

# Application of Ionic Liquids as Mobile Phase Modifier in HPLC

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**Abstract** Ionic liquids are receiving an upsurge of interest as 'green' solvents; primarily as replacements for conventional media in chemical processes. Although ionic liquids are rather "young" modifier, their great potential in high-performance liquid chromatography (HPLC) has already been demonstrated. This review presents an overview of the applications of ionic liquids as mobile phase modifiers in HPLC.

**Keywords:** ionic liquids, organic modifier, mobile phase, HPLC

## INTRODUCTION

Ionic liquids (ILs) are now widely recognized as important components of 'green' chemistry. There has been an increase in the attention paid to ILs, as reflected by the articles in both the scientific and popular scientific literature [1,2]. This attention in ILs has been largely driven by the perceived opportunities to improve industrial processes using 'green' chemistry ideology [3]. The basic concepts behind both 'green' chemistry and ILs is not new; however, the increased interest in ILs, coupled with the influence of novel minds, new approaches are generating original solutions and driving changes in industrial and commercial practices, proving that 'green' chemistry alternatives actually do exist.

ILs have been used in a variety of different areas within modern chemistry and shown to provide unique properties. Thus, it has been suggested they might also supply exclusive and interesting opportunities for application in separation science. However, it should be noted, when ILs are diluted or immobilized on a stationary support, they may not possess all their original properties. In some cases, they may keep several of their intermolecular interactions, which may be useful for chromatographic separations.

Without any doubt, chemically modified aqueous acetonitrile/water eluent systems have been widely used in the HPLC [4,5]. However, as was already noted above, in addition to buffer salts, various additives, such as ionic liquids, are often needed to give efficient performance.

The ready commercial availability of a wide range of ionic liquids is acting as a stimulus in the field – creating the correct climate for innovations in the area of high-performance liquid chromatography (HPLC). The quantity of scientific publications is steadily growing year on

year (Fig. 1), implying that ILs possess enormous advantages and potential in the liquid chromatography. This review will focus on some of the properties of ILs and their potential applications as modifiers in liquid chromatography.

## Physical Properties of ILs

ILs, sometimes referred to as "molten salts", are ionic substances with melting points at or close to ambient temperature. The easy definition of an IL is a liquid that is composed entirely of ions, melts at low temperatures (generally below 373K) and may be thought to resemble a molten ionic substance at high temperature (1073~1273K). This phenomenon can be explained as follows; while ILs are composed of several ions, the presence of bulky organic cations interrupts the crystal packing and lowers the melting temperature.

Typically, these compounds consist of nitrogen-containing organics and any of an array of inorganic anions (e.g., chloride, bromide, tetrafluoroborate, hexafluorophosphate), as well as ammonium [6], imidazolium [7], pyridinium [8], pyrrolidinium [9], and isoquinolinium [10], each with the probability for attaching different alkyl groups to the heterocyclic ring or quaternary cation. Depending on the kind of cation under investigation and the length of the alkyl chain, the resulting ionic liquid can have a melting point above ambient temperature (293K).

General cations and anions that can be grouped to prepare different ILs are demonstrated in Table 1. Depending on the qualitative array, the resulting ionic liquid may have hydrophobic or hydrophilic properties. Ions are present in the liquids, with an exactly equal number of positive and negative ions; therefore, the whole liquid is electrically neutral. Some important physical properties (melting point and densities) of the most common ILs are presented in Table 2. As seen from this table, the melting points for the majority of ILs can vary over a wide range of temperatures. These physicochemical pro-

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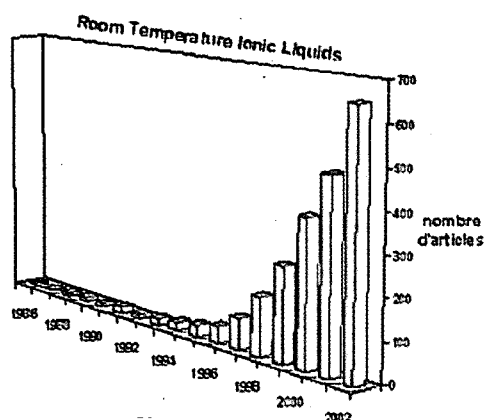
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**Table 1.** Several building blocks of ionic liquids

