REVIEW

CHIRAL IONIC LIQUIDS IN CHIRAL ELECTROPHORETICAL SEPARATIONS

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Abstract

Chiral ionic liquids (CILs) are an important class of chiral selectors that in the last years gained a lot of importance. Their utility in several separation techniques and the relative simple synthesis, allowed them to have multiple applications. Capillary electrophoresis (CE) is an electromigration based technique allowing the analysis of smaller amounts of samples compared to other classical techniques, such as HPLC. Chiral ionic liquids are used in various chiral electromigration techniques for enantioselectivity improvement, since 2006. CILs are rarely used as unique chiral selectors, but some researchers focus on achieving this aim.

Rezumat

Lichidele ionice chirale (CILs) sunt un tip de selectori chirali care, în ultimii ani au căpătat o importanță crescută. Prin utilizarea lor în mai multe tehnici de separare, dar și prin sinteza relativ simplă, acestea devin un domeniu cu o gamă largă de aplicații. Electroforeza capilară este o tehnică ce permite separarea unor cantități mici de substanțe analizate prin comparație cu alte tehnici clasice, cum ar fi HPLC. Lichidele ionice chirale sunt utilizate în diverse tehnici de electromigrare pentru îmbunătățirea enantioselectivității separărilor, încă din 2006. CILs sunt utilizate mai puțin ca selectori chirali unici, dar unii cercetători încearcă să îndeplinească aceste cerințe.

Keywords: chiral ionic liquids, enantioseparations, capillary electrophoresis

Introduction

Ionic liquids - IL ionic liquids are organic salts with a low melting point (below 100°C); if the melting point is below room temperature, the ionic liquids are room temperature ionic liquids (RTIL) [3]. They have the ability to dissolve both polar and non-polar species. The most important property is that they do not evaporate at high temperatures [26]. They are also relatively non-volatile and non-flammable [7] and they also have high thermal stability and recyclability [3]. They were first reported in literature in 1914 by the chemist Paul Walden [24].

If the anion and/or cation or both, are chiral, ionic liquids are chiral ionic liquids (CILs).

They often contain in their structure cations with a high percentage of organic nitrogen, such as substituted heterocyclic alkyl moiety and [26] bulky inorganic anions.

The first chiral ionic liquid was synthesized in 1999, by Seddon *et al*., the lactate of 1-butyl-3-methyl imidazolium [7]. It was obtained by ion exchange from chloride of 1-butyl-3-methyl imidazolium and (S)-2-hydroxypropionate solution. It was used as Lewis acid in Diels-Alder reactions [9].

CILs are an important class with a plethora of applications: chiral media in asymmetric reactions, solvent extraction, membrane separation, NMR spectroscopy, IR spectroscopy, luminescence spectroscopy, fluorescence spectroscopy, gas chromatography, liquid chromatography, and capillary electrophoresis (CE) [12].

A relatively new analytical method of separation is capillary electrophoresis [1] which has several important advantages, such as small volume injection and low consumption of substances compared to high performance liquid chromatography (HPLC) [8]. This technique can be used in chiral separations and the chiral discrimination is due to different mobilities of the resulting enantiomer-chiral selector complexes [40].

Unlike other selectors, as cyclodextrins, that form complexes with the analytes in different analytical techniques [33], CILs are seldom cited as sole chiral selectors [18, 26, 28, 34, 35, 45]. The CILs are mainly used as BGE (background electrolyte) additives, and secondly as chiral ligands and chiral selectors [12].

We have identified three main types of separation, with respect to the BGE and the method:

- separations in aqueous capillary electrophoresis with CILs as dual selectors or as sole chiral selectors;
- separations in aqueous ligand exchange capillary electrophoresis;
- separations in non-aqueous capillary electrophoresis.

In the last years, two reviews focused on separations using IL (ionic liquids): the first one gathers data between 2004 and 2014 on chromatographic and electrophoretic enantioseparations [12], the other covers recent advances in various electromigration techniques using IL [31].

