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Use of Amino Acid-Based Ionic Liquids in Capillary Electrophoresis

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1. Introduction

Ionic liquids (ILs) are unique solvents with melting points at or below 100 °C. They have drawn scientific interest due to their unique properties that involve good thermal stability, miscibility in different solvents, tunable viscosity, conductivity, negligible vapor pressure, non-flammability, and low toxicity. They have often been called *designer solvents* in order to indicate their large structural variability due to the cation or the anion of the salt. This, in turn, broadens their application area (chemical reactions, catalysis, electrochemistry, separation, etc.) [1-5].

In the last few years, a big number of chiral ILs (CILs), have been designed, synthesized and used for applications in electrophoretic and chromatographic chiral discrimination [4, 6]. They play a key role in enantioselective analysis because they combine the advantages of ILs with the properties of a chiral moiety, which can be anionic and/or cationic. Their utility in separation science as chiral selectors, additives, chiral ligands, and chiral stationary phases is becoming increasingly important [7].

Although a large number of reviews have been provided on the synthesis of CILs [8-10], a surprisingly limited number of articles have been published on their utility in analytical separations, and particularly in electrophoretic separations [7, 11]. Capillary electrophoresis (CE) has been extensively used in chiral separations by using various chiral selectors, such as cyclodextrins (CDs), cyclofructans, oligo- and polysaccharides, polymeric surfactants, and others [12]. Some of the problems that limit their use involve low solubility, instability at high temperatures and/or low pH values, time-consuming organic synthesis procedures and high cost. The use of CILs is considered a potential alternative because they can dissolve various polar and nonpolar analytes, they may provide chiral selectivity, and their synthesis procedure is simple. In CE, the CILs are mainly used as BGE additives, and secondarily as chiral ligands and chiral selectors.

A new class of CILs, called amino acid ester-based ILs (AAILs), was synthesized and characterized in 2005 by Tao *et al.* [13]. AAILs consist of cations, which are derived from amino acids or amino acid esters, such as glycine (Gly), alanine (Ala), alanine methyl ester (AlaC₁), and alanine ethyl ester (AlaC₂), and commonly used anions, such as nitrate (NO₃), tetrafluoroborate (BF₄), lactate (Lac) and bis(trifluoromethane)sulfonamide (NTf₂). Bwambok *et al.* [14] used a simple metathesis reaction to synthesize L- and D-alanine *tert* butyl ester-based ILs with several anions, and they studied their enantiomeric recognition properties by using fluorescence and nuclear magnetic resonance spectroscopy.

In this chapter, the ability of AAILs to be used as chiral and achiral media in CE is investigated. In particular, some representative studies that involve the utility of AAILs as background electrolyte (BGE) additives and as sole chiral selectors in electrophoretic separations are reported and discussed. These studies involve synthesis procedure, establishment of optimum separation conditions and method validation. The first part of this chapter involves the application of AAILs as BGE additives and the evaluation of their performance in both chiral and achiral analysis, while the second part demonstrates their chiral recognition ability for the enantioseparation of 1,1'-binaphthyl-2,2-dihydrogenphosphate (BNP).

2. AAILs as background electrolyte additives

Most of the applications of AAILs (Figure 1) in electrophoretic separations have been as additives in BGEs [15-23]. Hadjistasi *et al.* [15] were the first to report the use of an AAIL as an additive in the BGE to improve electrophoretic separations. In particular, the addition of the CIL D-alanine *tert*-butyl ester lactate (D-AlaC₄Lac) into the BGE improved the resolution of the pipecolic acid enantiomers. This CIL provided an increase in resolution from 1.41, obtained when β -cyclodextrin (β -CD) was used as the sole chiral selector, to 1.87, obtained when both chiral selectors were added in the BGE. In 2013, two novel AAILs, tetramethylammonium-L-arginine (TMA-L-Arg) and tetramethylammonium-L-aspartic acid (TMA-L-Asp), were applied, for the first time, in CE, with glycogen as the chiral selector, in order to evaluate their potential synergistic effect [16]. Glycogen is an electrically neutral and branched polysaccharide that has, over the years, proven its chiral recognition abilities for the enantioseparation of various basic and acidic drug compounds. In their study, it was observed that, when the TMA-L-Arg/glycogen and TMA-L-Asp/glycogen systems were applied, both resolution and selectivity were significantly improved in comparison to the single glycogen separation system. This, in turn, suggested the existence of the synergistic effect. In a more recent study, the vancomycin-based synergistic system with L-alanine and L-valine *tert* butyl ester bis(trifluoromethane)sulfonamide (L-AlaC₄NTf₂ and L-ValC₄NTf₂) as additives was evaluated in CE for the enantioseparation of five profens [18]. When the binary systems were applied, all enantioseparations were considerably improved, and the resolution values were greatly higher than in the case where vancomycin was used as the sole chiral selector. In this part of the chapter, two representative studies performed recently in chiral and achiral CE are briefly described [17, 22].

