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Chapter 12

IONIC LIQUIDS IN LIQUID CHROMATOGRAPHY

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ABSTRACT

Ionic liquids (ILs) as a new kind of media or materials have been widely studied in broad fields such as material science, catalysis, electrochemistry and analytical chemistry. Interests for ILs in academic and industrial technologies are still increasing due to their unusual chemical and physical properties. In separation science, ionic liquids have been used as extraction media, as mobile phase additives in high-performance liquid chromatography (HPLC), as buffer modifiers in capillary electrophoresis (CE), as stationary phases of gas chromatography (GC) and HPLC, etc. In this chapter, the general physicochemical characters of ILs will be introduced simply and a brief overview of recent developments of ILs in separation science is provided, with a special focus on their use as mobile phase additives and silica-supported IL stationary phases in liquid chromatography.

1. INTRODUCTION

Ionic liquids (ILs) [1] have a surprisingly long history since ethanolammonium nitrate (m.p. 52-55 °C) was found by Gabriel and Weiner in 1888 [2]. In 1914, the "first" room temperature ionic liquid (RTIL) is ethykammoium nitrate with melting point 12 °C reported by Walden [3]. In 1982, Wikes et al. found a new class of ILs that consist of alkylimidazolium chloroaluminate [4]. However, these chloroaluminate ILs are not stable due

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to their reactivity to moisture and many chemicals. Until 1992, Wilkes and Zaworotko [5] found that more hydrolytically stable anions could be used for ILs, and this field of research has increased significantly.

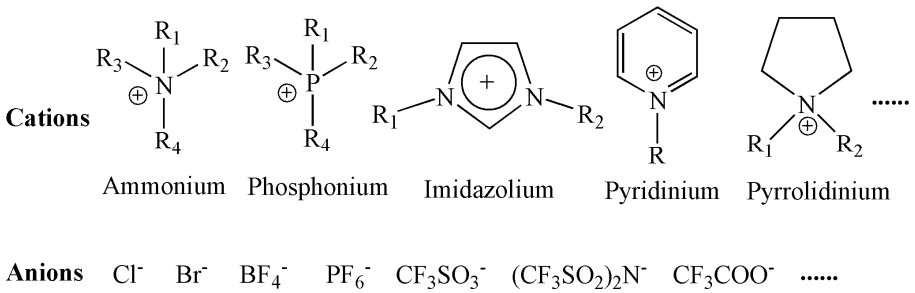


Figure 1. Examples for common cations and anions of ionic liquids.

Generally, ILs are defined as salts that melt at or below 100 °C to afford liquids. ILs are usually composed of bulky, nonsymmetrical organic cations such as imidazolium, pyrrolidinium, pyridinium, ammonium and phosphonium etc. Imidazolium is the most popular cations during the chromatographic separation until now. Anions could be inorganic, including Cl^-, Br^-, BF_4^-, PF_6^-, and more and more ILs consist of organic anions like trifluoromethylsulfonate ($CF_3SO_3^-$), bis(trifluoromethyl)sulfonylimide ($(CF_3SO_2)_2N^-$), trifluoroethanoate (CF_3COO^-) etc. The structures of some commonly used cations and anions are shown in Figure 1.

Compared commonly used organic solvents, ILs present unique physical and chemical properties such as negligible vapor pressure, high thermal stabilities, wide liquid range, good electrolytic conductivity and tunable viscosities etc. Another important feature is that ILs have a good designability, thus giving us the ability to manipulate the structure (with respect to the organic cation, inorganic anion and the length of the side chain attached to the organic cation) and consequently their properties. Consequently, combinations of a variety of cations and anions give rise to a tremendous number of available ILs for academic research and industrial application.

In recent years, ILs have been gained widespread recognition as alternatives to classical organic solvents and widely applied organic synthesis [6,7], catalysis [8-9], electrochemistry [10,11], liquid phase extraction [12,13], solid-phase microextraction [14,15] etc. ILs have also widely investigated as new media in chromatographic science mainly including electrolyte additives in capillary electrophoresis (CE), stationary phases in gas chromatography (GC), mobile phase additives and new stationary phases in high-performance liquid chromatography (HPLC). At the same time, there also some but few researches about that ILs used in countercurrent chromatography (CCC), thin layer chromatography (TLC) and supercritical fluid chromatography (SFC).