In this review we focus on the electrophoretic separations using CILs, starting with the first publication of this type, in 2006, until the beginning of 2015.

CILs in aqueous capillary electrophoresis

According to literature it is quite difficult to obtain a good enantioselectivity with a single chiral selector (i.e. chiral ionic liquid). There are some articles where CILs are cited as sole chiral selectors, but in the majority of the cases they act as partners in dual chiral selector systems or as BGE additives. The interaction mechanisms proposed in some papers, are important for understanding how CILs act as chiral selectors. CILs are selected as an alternative when cyclodextrins or other selectors cannot resolve the racemic mixtures. The third selector can also be added or an organic modifier is used for increasing the enantioselectivity.

Sole chiral selectors

A research group from Cyprus synthesized and studied several amino acid based chiral ionic liquids as sole chiral selectors in CE separation of 1,1' binaphthyl-2,2-diylhydrogenphosphate (BNP) isomers. L-AlaC_1Lactate, L-AlaC_2Lactate, L-AlaC_4Lactate, L- and D-AlaC_4NTf_2 synthesis was simple and implied ionic exchange. (C_4 stands for *tert*-butyl ester of the amino acid, C_1 for methyl ester, C_2 for ethyl ester and Ntf_2 is bis(trifluro-methane)sulphonamide). The pH of the analysis was set at 8 and because of the very low resolutions, Kaiser's resolution factor was calculated.

A separation mechanism consisting in three-point interactions was proposed. It probably requires simultaneous steric hindrance (the *tert* butyl group), electrostatic interactions (between the cation of the CIL and the negatively charged analyte) and hydrogen bonding (due to the phosphate group in BNP). The resolution increased with the length and bulkiness of the side chain. The presence of *tert* butyl group is favourable. The nature of the anion affects the separation, limiting the water solubility of the ionic liquid. L-AlaC_4Lac gave the best resolutions. With D-AlaC_4Lac, S-BNP eluted first, while with L-AlaC_4Lac the enantiomer migration order was reversed. The run-to-run and batch-to-batch reproducibility of the ILs was demonstrated [28].

The same group previously tested L-AlaC_4Lac as a chiral selector for the separations of 2-aryl propionic drugs (indoprofen, carprofen, ketoprofen, flurbiprofen, ibuprofen). However, no enantiomer separation was observed [19].

As sole chiral selectors, CILs are used in CZE (capillary zone electrophoresis), but also in micellar electrokinetic chromatography (MEKC). They can be chiral salts of amino acids obtained by a simple synthesis or chiral surfactants and polymers. A β cyclodextrin derivative was cited as a cyclodextrin (CD) ionic liquid and was used to separate several racemates. Table I presents actual experimental conditions used in CZE separation with CILs as sole chiral ionic liquids.

The only application in MEKC was reported by a research group from Georgia State University. They achieved the first chiral separation using CILs, cationic surfactants and polymers derived from amino acids (leucinol) and *N*-methylpyrrolidinol. The analytes were several herbicides, (±) α-phenoxypropionic acid, (±) (2-PPA) and (±) α-bromophenylacetic acid, (±) (α-BP-AA). At an optimum pH of 7.5 the monomers of the CILs above ensured better enantio-selectivity by comparison with their polymers.