Cation		Anion
Name	Abbreviation	
Imidazolium	[Im]	$\text{BF}_4^-$ , $\text{PF}_6^-$ , $\text{OH}^-$ ,
Alkylpyridinium	[Pyr]	$\text{CH}_3\text{COO}^-$ , $\text{COO}^-$ ,
1-Ethyl-3-hexyl imidazolium	[EHIm]	$\text{NO}_3^-$ , $\text{CN}^-$ ,
N-Ethylpyridinium	[NEPyr]	$\text{S}_b\text{F}_6^-$ , $\text{CF}_3\text{SO}_4^-$ ,
1-Butyl-3-methyl imidazolium	[BMIm]	$\text{F}_6\text{O}_4\text{S}_6^-$ , $\text{CF}_3\text{SO}_3^-$ ,
1-Hexyl-3-methylimidazolium	[HMIm]	$\text{Br}^-$ , $\text{I}^-$ , $\text{Cl}^-$ , $\text{PhSO}_3^-$ ,
1-Methyl-3-hexylimidazolium	[MHIm]	$(\text{CF}_3\text{SO}_2)_2\text{N}^-$ , $\text{Al}_2\text{Cl}_7^-$ ,
1-Ethyl-3-methylimidazolium	[EMIm]	$\text{CF}_3\text{CO}_2^-$ , $\text{AlCl}_4^-$ ,
1-Propyl-3-methylimidazolium	[PMIm]	$\text{CH}_3\text{SO}_4^-$ , $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2^-$

**Table 2.** Physical properties and applications of some ionic liquids

Cation	Anion	Melting point (K)	Density (g/L)	Application	Reference
[EMIm]	[BF <sub>4</sub> ]	279	1.240	Chromatography	13-16,19
[HMIm]	[BF <sub>4</sub> ]	207	1.290		14,16
[BMIm]	[Br]	201	1.440		14
[BMIm]	[BF <sub>4</sub> ]	192	1.170	Chromatography, Chemical synthesis, Enzyme media, Biological catalyst	12-14,17,18,24,25
[BMIm]	[PF <sub>6</sub> ]	289	1.363	Chemical synthesis, Enzyme media, Biological catalyst	25,26

**Fig. 1.** Number of papers published worldwide per year on the subject "ionic liquids" (2002 year estimate) (Chemical Abstracts, Current Contents and Medline databases).

properties of ILs strongly depend on both the nature and size of their cation and anion constituents. The viscosity and miscibility of ILs with water or organic solvents can be modified by changing the anions or the alkyl chains of the cations.

### ILs in Liquid Chromatography

ILs are relatively new modifiers of conventional mobile

phases for reversed-phase (RP) and normal-phase (NP) HPLC, so only a few studies have currently been made. Previously, the applications of ILs in liquid chromatography have mostly been as mobile phase modifiers, with their utilization having been rather limited. This has due to the fact several ILs are not UV transparent at wavelengths where analytes are detected. Furthermore, the density and viscosity of ILs is appreciably higher than usual HPLC solvents. For these reasons the mechanisms of their interactions with sorbates or stationary phases remain to be fully investigated and described [11].

### Mechanism of ILs as Additives on Separation

However, the chemical nature of ILs permit the conclusion that when used as mobile phase additives in HPLC, they exist in the mobile phase solution and are also coated onto the reversed-phase silica-based column. The probable mechanism of interactions can be described as follows. ILs cations can interact and compete with silanol groups (specific electrostatic interactions) on the alkyl silica base surface with the polar group of the analytes. At the same time, the nonpolar alkyl groups of the stationary phase can interact with different alkyl groups of the heterocyclic ring or quaternary cation (unspecific type of interactions and hydrophobic interactions) (Fig. 2) [12]. Therefore, this phenomenon can efficiently shield the residual silanols and improve the peak shapes, while also reduces the chromatographic retention times

of the sorbates. The relationship between the concentration of ILs modifier and the chromatographic retention may be qualified using the following factors. The significant addition of ILs modifier to a mobile phase leads to competition between IL cations and the polar groups of sorbates for the polar silanol groups on an alkylsilica surface, resulting in decreases in the retention times of substances. The retention of nonpolar sorbates will also be reduced, as the ILs modifier also disables the alkyl groups of the stationary phase, which leads to a sharp decrease in the possibility of dispersion interactions between the nonpolar sorbate and the alkyl groups of the stationary phase.