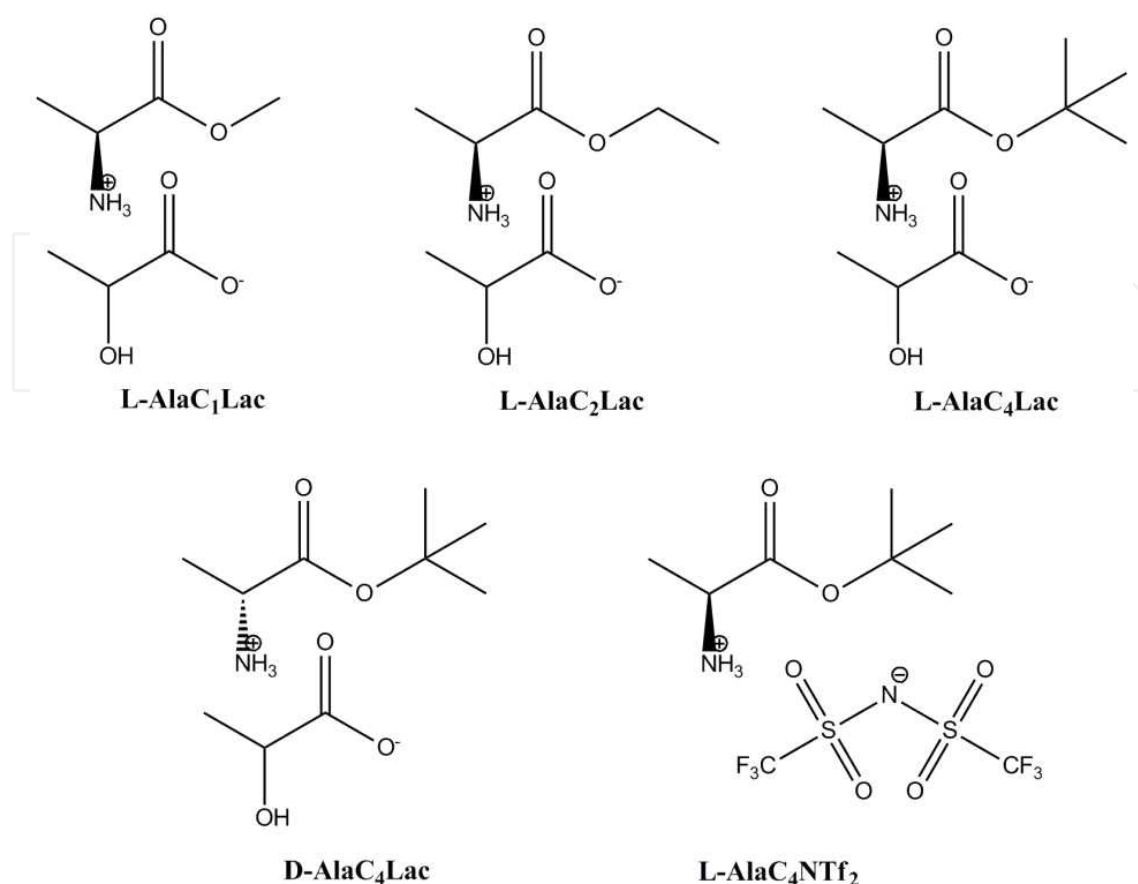


Figure 1. Structures of the AAILs L-AlaC₁Lac, L-AlaC₂Lac, L-and D-AlaC₄Lac, and L-AlaC₄NTf₂ [27].

2.1. Chiral analysis

The use of AAILs as BGE additives, for improved resolutions, selectivity factors, and efficiencies in chiral analysis, is demonstrated further here by providing a more in-depth analysis of a research work that was performed by Zhang *et al.* [17]. In their study, two AAILs (L-AlaC₄NTf₂ and L-ValC₄NTf₂) were applied as additives and β -CD derivatives (methyl- β -CD, hydropropyl- β -CD, glucose- β -CD) as chiral selectors in CE for the enantioseparation of six anionic racemic drug compounds.

The synthesis of both AAILs was accomplished by use of a one-step anion exchange reaction of the corresponding amino acid ester chloride and the bis(trifluoromethane)sulfonimide lithium salt [14]. Briefly, an appropriate amount of L-alanine and L-valine *tert* butyl ester hydrochloride and an equimolar amount of bis(trifluoromethane)sulfonimide lithium salt were separately dissolved in distilled water. Then, the two solutions were mixed and stirred for 2 h at room temperature. The mixture resulted in two layers, of which the lower layer was separated and dried under vacuum overnight. The resulted products were colorless liquids.

The main objective of their study was to evaluate the synergistic effect of the AAILs with the β -CD derivatives. It was proven to be significant for half of the analytes examined, and

particularly for naproxen, pranoprofen and warfarin. Figure 2 demonstrates the electropherograms obtained when β -CD derivatives were used as the sole chiral selectors [(a)], and when AAILs were used as additives [(b) and (c)].

