimental parameters as pH, background electrolyte, applied voltage, temperature, cyclodextrin and AAIL concentrations were studied.

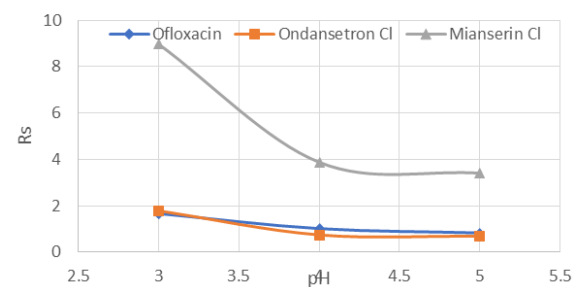
### Injection time

The samples were injected hydrodynamically; various injection times (1, 2 and 5 seconds) were tested and the injection time was set at 1 second as for larger injections, asymmetric peaks are obtained.

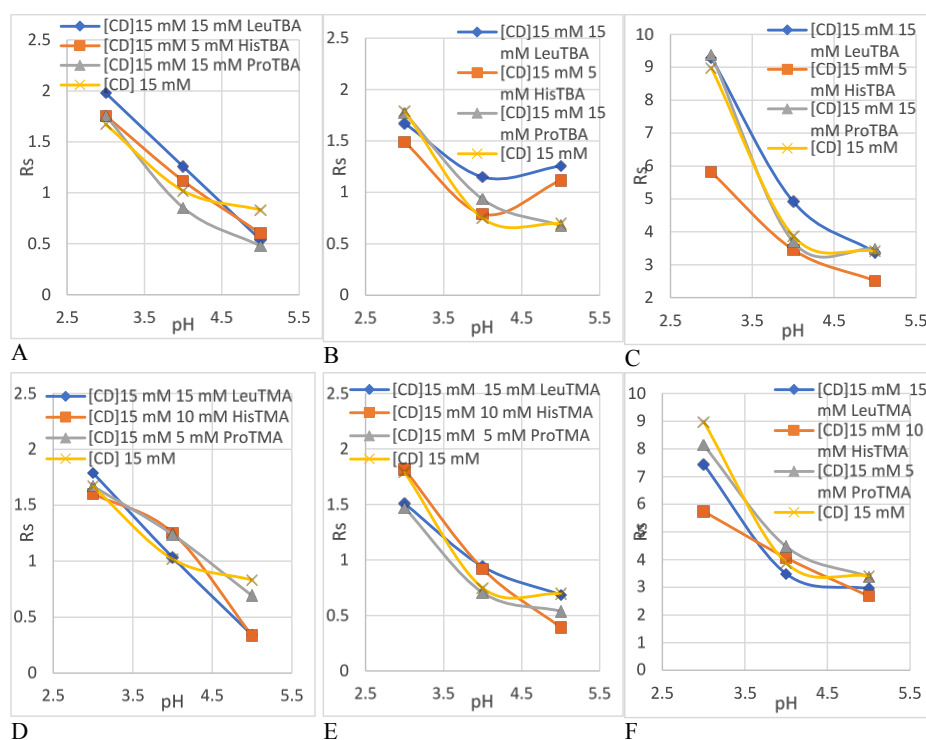
### pH

The experiments were made with background electrolyte with pH values from 3 to 5. The best resolution was obtained with HP $\beta$ CD as chiral selector at pH 3 (50 mM sodium citrate, as it can be seen in Figure 3). When both types of AAIL are added to the background electrolyte containing HP $\beta$ CD, it can be seen that the decreasing of the resolution with the increase of the background electrolyte pH is maintained (Figure 4), so we have selected pH 3 as working pH for further experiments. The addition of ionic liquids in the BGE containing cyclodextrins usually results in a decrease of effective mobilities of the enantiomers and EOF decreases with

the increase of the ionic liquid concentration (sometimes  $t_{EOF}$  is longer than 45 minutes). This decrease of the effective mobilities can probably be favourable (in the case of LeuTBA) for the separation.