With a further increase in the concentration of an ILs modifier, cations interact with the silanols groups through electrostatic interactions, producing a weak bilayer electronic structure, which repulses basic sorbates, and interacts with the alkyl groups through hydrophobic and non-specific interactions, so the retention of analytes decreases under due to these repulsive and hydrophobic interactions. If the concentrations of the ILs are slightly increased, their cation interactions with the silanol groups on the alkyl silica surface due to specific interactions or with the alkyl groups due to hydrophobic and non-specific interactions gradually strengthen, resulting in an increase in the carbon content of the stationary phase; thus, the retention times of substances are increased.

In view of the above, the mobilization of eluents by ILs is of great interest to researchers, and this procedure has been the object of much discussion over the decade. Realized investigations have shown that as mobile phase additives, ILs could play multiple roles, such as block the residual silanols groups, modify the stationary phase or to act as ion-pairing agents.

### Applications of ILs in RP-HPLC

Zhang and co-workers [13] used alkylmethylimidazole and *N*-butylpyridinium salts as mobile phase additives. The authors concluded that for the separation of catecholamines on a reversed phase column, the retention time decreased when ILs (2.8~45.0 mM) were present in the mobile phase. The peak tailing was also reduced, presumably because the ionic liquid ions coated the residual silanols groups, which reduced the interactions of substances with residual hydroxyl groups on the silica. The change in chromatographic retention time was dependent on the IL additive used. These authors carried out the separation of catecholamines with the addition of 1-butyl-3-methylimidazolium tetrafluoroborate to the mobile phase composition. Treatment of the mobile phase with 1-butyl-3-methylimidazolium tetrafluoroborate reduced the retention of catecholamines compared to water, but the addition of 1-ethyl-3-methylimidazolium tetrafluoroborate increased the retention. Thus, the potential exists to change the resolution of catecholamines by just changing the alkyl group on the imidazole. In the same paper, the separation of ephedrine on a C<sub>18</sub> column was obtained with the use of 1-butyl-3-methylimidazolium tetrafluoroborate (2.6~62.4 mM). In this case, improve-

ments in terms of decreased peak tailing, band broadening and pair's resolution were achieved.

The addition of ILs to an aqueous mobile phase also affect on the symmetry of chromatographic peaks. In the paper by He *et al.* [12] this phenomenon was attributed to the ILs coating the residual silanol groups of silica. The authors compared 1-butyl-3-methylimidazolium tetrafluoroborate and triethylamine as modifiers, with better peak resolution achieved for ephedrine with the use of the IL. Increasing the alkyl chain length of the ILs cation reduced the chromatographic retention of the substances and improved the peak shapes. He *et al.* noted the difference between triethylamine and ILs on the separation of ephedrine, and confirmed the ILs were superior additives.

An analysis of some amines and the interaction mechanism of ILs has also been reported [14]. The ionic liquids, ethyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-hexanyl-3-methylimidazolium bromide, have been used as additives in HPLC. The separation of phthalic acid isomers, *N*-ethylaniline, *N,N*'-dimethylaniline, benzidine, and benzylamine were performed with chromatographic silica gel column C<sub>18</sub>. Mobile phases were prepared as molar concentrations by dissolving known amounts of ILs in distilled water. The various effects, such as the concentration of 1-butyl-3-methylimidazolium tetrafluoroborate on the separation of the investigated amines and the length of the alkyl chain of the ionic liquids and its counter-ion on the separation, were discussed. Fig. 2 shows that with an increase in the lengths of the alkyl substituent of the imidazolium cation, from ethyl, butyl to hexyl, the retention factor (*k*) values of all the amines were greatly decreased. Ethyl-3-methylimidazolium tetrafluoroborate ionic liquid provides better resolution; however, the improvement is at the expense of a longer analysis time. In addition, ILs composed of organic cation with different inorganic counter-ions, *i.e.* tetrafluoroborate and bromide, was also examined. From Fig. 3, the change in the elution order of *N*-ethylaniline and *N,N*-dimethylaniline obtained with the use of ethyl-3-methylimidazolium tetrafluoroborate is very evident. This phenomenon can be explained by the formation of strong hydrogen bonding between the hydrogen atom on the C-2 carbon of the imidazolium cation and the counter-ion. This was confirmed by Wilkes and Zaworotko [15]; they reported that because of the cation-anion Coulombic attraction, with minimal hydrogen bonding, the inter-ionic interaction in ethyl-3-methylimidazolium tetrafluoroborate predominates.