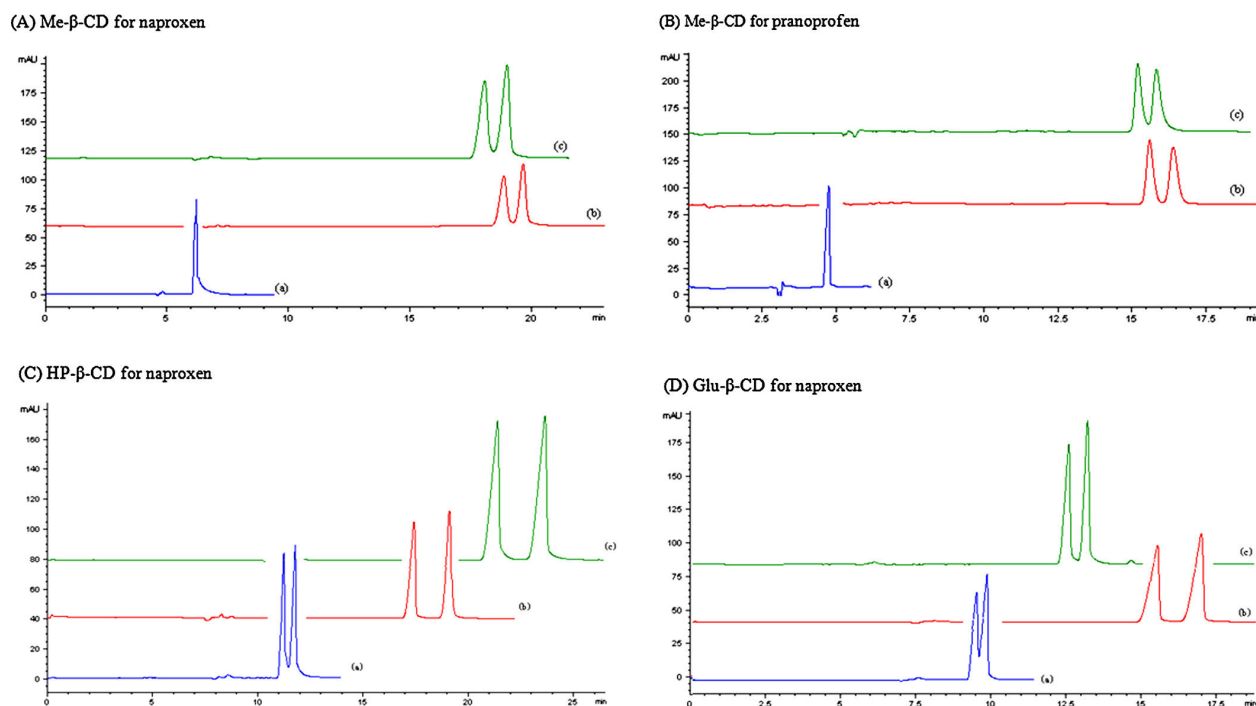


Figure 2. Electropherograms with the absence and presence of AAILs for enantioseparation with (A) methyl- β -CD for naproxen, (B) methyl- β -CD for pranoprofen, (C) hydropropyl- β -CD for naproxen and (D) glucose- β -CD for naproxen. Conditions: fused-silica capillary, 33 cm (24.5 cm effective length) \times 50 μ m id; 30 mM sodium citrate/citric acid buffer solution with 20% (v/v) ethanol for naproxen or 20% (v/v) acetonitrile for pranoprofen, containing (A) and (B) (a) methyl- β -CD; (b) 20 mM methyl- β -CD+15 mM L-AlaC₄NTf₂; (c) 20 mM methyl- β -CD+15 mM L-ValC₄NTf₂; (C) (a) 30 mM hydropropyl- β -CD; (b) 30 mM hydropropyl- β -CD+15 mM L-AlaC₄NTf₂; (c) 30 mM hydropropyl- β -CD+15 mM L-ValC₄NTf₂; (D) (a) 30 mM glucose- β -CD; (b) 30 mM glucose- β -CD+15 mM L-AlaC₄NTf₂; (c) 30 mM glucose- β -CD+15 mM L-ValC₄NTf₂; pH 5.0; applied voltage, 20 kV; capillary temperature, 25°C [17].

The novel synergistic system was optimized by using methyl- β -CD/AAILs as model systems. An important factor affecting the enantioseparation is the concentration of the chiral selector and the CIL, since both concentrations will determine the equilibria between the chiral selector, the CIL and the enantiomers. Initially, at a fixed concentration of 20 mM methyl- β -CD, as the concentrations of L-AlaC₄NTf₂ and L-ValC₄NTf₂ increased from 5 to 15 mM both resolution and effective selectivity factor improved. However, at the concentration of 20 mM, no peak was observed, even at 60 min. The electrophoretic mobility decreased dramatically, possibly due to the adsorption of the CIL cations onto the capillary walls.

The effect of the chiral selector's concentration on enantioseparation was also studied by varying the concentration of methyl- β -CD from 10 to 50 mM. In the single methyl- β -CD separation system, resolution values increased upon increasing the concentration, due to an increase in the complexation between the chiral selector and the enantiomers. On the other hand, in the methyl- β -CD/AAILs systems, resolution was initially increased, and then

decreased mainly due to the gradual complex saturation. Therefore, the optimum concentration of methyl- β -CD is lower with the presence of the synergistic effect in comparison to the single methyl- β -CD system.

The optimum BGE composition was also determined by examining two different systems (sodium sodium acetate-acetic acid and citrate-citric acid). The first BGE provided deformed peaks, while the use of the second one resulted in better peak shapes and better resolutions. The BGE pH was also investigated for the chiral recognition process, because the degree of protonation in the analytes and the AAILs depends on this parameter. It was observed that at pH values below 4.4 or above 5.6, resolutions of all the understudy chiral compounds were reduced. Therefore, a pH value of 5.0 was considered the optimum. According to the authors, at the particular optimum pH, in the present system of analyte/AAIL/methyl- β -CD, the following bindings occur: (a) hydrogen bonding among the hydroxyl function in methyl- β -CD, carboxyl group in the drug compounds and amino function in the AAILs and (b) ionic interactions between the carboxyl group in the analytes and the amino function in the AAILs.

Another important observation, in this study, was the improvement of both resolution and effective selectivity factor with the addition of an organic modifier, possibly due to a decrease in electroosmotic mobility, which, in turn, increases the interactions between the AAIL, methyl- β -CD and analyte. Other parameters, such as composition and pH of buffer system and applied voltage were also examined, and the optimum conditions included 15 mM AAIL, 20 mM methyl- β -CD, 30 mM sodium citrate/citric acid (pH 5), and 20 kV.

2.2. Achiral analysis

In a different study, the synergistic effect of sodium dodecyl sulfate (SDS) and L-alanine *tert* butyl ester lactate (L-AlaC₄Lac) was examined by Mavroudi *et al.* [22] for the separation of 2-arylpropionic acid non-steroidal anti-inflammatory drugs (Figure 3). The BGE was supported with either SDS, or an AAIL or SDS/AAIL, and their performance was evaluated by comparing migration times, efficiencies and %RSD values. Many analytical CE methods have, so far, been used for the separation of a wide variety of NSAIDs, by applying different modes of CE. These include micellar electrokinetic chromatography (MEKC) with SDS as an additive, capillary electrochromatography (CEC) with poly(stearyl methacrylate-divinyl benzene) monolithic columns and non-aqueous CE [24-26]. In the study reported by Maria *et al.*, the AAIL L-AlaC₄Lac was applied in CE as a sole additive for the simultaneous separation of NSAIDs for the first time. A very important consideration in this study was whether the use of an AAIL as a sole additive resulted in more effective separations than in the case of using a common surfactant. Therefore, a comparison was performed by adding L-AlaC₄Lac or SDS into the BGE (100 mM Tris/10 mM tetraborate decahydrate).

