

Application of ionic liquids as mobile phase additives and surface-bonded stationary phase in liquid chromatography

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Abstract—Ionic liquids (ILs) have gained wide recognition as novel solvents in chemistry. Their application in analytical chemistry, especially in separating analytes, is warranted due to their unique properties such as negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions. Recently, some new chromatographic separation media, formed by combining ILs on silica and polymer surface using covalently or non-covalently interaction, have been developed and applied to biological separation and environment analysis. This review will focus on some of the properties of ILs and their potential application as mobile phase modifier and surface-bonded stationary phase in high-performance liquid chromatography (HPLC) separation.

Key words: Ionic Liquids, Modifier, Surface Bonded Stationary Phase, Chromatographic Separation

INTRODUCTION

Ionic liquids (ILs) have been accepted as a new green chemical revolution. ILs are generally defined as salts that melt at or below 100 °C to afford liquids composed solely of cations and anions. In some cases, ILs are free-flowing liquids at room temperature, in which case they can be called ambient temperature ILs. They represent an interesting class of solvents because they exhibit properties such as negligible vapor pressure, good thermal stability, tunable viscosity, and primarily anion-dependent miscibility with water as well as various organic solvents. In recent years, ILs have been widely considered as alternatives to classical organic solvents and applied in organic synthesis, electrochemistry, liquid phase extraction and catalysis for clean technology [1-4]. Thus, it has also been suggested that they might supply exclusive and interesting opportunities for separation science in general, and chromatography, specifically.

ILs have been used as electrolyte additives in capillary electrophoresis (CE) [5,6]. The analytes may associate with either the IL cations that coat the inner wall of the fused silica capillary or with the free IL cations in the running electrolyte solution. If covalently bonded to the capillary inner walls, ILs provide a permanent positive charge, which in turn reverses the electroosmotic flow in CE.

Although ILs have been used as additives or coatings in CE, most of their applications in terms of analytical separations have been as stationary phases in gas chromatography (GC) [7,8]. Investigations of the physicochemical properties of ILs and solute/stationary phase interactions in GC stationary phases suggest that ILs may be useful as multi-modal media in chromatographic separations. Alkyl-imidazolium-based ILs have been used as a stable stationary phase for

GC. They show unusual selectivity with “dual nature,” separating polar compounds as if they were polar stationary phases and non-polar compounds as if they were non-polar stationary phases.

It has already been proven that ILs could be used as mobile phase additives in reversed-phase chromatography when mixed with other solvents of a low viscosity. Numerous experiments have been performed with water, methanol, acetonitrile, and their mixtures as the mobile phases.

Based on several years' research on ILs [9-16], we will introduce their physicochemical properties and give a brief overview of the application of ILs in liquid chromatography (LC) separation, with a special focus on their use as a mobile phase modifier and surface-bonded stationary phase.

PHYSICOCHEMICAL PROPERTIES OF IONIC LIQUIDS (ILs)

As a new green chemical solvent, ILs have the following unique properties compared with other solvents:

- They are generally colorless liquids with relatively low viscosities.
- They exhibit very low vapor pressures under ambient conditions and thus are effectively non-volatile.
- They are good solvents for a broad spectrum of inorganic, organic and polymeric materials and are immiscible with numerous organic solvents. Thus, applications in process intensification and as non-aqueous polar alternatives in biphasic systems are possible.
- Being composed of two parts, they exhibit a synthetic flexibility that is not possessed by single component molecular solvents.

The unique properties of ILs are determined by the structure and interaction of ions in the melt. ILs are usually composed of large asymmetric organic cations and inorganic or organic anions. The

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cations and anions that can generally be grouped to prepare different ILs are shown in Table 1. The most commonly employed IL anions are polyatomic inorganic species, such as PF_6^- and BF_4^- . The most prominent cations are pyridinium and the imidazolium ring with one or more alkyl groups attached to the nitrogen or carbon

atoms. The physical properties of some common ionic liquids are presented in Table 2.