Another successful example of the application of ILs in reversed-phase chromatography is represented in the paper by Kaliszan *et al.* [16]. The mobile phases, modified by several ILs (1-ethyl-3-methylimidazolium tetrafluoroborate, 1-methyl-3-hexylimidazolium tetrafluoroborate, and 1-hexyl-3-heptyloxymethylimidazolium tetrafluoroborate), were used for the separation of basic drugs, such as acetylsalicylic acid, salicylic acid, tiamenidethioridazine, trifluoropromazine, phenazoline, and naphazoline.

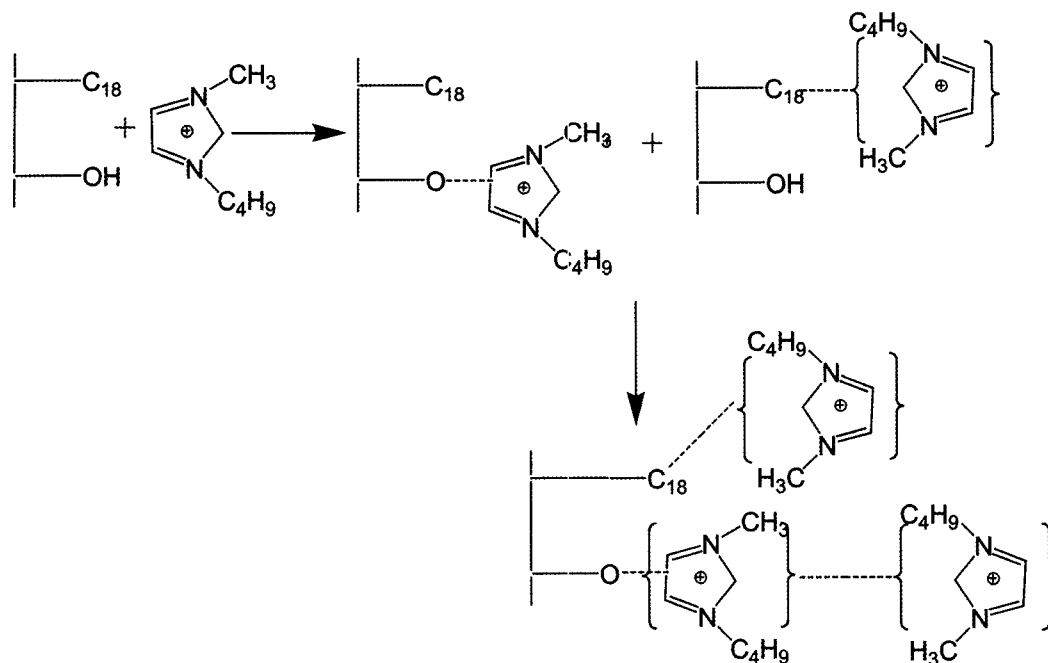


Fig. 2. Schematic drawing of the proposed interaction of [BMIm] with the modified silica based surface (Reprinted with permission from Ref. [12]).

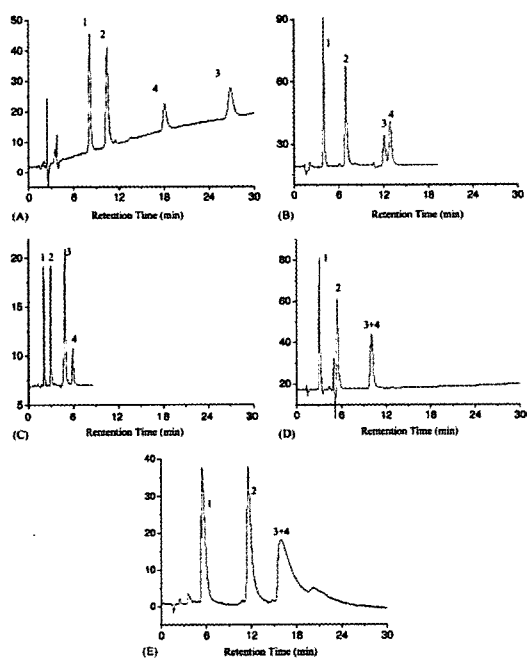


Fig. 3. Chromatograms of amines with mobile phases containing 30 mmol/L of (A) [EMIm][BF<sub>4</sub>], (B) [BMIm][BF<sub>4</sub>], (C) [HMIm][BF<sub>4</sub>], (D) [BMIm][Br], and (E) water; adjusted to pH 3 with HCl; chromatographic conditions: C<sub>18</sub> column (5 m, 150.0 × 4.6 mm i.d.); flow rate: 1.0 mL/min; detection wavelength: 254 nm. Peaks: (1) benzylamine; (2) benzidine; (3) *N*-ethylaniline; (4) *N,N*-dimethylaniline (Reprinted with permission from Ref. [14]).