Table I

CIL as sole chiral selectors in aqueous capillary electrophoresis

CIL	Racemates	BGE	Voltage	Reference and CE type
(R)-N,N,N-trimethyl-2-aminobutanol bis(trifluoromethanesulfon) imidate	Methylbenzylamine, Tryptophan, α-Phenyl-glycine, 1-Phenyl-1,2-ethanediol, Phenylalanine, 2-Phenyl-1-Propanol, 3-Benzyloxy-1,2-propane diol, Tyrosine, Propranolol, 1-(1-Naphthyl) ethanol, Di-O,O' p-toluyl tartaric acid, 1-Indanol, Trans-2-phenyl-1-Cyclohexanol, 2,3-o-Benzylidene-threitol, 1,1,2-Triphenyl-1,2-ethanediol	20 mM Na_2HPO_4-NaH_2PO_4 or 20 mM $Na_2B_4O_7$ Variable pH values 4-12.5	9 - 17 kV	CZE [35]
O-2-hydroxpropyltrimethyl-ammonium-β-cyclodextrin tetrafluoroborate [HPTMA-β-CD][BF_4]	chlorpheniramine ; brompheniramine ; pheniramine; tropicamide; bifonazole; promethazine; warfarin; liarozole	30 mM NaH_2PO_4 buffer pH 4 (5.0 for warfarin and 8.0 for bifonazole and pheniramine)	20 kV / -20 kV	CZE [34]
Tetramethylammonium-lactobionate (TMA-LA)	Atenolol hydrochloride, Metoprolol tartrate, Nefopam hydrochloride, Duloxetine hydrochloride, Bisoprolol fumarate, Propranolol hydrochloride	40 mM borax buffer 40% v/v methanol	20 kV	CZE [45]

The authors remark the important role structural features and electrostatic interactions are playing in the enantioseparation. The three-point interaction mechanism was proposed (e.g. the non-rigid structure of L-UCLB (L- undecenoxycarbonyl-L-leucinol bromide) might have resulted in favourable hydrogen-bonding interactions between the chloro group on the benzene ring and also the primary alcohol of the L-leucinol) allowing to confirm the interaction pattern [26].

Dual selectors or additives in BGE

As dual selectors or as additives (when cyclodextrins are chiral selectors) CILs can be used to separate racemates. Sometimes a third chiral selector has to be added in order to achieve enantioselectivity. Three types of CILs are usually used. The first group consists of amino acids (or derivatives) based IL (used in several separations along with cyclodextrins derivatives [16, 36-38, 41, 46,], glycogen [44] and vancomycin [42]. The second type of CILs includes methyl alkyl imidazolium derivatives [5, 47, 48]. Other CILs cited as dual chiral selectors or additives are quaternary ammonium derivatives [10, 17, 32]. There were several cases when the addition of an ionic liquid did not result in enantioresolution improvement. In some cases, a mechanism of interaction is proposed.

In 2009, Tran and Mejac synthesized *S*-[3-(chloro-2- hydroxypropyl)trimethylammonium] [bis((trifluoro-methyl)sulfonyl)amide] (*S*-$[CHTA]^+[Tf_2N]^-$). The authors used a ternary selector system: CIL, cholic acid and 1-S-octyl-β-d-thioglucopyranoside in order to improve enantioselectivity. The racemates separated were: atenolol, propranolol, warfarin, indoprofen, ketoprofen, ibuprofen and flurbiprofen. The three-point interaction explained the mechanism of the separation [32].

Other research groups proposed models for binding constant estimation using differences in electrophoretic mobility. The binding constants were estimated applying the concept of enzymatic reactions - competitive inhibition [38] or using a stoichiometric pattern of interaction [16]. However it is necessary that the binding constants with each chiral selector at a time to be calculated.

Zhang J. and colab. used a series of AAILs as electrolyte additives in CZE with promising results. They used AAILs (l-alanine and l-valine *tert* butyl ester bis (trifluoromethane) sulfonimide salts) and methyl-β-cyclodextrin (Me-β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and glucose-β-cyclodextrin (Glu-β-CD) were chosen as chiral selecting systems in separation of naproxen, pranoprofen, warfarin, carprofen, ibuprofen and ketoprofen. Significant synergistic effect was observed in the enantiomer separation of the first three analytes. The authors concluded that CILs added are part in the chiral recognition process, which is beneficial to chiral separations [41].

Other examples of CZE, NACE and MEKC separations using CILs in binary or ternary chiral selecting systems are presented in Table II.