Applications of ILs in analytical chemistry have been reviewed by Armstrong [16-18], Pandey [19,20], Jiang [21], Koel [22,23] and their coworkers etc. Applications of ILs in separation science were also reviewed separately [24-27]. In addition, several reviews only concentrated in the application of ILs in chromatographic technologies [28-33] and CE [32-34]. IL used as additives in capillary zone electrophoresis (CZE), non-aqueous CE (NACE), and micellar electrokinetic chromatography (MEKC) and as support coatings of the capillary

wall in electrochromatography was described [34]. Poole reviewed the determination of solvent properties of room temperature ionic liquids using chromatographic and spectroscopic methods [35]. The separation and analysis of ionic liquids using chromatographic and electromigration technologies can also be found in some review [23,28].

Table 1. Some cations and anions of ILs and their abbreviations

Cations	Abbreviation	Anions	Abbreviation
1-Ethyl-3-methylimidazolium	[EMIm]	Chloride	Cl
1-Propyl-3-methylimidazolium	[PMIm]	Bromide	Br
1-Butyl-3-methylimidazolium	[BMIm]	Tetrafluoroborate	BF_4
1-Hexyl-3-methylimidazolium	[HMIm]	Hexafluorophosphate	PF_6
1-Octyl-3-methylimidazolium	[OMIm]	Methylsulfate	MS
1-Butyl-2,3-dimethylimidazolium	[BMMIm]	Tosylate	Ts
1-Decyl-3-methylimidazolium	[C_{10}MIm]	*L*-Proline	Pro
1-Dodecyl-3-methylimidazolium	[C_{12}MIm]	*L*-Leucine	Leu
1-Tetradecyl-3-methylimidazolium	[C_{14}MIm]	*L*-Alanine	Ala
1-Butylpyridinium	[BPy]	*L*-Valine	Val
1-Vinyl-3-octadecylimidazolium	[C_{18}VyIm]	Methyl orange	MO

In this chapter, we will focus on the recent development of ILs used in HPLC including the application of ILs in mobile phase additives and surface-confined ILs (SCIL) as novel stationary phases for HPLC. Otherwise, ILs used in TLC, CCC, SFC and monolithic column was also introduced briefly. Some classical cations and anions of ionic liquids used in this chapter and their abbreviations were shown in Table 1.

2. HPLC MOBILE PHASE ADDITIVES

In 1986, Poole et al. [36,37] reported that ionic liquids were used as organic modifiers for mobile phase in LC separations. Alkylammonium nitrate or thiocyanate salt was mixed with another solvent of low viscosity and used as mobile phase. They also investigated the solvent properties of six ILs used in micro-column based reversed-phase liquid chromatography (RPLC). Waichigo et al. tried to replace traditional organic modifiers by ILs used in LC and published a series of papers [38-41]. However, some disadvantages were found during the use of ILs instead of common organic solvents as modifiers in mobile phase: 1. ILs usually are high viscious so as to producing high back pressure; 2. ILs always have more strong UV absorption, the detection limit would be influenced; 3. Most of ILs are still expensive compared common used solvents; 4. Some ILs may corrosive the instrument. Considered these disadvantages, although ILs have environmental-friendly potential effect, currently they still cannot replace common organic solvents such as methanol and acetonitrile.

In 2009, methylammonium formate (MAF) IL was considered as an effective replacement for methanol in RPLC in part because of the viscosity of MAF is lower as compared to other ILs such as ethylammoniumnitrate and ethylammoniumformate [42]. Very recently, Ohno et al. [43] demonstrated the first case of HPLC with an IL as an eluent for the separation of celluloses in size exclusion chromatography (SEC) mode. The called HPILC

method described here is a potentially powerful tool in view of its adaptability and range of application. However, as far as we know, because of the viscosity of ILs, there is still no report that pure ILs directly used as mobile phase in reversed-phase or normal-phase HPLC until now.