In addition to the interactions existing in conventional organic solvents (hydrogen bonding, dipole-dipole and van der Waals interactions), ILs also have ionic interactions (mutual electrostatic attrac-

Table 1. Several building blocks of ionic liquids

Cation		Anion
Name	Abbreviation	
Imidazolium	[Im]	BF_4^- , PF_6^- , OH^-
Alkylpyridinium	[Pyr]	CH_3COO^- , COO^-
1-Ethyl-3-hexyl imidazolium	[EHIm]	NO_3^- , CN^-
<i>N</i> -Ethylpyridinium	[NEPyr]	S_6F_6^- , CF_3SO_4^-
1-Butyl-3-methyl imidazolium	[BMIm]	$\text{F}_6\text{O}_4\text{S}_6^-$, CF_3SO_3^-
1-Hexyl-3-methylimidazolium	[HMIm]	Br^- , I^- , Cl^- , PhSO_3^-
1-Methyl-3-hexylimidazolium	[MHIm]	$(\text{CF}_3\text{SO}_2)_2\text{N}^-$, Al_2C_7^-
1-Ethyl-3-methylimidazolium	[EMIm]	CF_3CO_2^- , AlCl_4^-
1-Propyl-3-methylimidazolium	[PMIm]	CH_3SO_4^- , $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2^-$

Table 2. Physical properties of some common ionic liquids

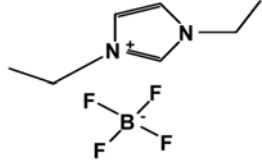
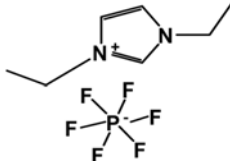
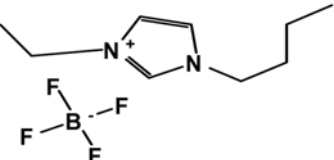
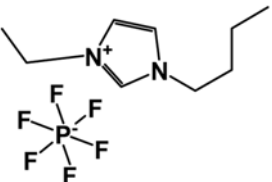
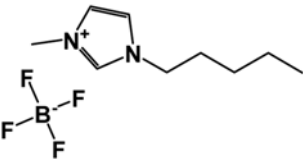
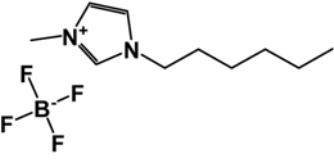
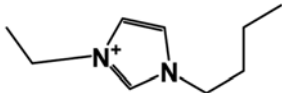
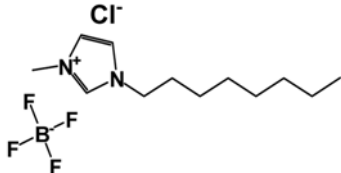
Systematic name	Abbreviation	Melting point, °C	Chemical formula
1-Ethyl-3-ethylimidazolium tetrafluoroborate	[EEIm][BF_4]	15	
1-Ethyl-3-ethylimidazolium hexafluorophosphate	[EEIm][PF_6]	58-60	
1- <i>n</i> -Butyl-3-ethylimidazolium tetrafluoroborate	[BEIm][BF_4]	-82	
1- <i>n</i> -Butyl-3-ethylimidazolium hexafluorophosphate	[BEIM][PF_6]	-8	
1- <i>n</i> -Amyl-3-methyl-imidazolium tetrafluoroborate	[AMIm][BF_4]	-88	
1- <i>n</i> -Hexyl-3-methylimidazolium tetrafluoroborate	[HMIm][BF_4]	-82	

Table 2. Continued

Systematic name	Abbreviation	Melting point, °C	Chemical formula
1- <i>n</i> -Butyl-3-ethylimidazolium chloride	[BEIM]Cl	65	
1- <i>n</i> -Octyl-3-methylimidazolium tetrafluoroborate	[OMIm][BF ₄]	-79	

tion or repulsion of charged particles), which makes them very miscible with polar substances [17]. At the same time, the presence of the alkyl chain on the cation determines their solubility in less polar fluids. Hydrogen bonding in ILs is thought to exist between an oxygen or halide atom on the anion and the hydrogen atoms on the imidazolium or pyridinium ring of the cation. This is particularly important in separation and catalysis, where trace impurities can make a huge difference to the outcome of a reaction.