Table II

CIL as dual chiral selectors in aqueous capillary electrophoresis

CILs and dual system	Racemates	BGE	Voltage	Reference and CE type
(*R*)(−)2-Hydroxy-*N*,*N*,*N*-trimethyl-1-phenylethan-aminium (PhChol NTf_2) (*R*)(−)1-hydroxy-*N*,*N*,*N*-trimethylbutan- 2-aminium bis (trifluoromethylsulfonyl)imide (EtChol NTf_2) Other selectors: DM-β-CD or TM-β-CD (dimethylated or trimethylated derivatives of β cyclodextrin)	carprofen, suprofen, naproxen, ketoprofen, indoprofen and ibuprofen	acetic acid / sodium acetate (5 and 60 mM) pH of 5.0 90:10 and 75:25 (v/v) water–MeOH	25 kV	CZE/ NACE [10]
(α-CD,β-CD, γ-CD, hydroxypropyl-α-cyclodextrin (HP-α-CD), HP-β-CD, HP-γ-CD and trimethyl-β-CD (TM-β-CD). Secondary selectors: R-Ethyl choline NTf_2 (R-Ethyl choline bis((trifluoromethyl) sulfonyl)amide) or S-tetrabutylamonium-camphorsulphonate	6-bromo-1-(2-hydroxy-naphthalen-1-yl) naphthalen-2-ol (BN2); 1-(2-(allyloxy)naphthalen-1-yl) naphthalen-2-ol (BN3); 1-(2-(benzyloxy)naphthalen-1-yl)naphthalen-2-ol (BN4); 1-(2-methoxy-naphthalen-1-yl)naphthalen-2-ol (BN5)	Phosphate electrolyte 10.3-10.8	10 kV	CZE [17]
N-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB) Secondary selector: 2,3,6-tri-O-methyl-β-cyclodextrin (TM-β-CD)	ibuprofen, fenoprofen, indoprofen, suprofen, ketoprofen	5 mM CH_3COONa 2.63 mM CH_3COOH (pH 5.0)	30 kV	MEKC [37, 38]
[EMIM] [L-lactate] 1-ethyl-3-methylimidazolium-L-lactate Secondary selector: βCD - β cyclodextrin	zopiclone, repaglinide, chlorphenamine maleate, brompheniramine maleate, dioxopromethazine hydro-chloride, promethazine hydrochloride, liarozole, carvedilol, homatropine hydrobromide, homatropine methylbromide, venlafaxine, sibutramine hydrochloride	30 mM Tris-H_3PO_4 pH 2.5 (except for homatropine methylbromide, pH 2.0)	20 kV	CZE [48]