**Figure 3.**  
pH influence on resolutions of separations, 15 mM HP $\beta$ CD, 20 kV



**Figure 4.**  
pH influence on enantioseparations of the cited racemic mixtures with tetraalkylammonium CIL - 15 mM HP $\beta$ CD for Ofloxacin (A & D), Ondansetron (B & E) and Mianserin (C & F)

### AAIL type and concentration

The AAIL concentration range in the background electrolyte was 5 - 15 mM. The effect of the AAIL added was studied against the separations obtained when using HP $\beta$ CD as sole chiral selector or with tetrabutylammonium hydroxide (TBAOH) added.

Several set of data were analysed, for a concentration of AAIL (5 mM to 15 mM) added to a fixed cyclodextrin concentration (5 mM, 10 mM and 15 mM), which means 9 experiments in the starting experimental conditions for each racemate drug.

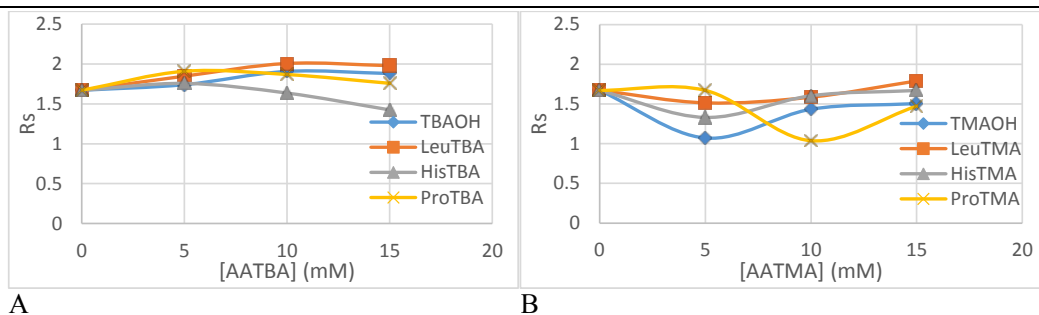


Figure 5.

Separation of enantiomers of ofloxacin with 15 mM HP $\beta$ CD-AAILs: (A) - AATBA, (B) - AATMA, 20 kV

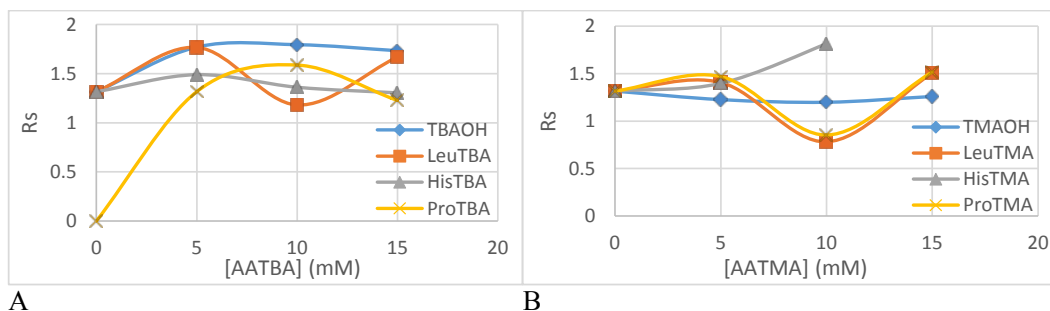


Figure 6.

Separation of enantiomers of ondansetron with 15 mM HP $\beta$ CD-AAILs: (A) - AATBA, (B) - AATMA, 20 kV

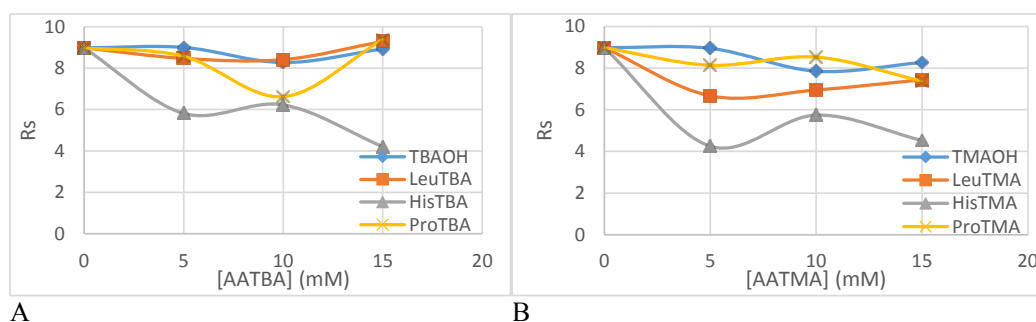


Figure 7.