As described in the review [16], compared to working as an alternative for organic modifiers in HPLC, ILs as low concentration additives were found to be more useful. In 2003, He et al. [44] reported 1-alkyl-3-methylimidazolium ILs were used as mobile phases additives for the separation of four ephedrines (norephedrine, ephedrine, pseudoephedrine and methylephedrine) on a C_{18} column. The effects of several imidazolium ILs with different alkyl chains on the cations or with different counterions used as the mobile phase additives were compared. And different concentrations of 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids as the eluent at pH 3.0 were studied as shown in the Figure 2. The retention times of the analytes increase at first and then decrease with the increase in the concentration of ILs.

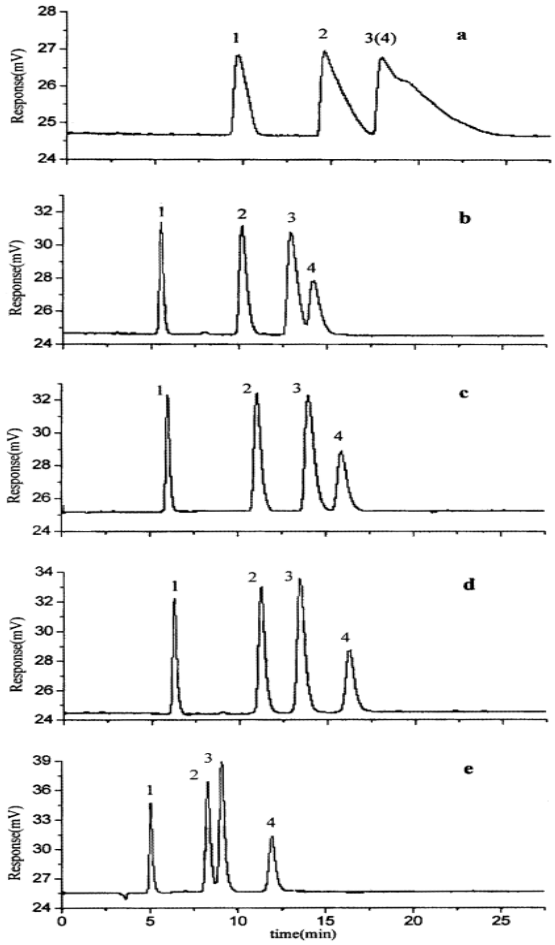


Figure 2. Chromatograms of ephedrines with a mobile phase containing different concentrations of $[BMIm]BF_4$ at pH 3.0. (a) 0, (b) 2.6, (c) 5.2, (d) 20.8, and (e) 62.4 mM. Chromatographic conditions: column: C_{18} (5 mm, 100 × 4.6 mm I.D.); rate-flow: 1.0 ml min^{-1}; detection: 252 nm. Peaks: (1) norephedrine, (2) ephedrine, (3) pseudoephedrine, and (4) methylephedrine [44].

The addition of ILs has great effects on the separation of these basic compounds: decreasing band tailing, reducing band broadening, and improving resolution. This effect may be attributed to the competition between imidazolium cations and the polar groups of the analytes for the silanol group on the alkylsilica surface, and also to the formation of weak bilayer electronic structure on the C_{18} column as shown in Figure 3.

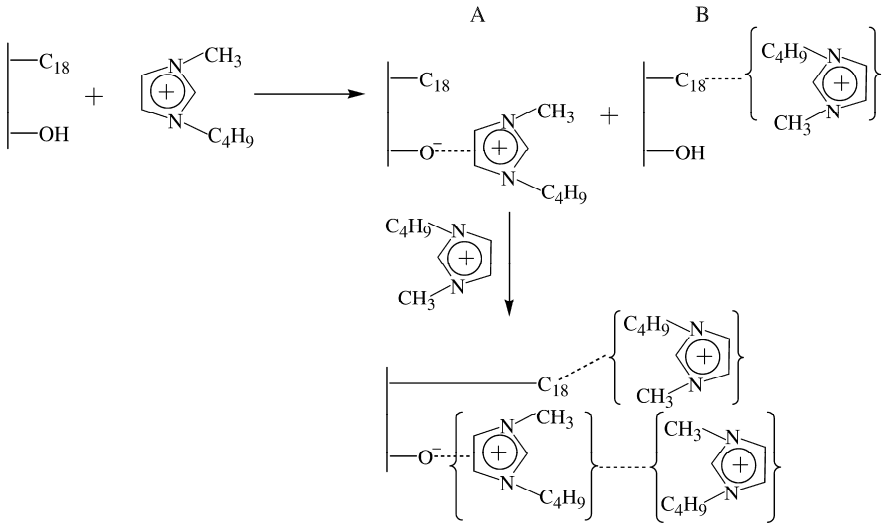


Figure 3. Proposed scheme of interaction for [BMIm]BF_4 on modified silica surface [44].