For the synthesis of L-AlaC₄Lac, appropriate amounts of the corresponding amino acid ester hydrochloride and silver lactate were separately mixed with methanol. The amino acid ester solution and the suspended silver lactate were then mixed and stirred. Subsequently, the precipitate was filtered and removed, and the remained solution was evaporated *in vacuo* and purified by being crystallized in methanol/ether [14].

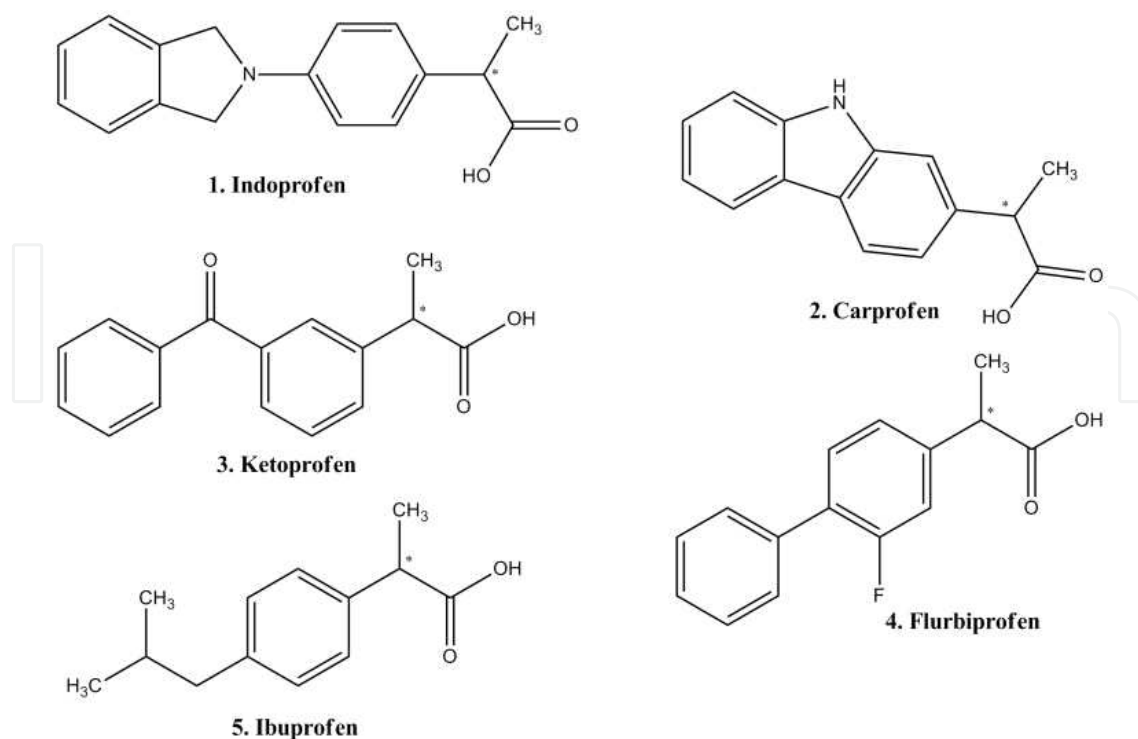


Figure 3. Structures of the five NSAIDs used in this study.

In the single SDS system, separation is achieved by differential partitioning of analytes between the hydrophilic core of the surfactant and the bulk aqueous phase via electrostatic and hydrophobic interactions, and hydrogen bonding. SDS was initially examined as a sole additive, by varying its concentration from 10 to 30 mM. It was observed from Figure 4 that, even though the migration times of all analytes increased along with the increase of SDS concentration, resolution values decreased dramatically. At 30 mM, a coelution of indoprofen and ketoprofen was observed, and carprofen did not elute, even at 30 min. This is because the predominant population, at pH 8, is in the anionic form, which is expected to be repelled by the negatively charged headgroup of the anionic surfactant.

For comparison purposes and for further optimization of the separation, an AAIL was used as an additive. The effect of its concentration on the separation of NSAIDs was first examined. The optimum concentration was determined according to resolution, efficiency and analysis time. As demonstrated in Figure 5, when a 20-mM concentration was used, the R_s values for the peak pairs carprofen-ketoprofen and flurbiprofen-ibuprofen were 1.3 and 1.2, respectively. Concentrations above 30 mM provided baseline separations with R_s values higher than 1.5. In addition, the total analysis time, in the cases of 20, 30 and 40 mM L-AlaC₄Lac, was not altered (~ 9.5 min), while, from 50 to 70 mM, it was increased to ~ 12 min. Efficiency was determined by calculating the number of theoretical plates (N) for all peaks. It was observed that at a concentration of 40 mM, N for all peaks was very high, in comparison to the ones obtained when the other concentrations were applied (Figure 6). Another important observation involved the elution order of NSAIDs, which was different from the elution order observed when the SDS was added in the BGE, probably due to the different types of interactions