Separation of enantiomers of mianserin with 15 mM HP $\beta$ CD-AAILs: (A) - AATBA, (B) - AATMA, 20 kV

Best separations (higher resolutions) for ofloxacin enantiomers were obtained for LeuTBA. When ProTBA and HisTBA are added to the background electrolyte, the resolutions are lower than those obtained with the TBAOH-HP $\beta$ CD dual selecting system (Figure 5) Also, as it can be seen, AATMA added to the background electrolyte gives lower discriminating power when compared to AATBA (Figure 5).

Also, the selectivity and the resolution variability, strongly depend on the chain length in the cation: for AATBA the resolution increases with the increasing AAIL concentration, for AATMA an opposite effect of the increasing IL concentrations can be noticed (Figure 5).

Ondansetron enantiomers can be resolved with a HP $\beta$ CD-TMAOH or HP $\beta$ CD-TBAOH dual selecting system, as well as with a background electrolyte containing HP $\beta$ CD and AATMA or AATBA. It is

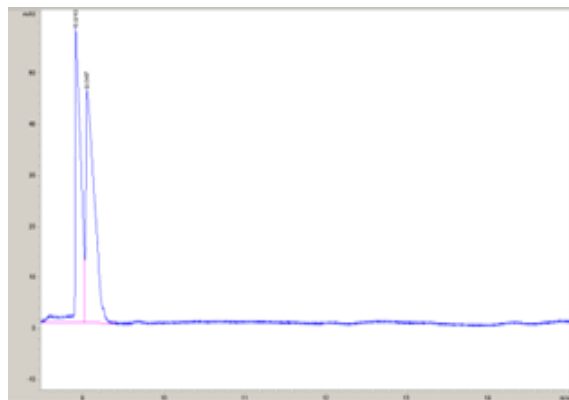
obvious that adding AATBA results in lower resolutions than with TBAOH. The Figure 6 shows also an inconstant effect for the separation systems containing AATBA IL.

The enantioselectivity of the system containing HP $\beta$ CD and AAIL decreases in the order TBAOH > LeuTBA > HisTBA > ProTBA.

When adding AATMA IL, the resolutions obtained are higher than those obtained with TMAOH. The effects of LeuTMA and ProTMA are almost the same. The system HP $\beta$ CD-HisTMA has a better enantiodiscriminating capacity than AATBA (Figure 6).

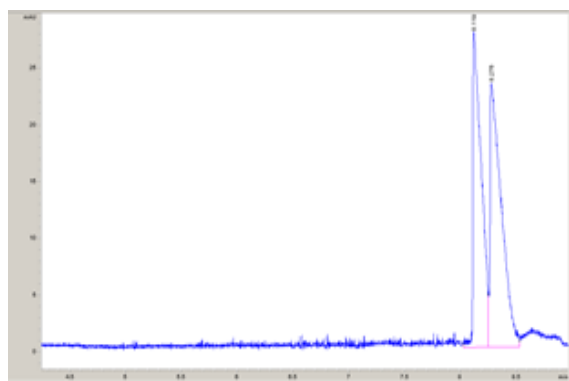
Mianserin enantiomers are fully resolved by cyclodextrin alone. The background electrolytes, such as, HP $\beta$ CD-TBAOH, HP $\beta$ CD-TMAOH, HP $\beta$ CD-AATMA and AATBA (tetraalkylammonium based AAIL) did not improve the separations with this experimental conditions; the resolutions decrease when CIL is

added (Figure 7). The enantioselectivity decreases in the order LeuTBA > ProTBA > TBAOH > HisTBA. When the concentration of tetraalkylammonium hydroxide increases, the resolutions' decrease is more pronounced for TMAOH.