ILs AS MOBILE PHASE MODIFIERS

In general, tailing problems in association with the poor separation reproducibility of basic analytes on chemically modified bonded-silica phases are often observed in high-performance liquid chromatography (HPLC). Several groups have reported using ILs as mobile phase modifiers to improve LC separations. The first time ILs were used as mobile phase additive was to study the chromatographic behavior of ephedrine and catecholamines [18,19]. The result showed that the IL addition could decrease the band tailing, reduce the band broadening, and improve the resolution, which was partly attributed to the competition between imidazolium cations and the polar groups of the analyte for the silanol group on the alkyl silica surface along with the formation of a weak bilayer electronic structure on the C₁₈ column. Therefore, it can effectively shield residual silanols and improve the peak shapes, while also decreasing the retention times of the analytes.

Another experiment [20] was undertaken to suppress silanophilic interactions by 1,3-dimethylimidazolium methyl sulfate ([MMIm][MeSO₄]) additive to eluent in reversed-phase HPLC. Chromatograms illustrating the influence of [MMIm][MeSO₄] added to the mobile phase composed of 50 : 50 acetonitrile : phosphoric buffer pH 3.0 (v/v) on the separation of the mixture of six basic drugs are presented in Fig. 1. Comparing the chromatograms (a-c) in Fig. 1 indicates that increasing the percentage of IL in the mobile phase decreases the retention and improves the separation of all the tested analytes. Obviously, the silanol-suppressing properties of alkylimidazolium-based ILs improve the peak shape and remove the peak tailing observed in the reference system.

Analyses of some nucleic and amino acids and the interaction mechanism of ILs have also been reported. In the papers by Row et al. [9-11], the effect of the concentration of the IL 1-butyl-3-methylimidazolium [BMIm][BF₄] as a mobile phase modifier on the retention of the nucleic (cytosine, cytidine, and thymine) and amino

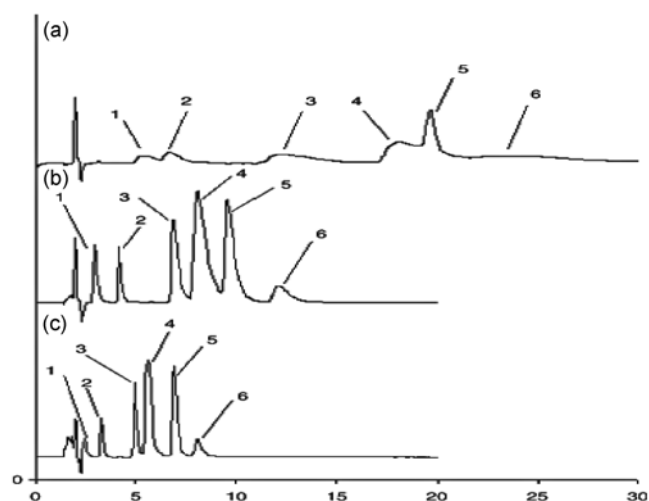


Fig. 1. Chromatograms of the separation of naphazoline (1), phenazoline hydrochloride (2), fluphenazine (3), chlorpromazine hydrochloride (4), trifluorpromazine (5) and thioridazine hydrochloride (6).

(a) acetonitrile : phosphoric buffer (pH 3.0) 50/50 (v/v) as the mobile phase; (b) acetonitrile : phosphoric buffer (pH 3.0) 50/50 (v/v) with the addition of 0.05% (v/v) 1,3-dimethylimidazolium methyl sulfate; (c) acetonitrile : phosphoric buffer (pH 3.0) 50/50 (v/v) with the addition of 0.5% 1,3-dimethylimidazolium methyl sulfate.

(N-CBZ-D-phenylalanine and D-tryptophan) acids was studied. The IL contents affected the resolution between the D-tryptophan and N-CBZ-D-phenylalanine in the mobile phase. The resolution was improved with increasing IL concentration. Chromatograms generated by using the IL in the mobile phase generally showed more symmetrical peaks and faster analysis times. This experiment also showed that the shape of peaks (asymmetry and peak tailing parameters) could be improved at the mobile phase by using the IL [BMIm][BF₄] as an additive.