[EMIM] [L-lactate] Secondary selector: HP-β-CD – Hydroxyproyl-β-cyclodextrin (CIL-no enantioselectivity)	ofloxacin, propranolol hydrochloride, dioxopromethazine hydrochloride, isoprenaline hydrochloride, chlorpheniramine maleate, liarozole, tropicamide, amlodipine benzenesulfonate, brompheniramine maleate, homatropine methylbromide	50 mM NaH_2PO_4-H_3PO_4 buffer (pH = 2.75)	20 kV	CZE [5]
L-AlaC_4NTf_2 and L-ValC_4NTf_2 L-alanine and L-valine tert butyl ester bis (trifluoromethane) sulfonimide Secondary selector: Me-β-CD, HP-β-CD and Glu-β-CD (glucose- β-cyclodextrin)	naproxen, pranoprofen, warfarin, carprofen, ibuprofen, ketoprofen	citrate buffer pH 5.0 20% organic modifier: 20% (v/v) ethanol for naproxen and warfarin 20% (v/v) acetonitrile for pranoprofen	20 kV	CZE [41]
TMA-L-Arg (Tetramethylammonium L-arginate) TMA L-Asp (Tetramethylammonium L-aspartate) Secondary selector: Glycogen	nefopam hydrochloride, citalopram hydrobromide, duloxetine hydrochloride	36.67 mM TRIS - adjusted to pH 3 with H_3PO_4	18.89 - 19.2 kV	CZE [44]
[TBA][L-Asp] Tetrabutyl ammonium L-aspartate Secondary selector: HP-β-CD	Cinchona alkaloids (quinine/ quinidine; cinchonine/ cinchonidine) from *Cinchona* sp. bark and Compound Quinine Injection, and Watsons Tonic Water	40 mM ammonium acetate background pH 3.5 - 5.5	15 kV	CZE [46]
L-AlaC_4Ntf_2 and L-ValC_4Ntf_2 L-alanine and L-valine tert butyl ester bis (trifluoromethane) sulfonimide Secondary selector: Vancomycin	naproxen, carprofen, ibuprofen, ketoprofen, and pranoprofen	50 mM phosphate buffer, pH 6.5-7, 20% organic modifier MeOH	20 kV	CZE [42]
[TBA][L-Asp] Secondary selector: β-CD	DL-phenylalanine DL-tryptophan	15 mM sodium tetraborate pH 9.5	10 kV	CZE [36]
N-undecenoxy-carbonyl-l-alaninolbromide (l-UCAB), N-undecenoxy-carbonyl-l-valinol bromide(l-UCVB), N-undecenoxy-carbonyl-l-leucinol (l-UCLB) bromide,N-undecenoxy-carbonyl-l-isoleucinol (l-UCILB) bromide and N-undecenoxy-carbonyl-l-ephedrine bromide (l-UCEB) Secondary selector: TM-β-CD	1.1'-bi-2-naphthol (BOH), 7,8,9,10-tetrahydro-benzo [a]pyren-7-ol (THBP), 2,2,2-trifluoro-1-(9-anthryl)-ethanol (TFAE), trans-stilbene oxide (TSO)	For BOH, TSO TFAE 10 mM CH_3COONa/ CH_3COOH, pH 5 For TSO NaH_2PO_4/Na_2HPO_4 10 mM pH 7	30 kV	MEKC [16]
[EMIM][L-lactate], *N*-methyl-*N*-ethylpyrrolidinium tetrafluoroborate (P12BF4) dodecyl trimethyl ammonium chloride (DTAC) (DTAC-not a CIL) Secondary selector: HPβCD	Miconazole, econazole, ketoconazole, and itraconazole	50 mM NaH_2PO_4/H_3PO_4 buffer (pH 3.5)	20 kV	MEKC [47]

LECE

The principle of ligand exchange (LE) was established in the late 1960s by Davankov. The basic mechanism implies a metallic ion in the middle of a heterogeneous complex with the enantiomers and the chiral selector. The complex must be kinetically labile and form and dissociate in a fast rate. The central metal ion has defined positions within its coordination (six positions for copper) and each can be occupied by a single pair of electrons from organic groups, such as amino groups, carboxyl, hydroxyl, amide and thioalcohols, or water molecules [2].

When used in chiral separations, the chiral selector is an amino acid derivative or other chiral bidentate analogue. Chiral recognition is based on dipole-dipole or steric interactions with the selector [2]. The ligand exchange separation is therefore based on the formation of a diastereoisomerically ternary metallic complex between the ligand, the analyte and the chiral selector. Enantiomer separation is due to the difference in analyte-enantiomers complexes stability. The following equilibria are considered (Sel: selector, A: analyte [11]):

$$Cu\ (L\text{-}Sel)_2 + S\text{-}A \leftrightarrow Cu\ (L\text{-}Sel)\ (S\text{-}A) + L\text{-}Sel;$$

$$Cu\ (L\text{-}Sel)_2 + R\text{-}A \leftrightarrow Cu\ (L\text{-}Sel)\ (R\text{-}A) + L\text{-}Sel.$$

Chiral ligand exchange can be used both in HPLC and the CE. The first LECE separation was made in 1985, when Gassman, Kuo and Zare separated several racemic mixtures of amino acids using a Cu^{2+}- dansylated L-histidine complex [40]. The commonly used chiral ligands include amino acids (L-arginine, L-lysine etc.), branched amino acids (L-4-hydroxyproline,N-(2-hydroxyoctyl)-L-4-hydroxyproline etc.) and other organic acids (L-tartrate, D-saccharic acid etc.).