The properties of tetraalkylammonium nitrate and thiocyanate ionic liquids in gas and liquid chromatography were studied by Poole and co-authors [17,18]. These salts demonstrated very strong orientation and proton acceptor interactions, with weak proton donor capacity, which could be used within temperature ranges from around room temperature to 423–453K, at which temperature they exhibited significant vapor pressures. Their viscosity was also easy to control by operating at elevated temperatures or through dilution with a co-solvent. For HPLC, ionic liquid containing mobile phases were shown to rapidly deteriorate packing of the silica based column. They were also used as liquid mobile phase modifiers with unbounded silica packing. Recently, the use of butyl-3-methylimidazolium tetrafluoroborate with pure water has been shown for the separation of polar basic drugs. The screening of the silanols groups by the imidazolium cation were not clearly different from that obtained with amines (*e.g.* triethylamine).

#### Application of ILs in NP-HPLC

Another investigation [19] used an ionic liquid as a modifier in normal-phase thin-layer liquid chromatography, with the behavior of some peptides within the thin-layer chromatography system, both with and without the addition of 1-ethyl-3-methylimidazolium tetrafluoroborate to the eluent, compared. It was observed that addition of such IL blocked silanols groups and provided efficient separations of investigated substances using thin layer chromatography.

## Applications of ILs in Other Areas

ILs are being investigated for their application in almost every field of chemistry (Table 2). Firstly, it should be noted that increases in both the interest and available variety of ILs result from electrochemical investigations on molten salts [20]. ILs can be utilized as alternatives to high temperature molten salts, and as low melting organic ionic electrolytes. At the present time, electrochemical studies continue to be highly active, which make use of substances from electro synthesis, electroplating and electronic productions [20,21].

ILs are being investigated as replacements for volatile organic compounds, which are usually used as diluents. Multiple chemical applications of ILs have focused on the properties, which has led to their wide adoption in other chemical and biological [22] fields also.

ILs have been successfully used as solvents and/or catalysts in modern organic chemistry processes. Several efficient proposed examples include Diels-Alder, Friedel-Crafts, Heck, and nickel-catalyzed olefin dimerization reactions [23,24].

Numerous ILs are immiscible with several organic solvents and/or water, ensuring alternate multiphase systems for liquid-liquid extractions [25]. Some hydrophobic ILs have been designed for the extraction of substituted-benzenes from water [23] and of inorganic ions (e.g., cadmium, mercury) from aqueous solutions [26].

## SUMMARY

This review has demonstrated the wide applicability of ILs to different areas of liquid chromatography. The results reported thus far have clearly shown the potentials of using ILs as modifier in HPLC. They provide promising separation interactions for a wide variety of substances, such as aromatics, polyphenols and peptides. Most work on the use of ILs has focused on chromatography, with the emphasis having been on investigation of the physical properties of analytes and between solvent and solutes interactions. The use of ILs makes possible the separation of many solutes (both polar and apolar in character); it would seem that in the future they may be especially suitable for the separation of positional and optical isomers. The resolution and selectivity of analytes were dependent on the concentration and type of ILs or organic solvent used. Good separation of solutes with the use of ILs as modifier has sometimes been achieved; in other instances, the ILs exert considerable impact on chromatographic retentions. Although the use of ILs as modifiers is in its infancy, they have already demonstrated great potential in HPLC. Similar studies on ILs within chromatographic science have been the latest addition to their uses, with the majority of papers in this area only having been published recently.

Unfortunately, the influence of ILs modifiers on chromatographic retention is currently unclearly. The latter fact, together with the already existing successful examples of the application of ILs for chromatographic separ-

ations, is a powerful stimulus for further studies in this direction. Thus, many opportunities for the exploration of their applications exist in all areas of chromatographic separation.

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