Other researchers [12-16] have used different ILs as a mobile phase modifier to study the effect of IL concentration, different alkyl imidazolium group and different cation counterion on the analyte retention. They found that the retention factor is claimed to depend on the hydrophobic nature or chaotropic character of the IL anion. In a similar study [21], the researchers separated some amines, including benzidine, benzylamine, *N*-ethylaniline and *N,N*-dimethyl-

laniline, using the ILs ([EMIm][BF₄]), 1-butyl-3-methyl-imidazolium1-tetrafluoroborate1-([BMIm][BF₄]), 1-hexanyl-3-methylimidazolium tetrafluoroborate ([HMIm][BF₄]) and 1-butyl-3-methyl-imidazolium bromide ([BMIm][Br]) as the mobile phase additive, respectively. All amines become less retained when ILs were added and most of their retention factors decreased with increasing IL concentration. With the increase in the length of the alkyl substituent of the imidazolium cation from ethyl, through butyl, to hexyl, all *k* values of amines are markedly decreased.

ILs are not only used in reversed-phase HPLC but also in normal HPLC. Marszałł [22] used ILs as the mobile phase additives to demonstrate distinct differences in retention and selectivity in both normal and reversed-phase HPLC. The four ILs tested showed very similar trends in both normal and in reversed-phase LC systems. However, the differences in retention of individual analytes in specific LC systems allow practically useful changes in the separation of basic analytes. The silanol-suppressing potency of ILs strongly surpasses that of the standard alkylamine additives. The use of ILs to replace the less effective and environmentally harmful alkylamine additives in LC will enable the separation efficiency and laboratory safety to be improved.

The separation of chiral compounds has been of great importance in both research and industry, particularly in the pharmaceutical industry. The search for novel chiral selectors is an open field still attracting the creativity and efforts of researchers all over the world. Yuan et al. [23] used chiral IL (R)-N,N,N-trimethyl-2-aminobutan-1-ol-bis (trifluoromethanesulfon)imide as the chiral selector in chromatography to separate eight racemates for the first time and got better resolution than no chiral IL. Their work indicates that the chiral IL could soon become very attractive as a new chiral selector in the chromatography.

These investigations showed that as mobile phase additives, ILs could play a multiplicity of roles, such as blocking residual silanols groups, modifying the stationary phase, and acting as active ion-pairing agents. If the basic compounds are polar and lightly retained, a polar IL additive with a strongly chaotropic anion is recommended. With less polar and hydrophobic amines, a less polar IL additive with a cosmotropic anion may be a good choice.

ILs AS SURFACE-BONDED STATIONARY PHASES

In addition to acting as a mobile phase modifier, stationary phase-containing, covalently bound, surface confined IL (SCIL) groups

have also been reported in the literature. Their ability to separate individual classes of compounds under reversed-phase conditions and the physical characterization of these SCIL phases have been the primary focus of these reports.

Several new IL bonded-surface stationary phases have been synthesized and the obtained columns were used to separate a variety of solutes wide enough to enable linear solvation energy relationship studies [24,25]. It was found that the 1-butyl-3-heptylimidazolium bromide stationary phase was similar to conventional phenyl-based stationary phases under reversed-phase conditions for the separation of a group of neutral aromatic solutes. The excellent correlation of the global fit between the experimental and calculated retention across the mobile phase composition range used in these studies, despite the complexity of the novel stationary phase, supports the utility of the LSER model to describe retention for this limited set of solutes under normal phase conditions.

A new anion-exchange stationary phase was made based on *N*-methylimidazolium through the reaction of activated silica with 3-chloropropyltrimethoxysilane and subsequently with *N*-methylimidazole [26]. Using this new stationary phase, familiar inorganic anions, organic anions and some other organic compounds were separated successfully. High column efficiency and satisfactory resolution were obtained. The phase based on *N*-methylimidazolium is an anion-exchange phase, but with multi-modal retention properties because it also has reversed-phase interactions and a hydrogen bonding interaction. The study supported the capability of the new phase to separate some biological samples such as amino acids and proteins.