At the same time, Zhang et al. developed a new method for the separation of catecholamines [45] and nucleotides [46] using 1-alkyl-3-methylimidazolium-based ILs as additives by RP-HPLC. At the separation of five nucleotides, the results show that they are baseline separated by using ionic liquids as additives on a common reversed-phase C_{18} column without gradient elution and any other modifiers. The addition of RTIL provides better resolution and peak symmetry. Some amines including benzidine, benzylamine, *N*-ethylaniline and *N,N*-dimethylaniline are separated using ILs as additives by Xiao et al. [47] After they compared the differences between ionic liquids and tetrabutylammonium bromide (TBA) on the separation of *o*-, *m*-, *p*-phthalic acids, the results showed that ionic liquids are ion-pair reagents in essence, although their hydrophobicity and hydrogen bonding also play important roles.

Kaliszan and coworkers [48-50] also published several papers using ILs as additives to reduce silanophilic interaction when separating basic drug analytes. Berthod et al. [51] used nine different 1-alkyl-3-methylimidazolium ionic liquids with different alkyl chain length and chloride or BF_4^- or PF_6^- anions as additives in RPLC separation of some cationic basic solutes on a Kromasil C_{18} column. They found that both the anion and the cation of ILs contribute to solute retention and peak efficiency extending beyond simple "salting-out" or ion-pairing effects.

Later, Ruiz-Angel and coworkers [52] reported that a comparative study of peak shape, elution behavior, elution strength and resolution of seven β-blockers (acebutolol, alprenolol, labetalol, metoprolol, nadolol, pindolol and propranolol) with aqueous-organic mobile phases containing [BMIm]BF_4 or triethylamine (TEA) additives is performed in RPLC. [BMIm]BF_4 was found clearly superior to the classical TEA additive for efficiency as well as peak shape

enhancement. The role of the dual nature of ionic liquids (with a cationic and anionic character) as mobile phase additives was systematically studied [53]. The cations and anions both could be adsorbed on the surface of the stationary phase. Mobile phases without additive and containing a cationic TEA or anionic (sodium dodecyl sulphate, SDS) additive were used as references for the interpretation of the behaviours. The silanol suppressing potency of the additives, and the association constants between the solutes and modified stationary phase or additive in the mobile phase, were estimated. They found that [HMIm]BF_4 IL and SDS are the best enhancers of chromatographic peak shape among those studied.

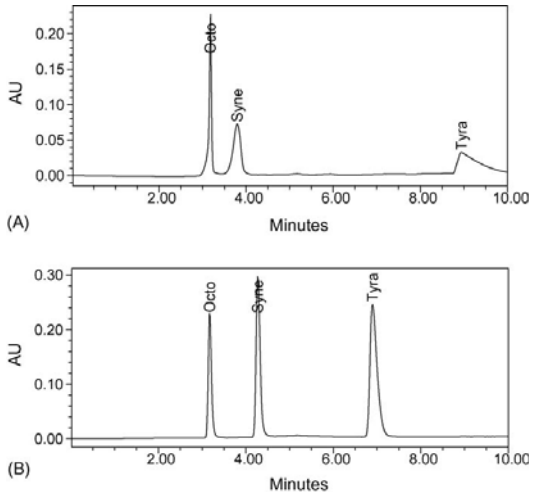


Figure 4. Chromatograms of adrenergic amines with different mobile phases at pH 4.0. (A) Water; (B) aqueous solution containing 32 mM [EMIm][BF_4] [54].