AAILs (amino acid ionic liquids) can be used as selectors in LECE, especially in the enantiomers separation of underivatized amino acids. The metallic ion is, usually, Cu^{2+} (sometimes Zn^{2+} or Mn^{2+}). As the racemates separated are amino acids, D- and L-Amino acid oxidase activity can be estimated by LECE [20], and also Michaelis–Menten equation's parameters [30] or tyrosinase inhibition [29].

Table III

LECE

CIL and metallic salt	Racemates	BGE	Voltage	Reference and other CE type
[C_4mim] [L-Orn] 1 butyl 3-methyl imidazolium L-ornithine and $ZnSO_4$	Dns-D,L-Ser, Dns-D,L-Met, Dns-D,L-Ile, Dns-D,L-Phe, Dns-D,L-Tyr, Dns-D,L-Cys, Dns-D,L-Asn, Dns-D,L-Arg, Dns-D,L-Ala, Dns-D,L-His, Dns-D,L-Thr, Dns-D,L-Asp, Dns-D,L-Leu, Dns-D,L-Lys, Dns-D,L-Met-test analyte/ Inhibition screening for D-Amino acid oxidase (DAAO)	100.0 mM boric acid, 5.0 mM ammonium acetate adjusted to pH 8.4 with TRIS	-20 kV	[20]
[L-Pro][CF_3COO], [L-Pro][NO_3], [L-Pro]$_2$[SO_4], [L-Pro][BF_4] (L-proline salts: trifluoroacetate, nitrate, sulphate, tetrafluroborate) [L-Pro][CF_3COO]: model AAIL Cu^{2+} salts	Dns-D,L-Ala, Dns-D,L-Asn, Dns-D,L-Asp, Dns-D,L-Ile, Dns-D,L-Met, Dns-D,L-Ser, Dns-D,L-Phe, Dns-D,L-Thr, Dns-D,L-Tyr	25.0 mM $Cu(Ac)_2$, 50.0 mM AAIL 20% (v/v) methanol pH 4.0	20 kV	[21]
[1-ethylpyridinium][L-lysine], [1-butylpyridinium][L-lysine], [1-hexylpyridinium][L-lysine], [1-[octylpyridinium][L-lysine] and Zn^{2+} salts	Dns-D,L-Ala Dns-D,L-Asn Dns-D,L-Asp Dns-D,L-Ile Dns-D,L-Leu Dns-D,L-Met Dns-D,L-Phe Dns-D,L-Ser Dns-D,L-Thr Dns-D,L-Trp Dns-D,L-Tyr (L-Amino acid oxidase -LAAO)	100.0 mM H_3BO_3 5.0 mM CH_3COONH_4 pH 8.4	21 kV	[30]
[TMA][L-OH-Pro] (Tetramethyl-ammonium L-hydroxyproline salt) and Cu^{2+} salts	Trp, Phe Hys,Tyr, 3,4-dihydroxyphenylalanine (DOPA)	60 mM AAIL containing 30 mM Cu^{2+}, pH 4.5	22 kV	MEKC [15]
[BMIM][L-Ala] 1 butyl 3-methyl imidazolium L-alanine, Mn^{2+} and βCD	Dns-D,L-Thr, Dns-D,L-Val, Dns-D,L-Tyr, Dns-D,L-Leu, Dns-D,L-Ile, Dns-D,L-Pro, Dns-D,L-Met, Dns-D,L-Ser, Dns-D,L-His, Dns-D,L-Phe, Dns-D,L-Ala, Dns-D,L-Asn, Dns-D,L-Trp, Dns-D,L-Gln, Dns-D,L-Orn, Dns-D,L-Glu, Dns-D,L-Arg, Dns-D,L-Asp, Dns-D,L-Cys, Dns-D,L-Lys	100.0 mM boric acid, 5.0 mM ammonium acetate, pH 8.3 ajusted with TRIS	23 kV	[29]

The first separation using chiral AAILs in capillary electrophoresis and high performance liquid chromatography was first reported in 2009. Several imidazolium AAILs were used as chiral selectors for the separation of underivatised amino acids (DL-Phe as model analyte, DL-Trp, DL-Tyr, DL-His), providing higher enantioselectivity compared to conventionally used amino acid ligands.