Another successful use of IL bonded-surface stationary phase is to separate different analytes. For example, Qiu et al. [27] synthesized a new zwitterionic stationary phase based on silica bonded with 1-alkyl-3-(propyl-3-sulfonate) imidazolium. The synthesis steps are shown in Fig. 2. The materials were confirmed and evaluated by elemental analysis, thermogravimetric analysis and X-ray photoelectron spectroscopy. Potassium and calcium were separated simultaneously with several common inorganic anions, including iodate, chloride, bromide, nitrate and iodide on the phase. The chromatogram of the inorganic anions is shown in Fig. 3.

Liu et al. [28] made a new and effective, IL-based stationary phase and applied it to the separation of ephedrine in HPLC. The separation results indicated the high efficiency and reproducibility of the stationary phase. The electrostatic and ion-exchange interactions between the solutes and the stationary phase were considered to account for the effective separation. Moreover, the free silanols on

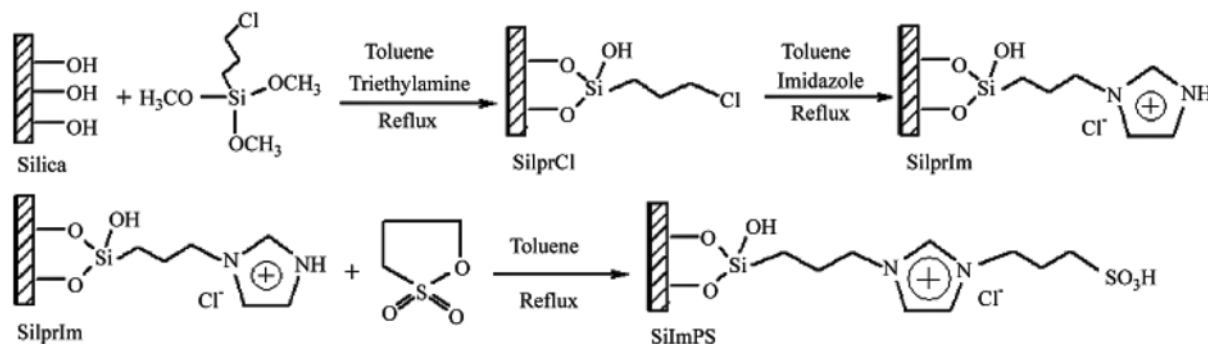


Fig. 2. Synthesis steps used in the preparation of zwitterionic stationary phase.

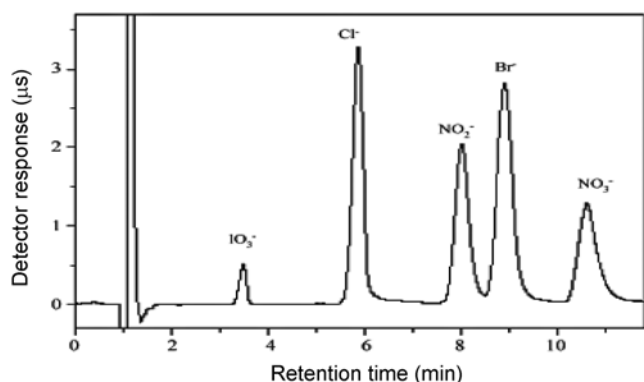


Fig. 3. Chromatogram of inorganic anions (Column, SiImPS stationary phase; mobile phase, 4 mM potassium hydrogen phthalate (pH 4.0); flow rate: 1 mL/min; detection, conductivity detector; analytes, 2 mM each of potassium iodate, potassium chloride, sodium nitrite, lithium nitrate and 1.6 mM potassium bromide).

the surface of the silica were effectively masked by the immobilized IL, resulting in the decrease of the non-specific absorption.

More recently, two new stationary phases based on [PMIm]Br and [BMIm]Br ILs were studied for the separation of a group of organic acids [29]. The hydrophobic and ion-exchange interactions seemed to be the major factors that contributed to the retention. One of the main advantages of the use of surface-immobilized IL stationary phases is that effective separations can be achieved with aqueous mobile phases with no or only very little organic solvent.

ILs IN CHROMATOGRAPHIC SEPARATION

ILs have been used in a variety of chromatographic methods as novel materials. The unusual properties of these neoteric solvents may impart potential benefits to many areas of separation science.