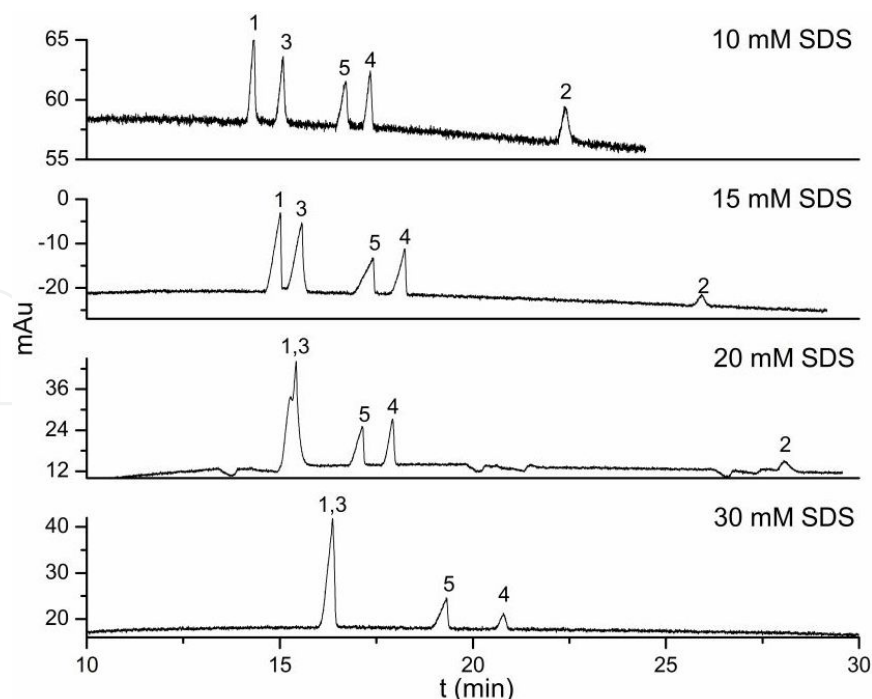


Figure 4. Effect of SDS concentration on the simultaneous separation of NSAIDs. Conditions: 100 mM Tris/10 mM tetraborate decahydrate pH 8, applied voltage 30 kV, temperature 20 °C, detection wavelength 200 nm [22].

between the additive and the analytes. In the case of SDS, the interactions are based on hydrophobicity, while in the case of the additive L-AlaC₄Lac, the separation is based on electrostatic interactions.

pH is another important parameter that is necessary to be optimized because, firstly, alterations in pH can affect the analyte charge and, secondly, the primary amine group of the cation of the AAIL can be positively charged or neutral in several pHs. An increase in pH from 8.0 to 8.5, the resolution decreased from 2.9 to 1.5 for peaks carprofen-ketoprofen and from 1.7 to 1.3 for peaks flurbiprofen-ibuprofen. A further increase in pH (9.0 and 9.5) resulted in two coelutions and shorter migration times due to fewer electrostatic interactions between the AAIL and the negatively charged analyte, since the amount of the positively charged amino group is decreased. In addition, at high pH values, the AAIL may undergo ester hydrolysis, which results in the lack of the *tert* butyl group in the cation.

The reproducibilities were also evaluated and compared by calculating the relative standard deviation (RSD) values of the electroosmotic flow (EOF) and the migration times of all the analyte peaks. In particular, in both SDS and L-AlaC₄Lac cases, the run-to-run RSD values were obtained from 10 consecutive electrophoresis runs. In the case of SDS, the RSD of the EOF was 2.1%, and the RSD values of the analytes ranged from 2.8% to 11.7%. In the case of L-AlaC₄Lac, the RSD of the EOF was 0.4% and the RSDs of the NSAIDs ranged from 1.2% to 1.3% (Table 1). In the same table, a comparison between the two additives in regard to efficiency is also demonstrated. The efficiency of all analyte peaks was above 102,000 for L-AlaC₄Lac, in comparison to SDS, which provided efficiency values between 47,000 and 76,000 theoretical plates.

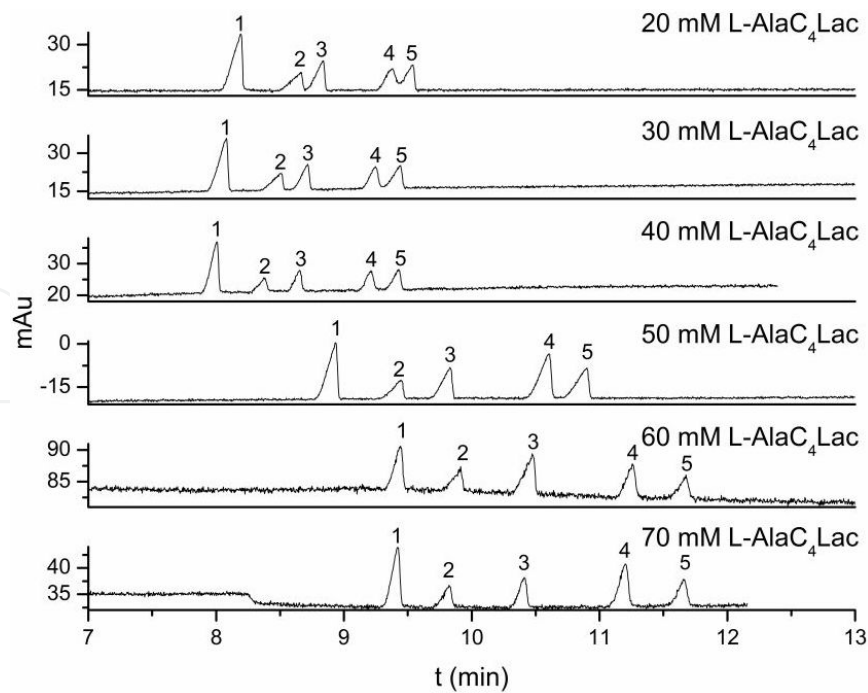


Figure 5. Effect of L-AlaC₄Lac concentration on the simultaneous separation of NSAIDs. Conditions: 100 mM Tris/10 mM tetraborate decahydrate pH 8, applied voltage 30 kV, temperature 35 °C, detection wavelength 200 nm [22].