1-hexyl-3-methylimidazolium proline salt (C_6mim Pro) was selected as the most efficient chiral selector in CE separations, with 30% organic modifier added (methanol). Co^{2+}, Ni^{2+} and Zn^{2+} were tested, but copper provided better results in ligand exchange chiral separations [14].

Zhang H. and colab. have optimised a separation method with[C_6mim][L-Lys] with Zn^{2+} salts for seven pairs of dansylated amino acids. The ratio between the ionic liquid and the zinc ion was 2:1 taking in account a shorter analysis time. Using the separation data, they explained the mechanism for the EOF change. The concentration effect on the electroosmotic flow mobility is based on the hydration equilibrium. The IL is hydrolysed in a ternary conjugate with three imidazolic cations, a HO^- ion and three lysine anions. With the increase in concentration of IL, the ternary conjugate was more abundant and the EOF increased. At higher concentrations, the adsorption of imidazole cation on the capillary wall is low and no decrease in the EOF is observed, though higher concentration of IL lead to a gap in the mobility of the labelled amino acids, due to the ionization and hydration equilibrium [43].

Several AAILs, both cationic and anionic salts, were used as chiral selectors in various applications [14, 15, 20, 21, 29, 43,]; the metallic ions used are Cu^{2+} [14, 15, 21] and Zn^{2+} salts [20, 30, 43], one separation using Mn^{2+} was cited, too [29]. Details of these separations are presented in Table III.

NACE

Non aqueous capillary electrophoresis (NACE) is an useful alternative whenever aqueous capillary electrophoresis is not sustainable, though water is the solvent of first choice in chiral separations [22, 23, 39].

Electrophoresis in non-aqueous medium was introduced by Waldbrohl and Jorgenson, but only in 1990 its utility in chiral separations was revealed. One of the advantages is represented by the low conductances allowing higher voltages without Joule effect [13].

There are just a few papers presenting CILs use in NACE. Separations in non-aqueous medium were cited as an alternative for aqueous media, but a second chiral selector may be necessary.

Ephedrine-based chiral ionic liquid, (+)-*N*,*N*-dimethylephedrinium bis(trifluoromethanesulfon) imidate ($[DMP]^+[Tf_2N]^-$) was used as unique chiral selectors for the chiral separations of some racemic sulfoxides acting as proton pump inhibitors (rabeprazole and omeprazole) in an acetonitrile–methanol mixture (60:40 v/v) [10]. The electroosmotic flow was inversed with the addition of $[DMP]^+[Tf_2N]^-$. The separation is achieved based on different mobilities of the free forms of the analytes and the ion pairs formed between the IL cations and negatively charged enantiomers. Hence, the main mechanism for enantioseparation should be ion-pairing and hydrogen bonding [18]. The ephedrine-based chiral IL was also used as a stationary phase in gas chromatography for enantioseparation of alcohols, diols, sulfoxides, epoxides and acetylated amines by Ding *et al*. [6].
The enantiomers of a synthetic intermediate of new 3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans, 4-amino-2,2-dimethyl-6-ethoxycarbonylamino-3,4-dihydro-2H-1-benzopyran, were separated with heptakis-(2,3-di-O-methyl-6-O-sulfo)-β-CD and ethylcholine bis(trifluoromethylsulfonyl)imide. The CIL addition improves the separation. The method was validated and the optimized method was useful for the enantiomeric excess estimation [27].

Conclusions

CILs are chiral selectors drawing attention in the last years, because they are, "tailor made" selectors. Their synthesis is relatively simple and they are used in chiral separations in CZE along with cyclodextrins, chiral surfactants, antibiotics or glycogen in the separations of several racemic drugs. They may be used as unique chiral selectors and can separate racemic mixtures with good enantio-selectivities, either in aqueous or, sometimes, in non-aqueous medium.
Using CILs as sole chiral selector could provide a better understanding of the chiral separation mechanism in CE, which can be resumed to the three-point interaction and the interaction pattern based on the structural features of the selector. Electrostatic interactions should also be taken into account.

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