The application of ILs for the separation of various compounds in real samples by CE has recently been recognized. The application of 1-alkyl-3-methylimidazolium as a background electrolyte in CE for the simultaneous determination of five anthraquinones in Rhubarb was reported by Tian et al. [30]. The effective separation of five anthraquinone derivatives in Rhubarb species was achieved by using only ILs as the background electrolyte. In another study, Warner and coworkers reported the use of ILs as modifiers in the separation of chiral analytes in micellar electrokinetic chromatography, along with polymeric surfactants [31]. ILs may be able to assist in the separation of hydrophobic mixtures while maintaining adequate background current.

As previously mentioned in this review, because ILs can reduce the residual silanol groups, they can be used as mobile modifiers and bonded-surface stationary phase in LC and have been used to separate analytes in real samples. Chromatographic separation was achieved in a reversed-phase C_8 column using the IL [EMIm][BF₄] "green" mobile phases to determine octopamine, synephrine and tyramine in citrus herbs [32]. Comparing chromatograms (A, B) in Fig. 4, one will note that the addition of [EMIm][BF₄] decreased the band tailing, increased the retention and improved the resolution. Furthermore, [EMIm][BF₄] can suppress the influence of the

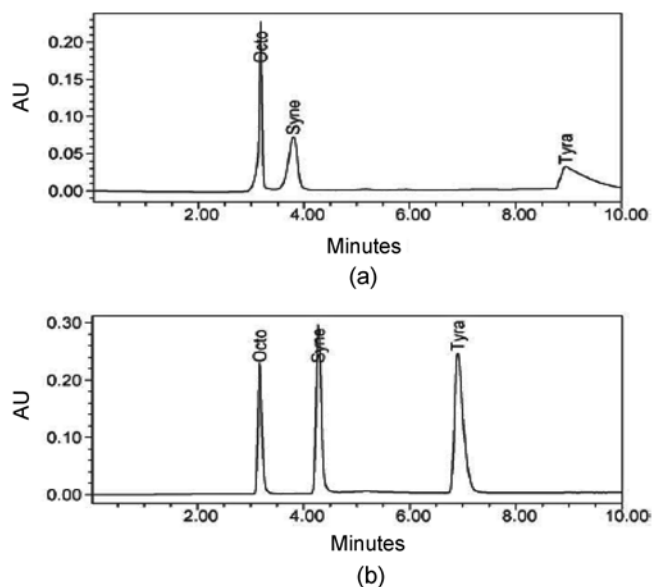


Fig. 4. Chromatograms of adrenergic amines with different mobile phases at pH 4.0 and 30 °C. (A) Water; (B) aqueous solution containing 32 mM [EMIm][BF₄].

pH shift on the HPLC separation. Recently Cruz-Vera and co-workers used IL as a silanol activity suppressor to improve the determination of antidepressants in urine samples by LC [33]. According to the presented results, the developed method is a useful tool for the determination and therapeutic monitoring of nine antidepressants in urine samples. Very recently, the ionic liquids as additives are applied to determine fluoroquinolone antibiotics in environmental samples [34]. Four ionic liquids differing in the length of the alkyl chain on the imidazolium cation and one ionic liquid containing tetraethylammonium, have been tested as mobile phase additives. The optimized method was successfully applied to the analysis of the fluoroquinolone antibiotics in different water samples.

CONCLUSIONS

The investigations showed that as mobile phase additives and surface-bonded stationary phases, ILs have been applied to many separation areas. However, there are several problems that limit their development. First, as organic salts, ILs do not have a simple retention mechanism in GC and HPLC. Mixed mechanisms involving ion pairing, ion exchange and hydrophobic interactions combine to produce retention of the anions and, possibly differently, of the cations. The matrix of a real sample is complicated as the retention of analytes may be disturbed by the interaction between the matrix and IL. Second, among the numerous ILs it is difficult to find the proper one to separate the specified analytes in a real sample. Third, the expense of the ILs increases the cost. So if these problems could be successfully resolved, ILs undoubtedly have much greater potential in future chromatographic separation.

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