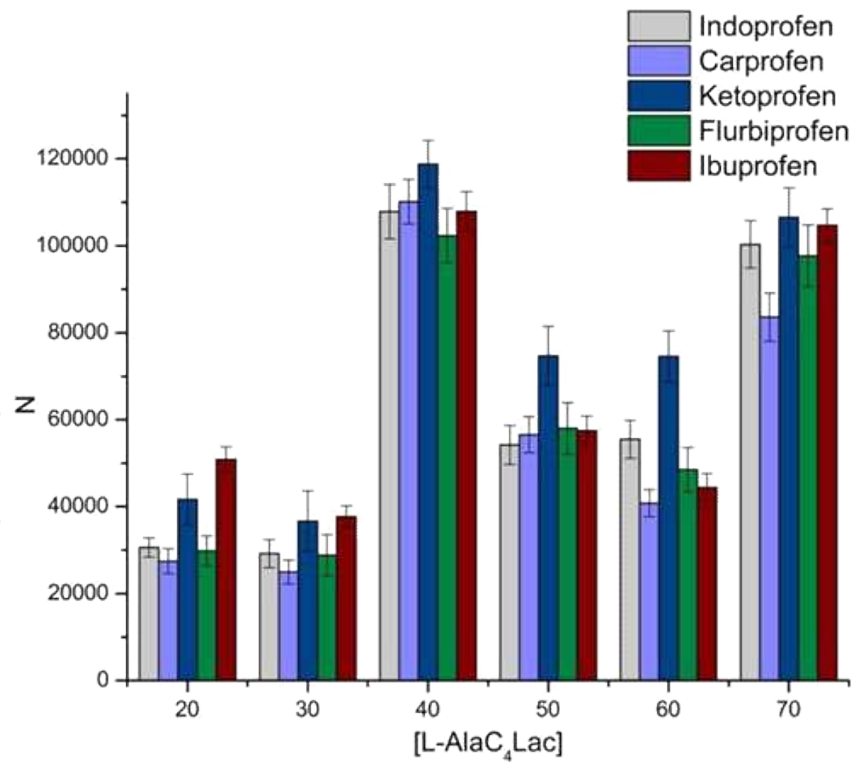


Figure 6. Effect of L-AlaC₄Lac concentration on *N*. Conditions: 100 mM Tris/10 mM tetraborate decahydrate pH 8, applied voltage 30 kV, temperature 35 °C, detection wavelength 200 nm [22].

		10 mM SDS			40 mM L-AlaC ₄ Lac		
Peak	Analyte	t (min)	RSD %	N	t (min)	RSD %	N
EOF	—	5.50	2.1	—	3.69	0.4	—
1	Indoprofen	14.33	3.0	64,722	8.03	1.2	107,863
2	Carprofen	22.39	11.7	51,868	8.48	1.2	110,156
3	Ketoprofen	15.09	2.8	60,805	8.78	1.3	118,785
4	Flurbiprofen	17.34	4.1	75,824	9.43	1.2	102,357
5	Ibuprofen	16.71	3.6	46,968	9.65	1.3	107,943

Table 1. Run-to-run reproducibility and efficiency of SDS and L-AlaC₄Lac for the simultaneous separation of NSAIDs. Conditions: 100 mM Tris/10 mM tetraborate decahydrate pH 8, applied voltage 30 kV, temperature 20 °C (for SDS) and 35 °C (for L-AlaC₄Lac), detection wavelength 200 nm [22].

A last consideration for this study involved the effect of the addition of both SDS and L-AlaC₄Lac into the BGE on the separation of NSAIDs. A concentration of 10 mM SDS and different concentrations of L-AlaC₄Lac were added into the BGE (1-40 mM). An increase in the concentration of L-AlaC₄Lac resulted in a more effective separation of NSAIDs, in regard to efficiency and resolution, probably due to the synergistic effect of the SDS/L-AlaC₄Lac system. However, the elution order and analysis time (~ 23 min) were similar to the ones obtained when the SDS was used as the sole additive. Therefore, it was clear from this study that the additive L-AlaC₄Lac is considered an effective alternative to SDS for a reproducible, baseline, high efficient and fast separation of NSAIDs.

3. AAILs as chiral selectors

Although numerous studies reported the use of AAILs in electrophoretic enantiomeric separation, only one study was performed by using AAILs both as co-electrolytes and chiral selectors [27]. However, other CILs, that are not amino-acid based, have been added into the BGE and used as sole chiral selectors for the enantioseparation of a number of analytes [28-31]. Yuan *et al.* [28] used the IL (R)-N,N,N-trimethyl-2-aminobutanol-bis(trifluoromethanesulfon)imide as the sole chiral selector in CE, GC and HPLC for the separation of fifteen enantiomeric compounds. It was used as an additive in a BGE of 20 mM Na₂HPO₄-NaH₂PO₄ or 20 mM Na₂B₄O₇. The resolution values varied from 0.60 for di-O,O' p-toluytl-tartaric acid to 6.80 for 3-benzyloxy-1,2-propane diol.

In another study by Tran and Mejac [29], the CIL S-[3-(chloro-2-hydroxypropyl)trimethylammonium] [bis((trifluoromethyl)sulfonyl)amide] (S-[CHTA]⁺[Tf₂N]⁻) was utilized as a sole

chiral selector for the enantioseparation of a number of pharmaceutical compounds. Even though S -[CHTA] $^+[\text{TF}_2\text{N}]^-$ can serve as a chiral selector, it was unable to be used as a sole chiral selector since no enantioseparation could be achieved. Therefore, more BGE chiral additives were used, such as the chiral anion sodium cholate and the neutral chiral 1- S -octyl- β -D-thioglucopyranoside (OTG).

Ma *et al.* [30] explored the potential of using an ephedrine-based CIL, (+)- N,N -dimethylephedrinium-bis(trifluoromethanesulfon)imide ($[\text{DMP}]^+[\text{TF}_2\text{N}]^-$) as both a chiral selector and a BGE in nonaqueous CE. The addition of $[\text{DMP}]^+[\text{TF}_2\text{N}]^-$ resulted in a reversed EOF (anodic flow); so, the experiments were performed in the reversed polarity mode by using acetone as the EOF marker. The enantioseparations of rabeprazole and omeprazole were achieved mainly due to the different ion-pair formation equilibrium constants between the ephedrine-based CIL cations and the negatively charged enantiomers, and the hydrogen bonding, and secondarily due to other interactions, such as π - π interactions and dipole-dipole interactions.

In 2013, Yu *et al.* [31] synthesized a novel CIL functionalized β -CD (6- O -2-hydroxypropyltrimethylammonium- β -CD tetrafluoroborate, $[\text{HPTMA-}\beta\text{-CD}][\text{BF}_4]$) and applied it as a chiral selector in CE for the enantioseparation of eight chiral drug compounds. The separation conditions were optimized by studying the effect of the CIL concentration and the BGE pH. The results obtained demonstrated the excellent chiral discriminating ability of $[\text{HPTMA-}\beta\text{-CD}][\text{BF}_4]$.