**Figure 8.**

Electropherogram of ondansetron enantiomers separation; experimental conditions: 15 mM HP $\beta$ CD 15 mM LeuTBA, 20 kV, 25°C



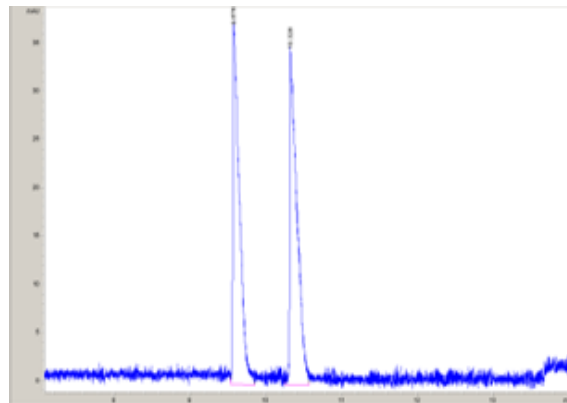
**Figure 9.**

Electropherogram of ofloxacin enantiomers separation; experimental conditions: 15 mM HP $\beta$ CD 15 mM LeuTBA, 20 kV, 25°C

The AATMA in the dual system leads to lower enantiodiscrimination capacity than TMAOH. The resolutions decrease in the order TMAOH > LeuTMA ~ ProTMA > HisTMA.

For a concentration of HP $\beta$ CD of 15 mM, at the addition of AAIL in the BGE at an optimum ratio between the selectors, the highest enantioresolution can be observed (Figures 8, 9 and 10). By increasing the concentration of IL above this upper limit the resolutions decrease. In general for almost

all the separations from the starting experimental conditions LeuTBA gives the best enantioselectivities. ProTMA gives better enantioselectivities at lower concentrations of 5 mM and LeuTMA at 15 mM. The histidine derivatives give the lowest resolutions.



**Figure 10.**

Electropherogram of mianserin enantiomers separation; experimental conditions: 15 mM HP $\beta$ CD 15 mM LeuTBA, 20 kV, 25°C

#### *Voltage influence*

The concentrations of selectors and the temperature of 25°C were the fixed experimental conditions. The concentration of HP $\beta$ CD was set at 15 mM for all the experiments. By decreasing the voltage from 20 kV to 10 kV, the time of interaction is higher and the resolutions' values increase in the majority of cases. In general, if the second chiral selector is added in the BGE, a lower voltage value can favour the enantiodiscrimination process. If the concentration range has been reached, a further decrease of the applied voltage would not increase the resolutions furthermore.

LeuTBA and HP $\beta$ CD in an equimolecular ratio of 15 mM give the highest enantioselectivities for ofloxacin at 15 kV and for ondansetron and mianserin at 10 kV. This dual system can be optimal for improved enantioselectivities.

The length of the chain of the AAIL is important; tetramethylammonium derivatives have lower enantiodiscrimination capacity than tetrabutylammonium derivatives with each of the applied voltage value. (Table IV, V, VI)

**Table IV**

Applied voltage influence on the separation of enantiomers of ofloxacin with 15 mM HP $\beta$ CD-AAILs

[HP $\beta$ CD] 15 mM	LeuTMA	LeuTBA	HisTMA	HisTBA	ProTMA	ProTBA	TMAOH	TBAOH
U (kV)	15 mM	15 mM	10 mM	5 mM	5 mM	10 mM	15 mM	15 mM
10	2.130	2.080	2.186	2.332	2.109	2.391	1.840	2.069
15	1.722	2.451	1.650	1.833	1.856	2.234	1.662	2.304
20	1.789	1.978	1.603	1.756	1.670	1.867	1.504	1.9063

**Table V**Applied voltage influence on the separation of enantiomers of ondansetron with 15 mM HP $\beta$ CD-AAILs

[HP $\beta$ CD] 15 mM	LeuTMA	LeuTBA	HisTMA	HisTBA	ProTMA	ProTBA	TMAOH	TBAOH
U (kV)	15 mM	15 mM	10 mM	5 mM	10 mM	15 mM	15 mM	15 mM
10	1.853	2.080	2.004	2.053	2.077	1.688	1.497	1.728
15	1.468	2.052	1.209	1.792	1.816	1.947	1.552	1.871
20	1.580	1.669	1.815	1.489	0.853	1.772	1.259	1.733