As mentioned earlier, the use of AAILs as sole chiral selectors has only been reported once by Stavrou *et al.* [27]. Their applications are demonstrated further here by providing a more in-depth and informative CE chiral analysis of BNP. In their study, five AAILs [L-alanine methyl ester lactate, L-alanine ethyl ester lactate, L- and D-alanine *tert* butyl ester lactate (L-AlaC₁Lac, L-AlaC₂Lac and L- and D-AlaC₄Lac), and L-AlaC₄NTf₂] (Figure 1) were synthesized and used as additives in the BGE in order to evaluate their chiral recognition ability by comparing the resolution values.

The optimum separation conditions were established by altering different important parameters, such as the alkyl ester group, the anion, the configuration and the concentration of the AAIL. In their first study, the influence of steric hindrance on the enantiomeric separation of BNP was examined by applying separately as sole chiral selectors the AAILs L-AlaC₁Lac, L-AlaC₂Lac and L-AlaC₄Lac at concentrations of 60 mM and 100 mM. It was observed that as the length and the bulkiness of the ester group increased, the resolution of BNP increased. In particular, the first AAIL did not demonstrate any enantioselectivity, while the second one was able to provide partial enantioseparation (R_s : 1.09). However, when L-AlaC₄Lac was used at both concentrations, a baseline separation was achieved with R_s values of 1.94 and 2.43 (Table 2). It is, therefore, concluded that the enantioseparation of BNP is favored in the presence of *tert*-butyl group, and steric hindrance is involved in the enantioseparation mechanism.

Two very important considerations in this study involved the effect of the anion and the configuration of the cation on resolution. Two different anions were used (Lac and NTf₂), which provided baseline separation (Table 2). In particular, the resolution obtained by use of L-AlaC₄NTf₂ was slightly lower (R_s =1.72) than the one obtained with L-AlaC₄Lac (R_s =1.94),

possibly due to the low solubility of the first in water, which provides fewer free cations and less interaction with the analyte molecules. In addition, the results obtained with NTf₂ were not reproducible and provided an unstable baseline. As far as the cation configuration is concerned, the D- and L-AlaC₄Lac were used at a concentration of 60 mM in order to compare their enantioseparation ability. It was observed that the R_s values obtained were very similar (1.95 and 1.94), while the elution order was different. As expected, the chiral center of the cation is considered the main active center of the chiral selector. Therefore, the elution order of the enantiomers of the analyte is based on the configuration of the CIL.

CIL	*[CIL]	t _{EOF}	t ₁	t ₂	R _s
L-AlaC ₂ Lac	60 mM	5.755	11.471	11.511	-
	100 mM	6.272	13.929	14.065	1.09
L-AlaC ₄ Lac	60 mM	6.207	12.702	12.922	1.94
	100 mM	6.650	13.064	13.377	2.43
D-AlaC ₄ Lac	60 mM	6.172	12.499	12.702	1.95
L-AlaC ₄ NTf ₂	60 mM	6.495	14.622	14.865	1.72

Table 2. Effect of the cation, the anion and the configuration of the CIL on R_s. Conditions: BGE, 100 mM Tris/10 mM Borate pH=8; applied voltage, 30 kV; capillary temperature, 25 °C; detection wavelength, 214 nm.

In another study for the optimization of the BNP enantioseparation, the effect of the concentration of L-AlaC₄Lac on resolution was investigated. As demonstrated in Figure 7, resolution increased significantly from ~ 0.4 to 2.43 with increasing the concentration from 20 to 100 mM. It was also observed that an increase in the AAIL concentration resulted in a decrease in the electroosmotic flow mobility, probably due to the coating of the AAIL cations onto the capillary wall Figure 8. The electrophoretic mobility, μ_{EOF} , was calculated according to:

$$\mu_{EOF} = \frac{lL}{t_{nm}V}$$

where L is the total capillary length, l is the effective capillary length (from the injection end to the detector), t_{nm} is the migration time of the neutral marker and V is the applied voltage.

The last parameter examined was the BGE pH, which was important, since the cation of the CILs used seems to be pH depended. Resolution decreased from 1.94 to 1.29, upon increasing the pH from 8 to 8.5, while at higher pHs (9 and 10), no enantioseparation was observed. As mentioned in Section 2.2, an increase in the pH decreases the amount of the positively charged amino group, which consequently reduces the electrostatic interactions between the AAIL and the negatively charged analyte. In addition, at high pH values, and particularly pH 9 and 10, the AAIL may undergo ester hydrolysis. This results in the lack of the *tert* butyl group in the cation, which is an important factor for the particular enantioseparation. Finally, according to the authors, it is concluded from the above-mentioned studies that the enantioseparation

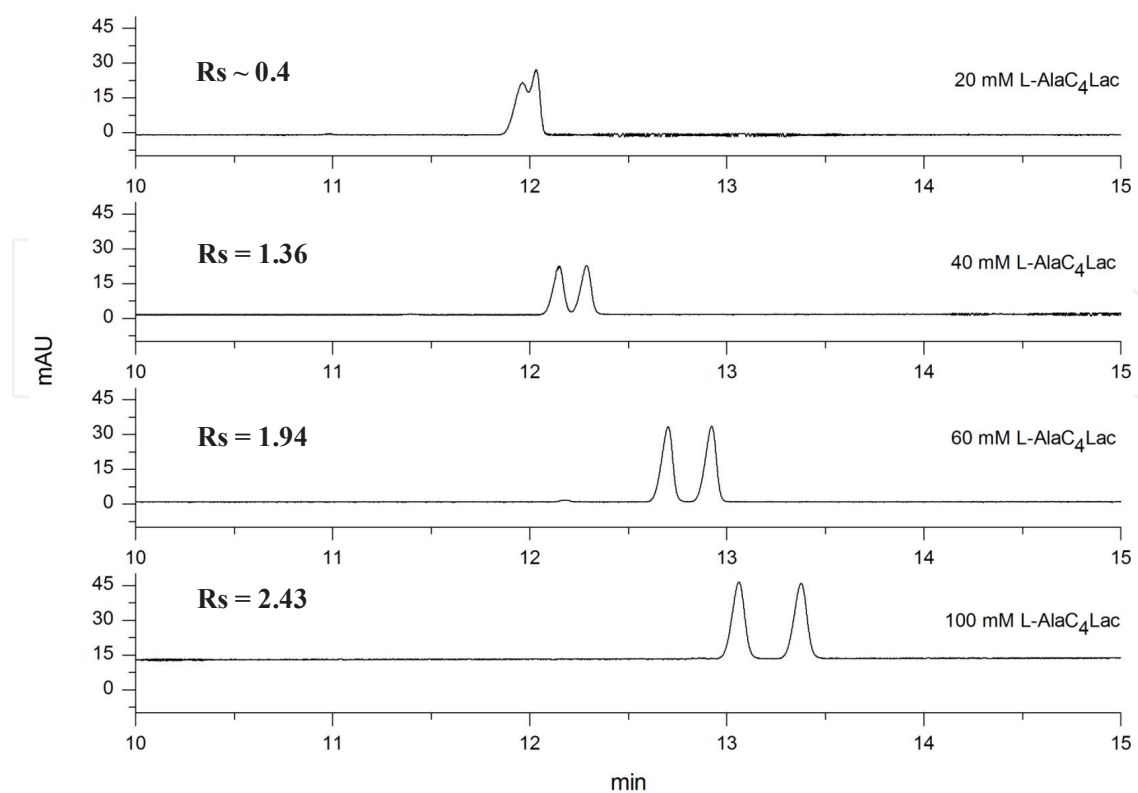


Figure 7. Effect of L-AlaC₄Lac concentration on the enantioseparation of BNP. Conditions: BGE, 100 mM Tris/10 mM Borate pH=8 applied voltage, 30 kV; capillary temperature, 25 °C; detection wavelength, 214 nm [27].

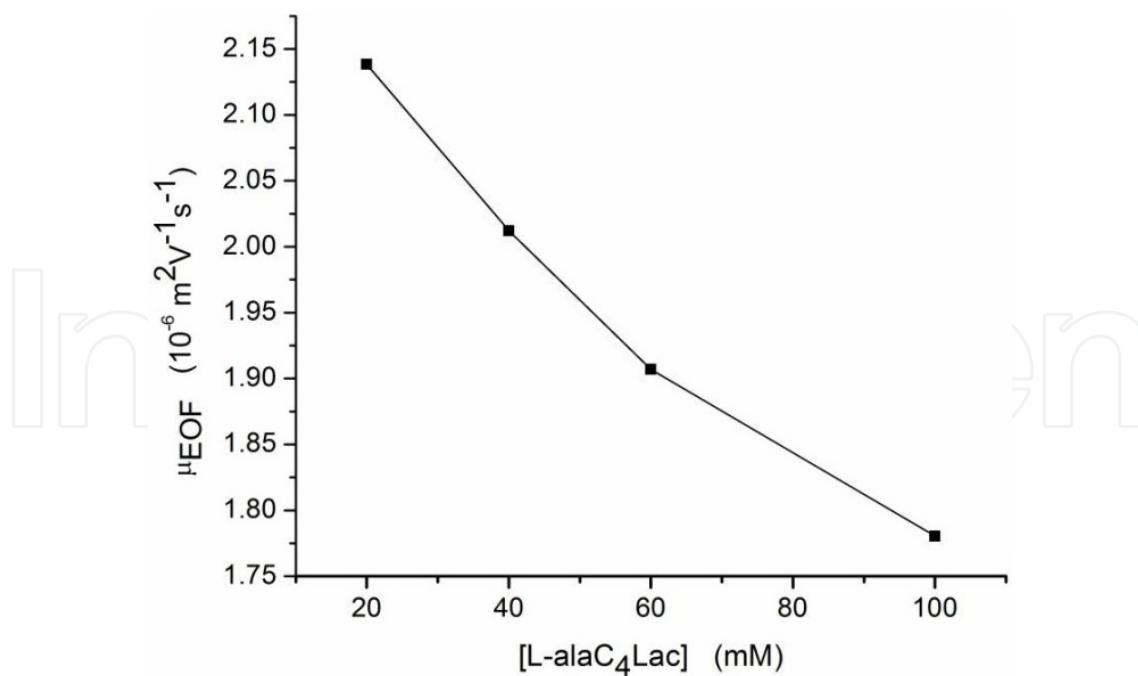


Figure 8. Effect L-AlaC₄Lac concentration on μ_{EOF} . Conditions: BGE, 100 mM Tris/10 mM Borate pH=8 applied voltage, 30 kV; capillary temperature, 25 °C; detection wavelength, 214 nm [27].

mechanism for this particular application is based on: (a) steric hindrance (*tert* butyl group), (b) electrostatic interactions (between the cation of the CIL and the negatively charged analyte) and (c) hydrogen bonding (hydrogen-bonding capability of the phosphate group in BNP).

4. Concluding remarks

In this chapter, the suitability of the AAILs in chiral and achiral CE analysis was evaluated. These new AAILs, which can be easily synthesized from commercially available reagents, proved to be efficient chiral additives for the enantioseparation of different analytes. Even though only a limited number of studies have, so far, applied AAILs as BGE additives and chiral selectors, it is easy to conclude from the data demonstrated in this chapter that the future of AAILs in separation science has a great deal of potential, and it is expected to expand significantly. Further research though is required in order to understand the chiral recognition mechanisms between the AAILs, the common chiral selectors and the enantiomers. This will, in turn, help us design even more effective AAILs for applications in chiral electrophoretic and chromatographic recognition.

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