**Table VI**Applied voltage influence on the separation of enantiomers of mianserin with 15 mM HP $\beta$ CD-AAILs

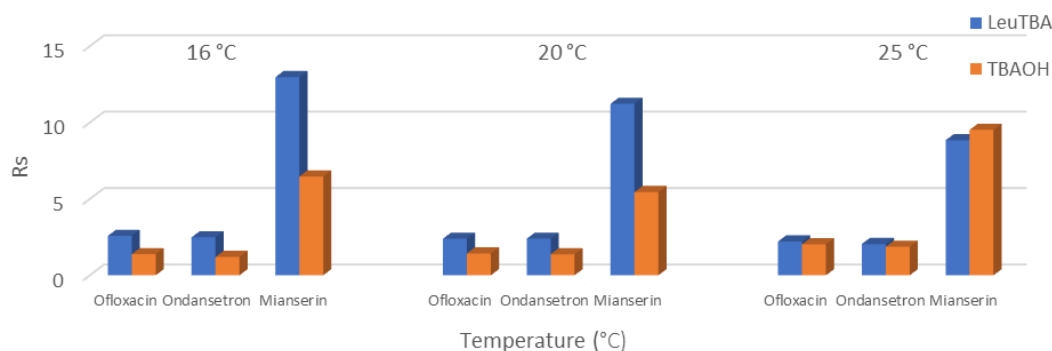
[HP $\beta$ CD] 15 mM	LeuTMA	LeuTBA	HisTMA	HisTBA	ProTMA	ProTBA	TMAOH	TBAOH
U (kV)	15 mM	15 mM	15 mM	15 mM	15 mM	15 mM	15 mM	15 mM
10	8.823	11.42	6.400	6.933	6.368	9.224	9.525	9.280
15	8.614	9.053	5.386	10	6.576	9.403	10.187	9.521
20	7.436	8.469	4.527	4.204	9.376	9.376	8.948	8.925

### Temperature

When the temperature of analysis is decreased the resulting increase of the viscosity can cause a change in the resulting enantioselectivities. The voltage of 15 kV, 15 mM HP $\beta$ CD and a working temperature of 25°C were selected as starting conditions. The temperature was decreased at 20°C and then at 16°C. Figure 11 shows the temperature effect on enantioseparation for a BGE containing HP $\beta$ CD 15 mM and LeuTBA 15 mM.

Decreasing the temperature of the analysis can favour a slightly increase in enantioselectivities in the case of ofloxacin and ondansetron. An equimolecular mixture of 15 mM of both selectors can improve the resolutions of the separation of the mianserin racemate at a low temperature.

For the other dual systems, the resolution change with the decrease of temperature was not so obvious.

**Figure 11.**

Temperature influence on the separation of enantiomers of ofloxacin, ondansetron and mianserin with 15 mM HP $\beta$ CD - 15 mM LeuTBA/TBAOH (15 kV)

### Conclusions

Amino acid ionic liquids are a class of chiral ionic liquids that can be successfully used as chiral selectors in dual system with different types of native or derivatives of cyclodextrins for separations of several racemic drugs in electromigration techniques. We chose the tetraalkylammonium derivatives of amino acids being relatively cheap and having a relative simple synthesis.

These derivatives have different enantiodiscrimination abilities when used as second chiral selectors in the system HP $\beta$ CD-AAILs by comparison with HP $\beta$ CD-tetraalkylammonium hydroxides in citrate acidic buffer. The tetrabutylammonium derivatives give higher enantioselectivities than the tetramethyl-

ammonium derivatives. The enantioselectivity increases with decreasing pH, the value of 3 being the optimum. Equimolecular mixtures 15 mM HP $\beta$ CD: 15 mM LeuTBA give the best enantioselectivities especially at a low voltage value, 15 kV for ondansetron and ofloxacin. This ratio can be favourable for the separation of the racemic mixture of mianserin for 16°C at 15 kV. In most cases CILs decrease the enantioselectivities of the racemic mianserin, but to some extent the decreasing voltage and/or temperature are favourable for an increase of the enantioselectivities for all the analytes tested. By increasing the concentration of ionic liquid for a fixed concentration of cyclodextrin, the effective mobilities decrease, this process can favour the separations when leucine derivative is used.



The experimental data support the hypothesis of a synergic effect of both CILs and CD used as chiral selectors in mutual mixtures.

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