Using a mobile phase containing 32 mM [EMIm][BF_4], the contents of octopamine (Octo), synephrine (Syne) and tyramine (Tyra) in Citrus herbs were accurately determined by Tang et al. [54] According to the report, the effect of [EMIm][BF_4] was the best in the six investigated ILs. The concentration of [EMIm][BF_4], mobile phase pH and column temperature, which influenced the chromatographic behaviors of the analytes, were investigated in detail. The addition of [EMIm][BF_4] resulted in decreasing band tailing, increasing retention and improving resolution as shown in Figure 4. A little of RTIL additives in water can become a satisfactory and environmentally friendly mobile phase.

Different kinds of ionic liquid additives used for HPLC mobile phase and the analytes separated in the investigated system were summarized in Table 2.

Later, Row and coworkers published a series of papers using ionic liquids as mobile phase additives to improve the separation of nucleotides [55], amino benzoic acids [56], and chlorophenols [57]. We used four different ILs, including [EMIm]BF_4, [BMIm]BF_4, [HMIm]BF_4, and [BMIm]Cl, as mobile phase additives to enhance the separation of bases (cytosine, thymine, adenine, 6-chlorouracil) and amino acids (*L*-histidine, *L*-tyrosine, *L*-phenylalanine, and *DL*-tryptophane) [58]. The separation of phenoxy acid herbicides and phenols using IL additives was also reported [59].

More applications of ionic liquids were reported recently. Martin-Calero et al. [60] have evaluated the suitability of ILs as mobile-phase additives for the determination of heterocyclic aromatic amines with fluorescence and UV detection in HPLC. Rodríguez-Delgado and

coworkers [61] described the use of [EMIm]BF_4 as additive in eluent for the HPLC analysis with fluorescence detection of a group of seven basic fluoroquinolone antibiotics (i.e. fleroxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin and difloxacin) in different milk samples. In this report, they found that [EMIm]BF_4 was found superior to [BMIm]BF_4 for the separation of the analytes from chromatographic interferences of the sample matrix. Separation of eight commonly used antiretroviral drugs was achieved on a hydrophobic monolithic column using as a mobile phase containing ILs in a gradient elution mode [62]. RTILs improved RP-HPLC on-line combined with inductively coupled plasma mass spectrometry (ICP-MS) was developed for selenium speciation [63].

Table 2. Ionic liquids used as additives in HPLC

Ionic liquids additives in mobile phases	Analytes	Ref.
0-62.4 mM [BMIm]BF_4, [EMIm]BF_4, [EMIm]BF_4, [EMIm]Cl at pH 3.0	Ephedrine	[44]
1-Alkyl-3-methylimidazolium and N-butyl-pyridinium salts	Catecholamines	[45]
23 mM [BMIm]BF_4 in aqueous solution	Nucleotides	[46]
30 mM [EMIm]BF_4, [BMIm]BF_4, [HMIm]BF_4, or [BMIm]Br at pH 3.0	Aromatic amines	[47]
Different ionic liquid additives in CH_3CN/H_2O (50:50 v/v)	Basic drug analytes	[48-50]
0-50 mM ILs in CH_3CN/H_2O (30/70, v/v) at pH 4.0	Cationic basic solutes	[51]
0-6 mM [BMIm]BF_4 in CH_3CN/H_2O (15/85, v/v) at pH 3.0	β-blockers	[52]
0-60 mM different ILs in CH_3CN/H_2O (15/85, v/v) at pH 3.0	Basic drugs	[53]
32 mM [EMIm]BF_4 in water at pH 4.0	Octopamine, synephrine and tyramine in Citrus herbs	[54]
0.5-13.0 mM [EMIm]BF_4, [BMIm]BF_4 or [EMIm]MS in CH_3OH/H_2O (90/10, v/v)	Nucleotides	[55]
[EMIm]BF_4, [BMIm]BF_4 [EMIm]MS, [OMIm]MS	Amino benzoic acids	[56]
3.0 mM [OMIm]MS or 1.0 mM [EMIm]MS	Chlorophenols	[57]
25 mM [EMIm]BF_4, [BMIm]BF_4, [HMIm]BF_4, [BMIm]Cl in water	Bases and amino acids	[58]
20 mM [EMIm]Cl, [BMIm]Cl, [C_{10}MIm]Cl	Phenoxy acid herbicides and phenols	[59]
1 mM [BMIm]BF_4 in CH_3CN/H_2O (18/82, v/v) at pH 3.6	Heterocyclic aromatic amines	[60]
3 mM [EMIm]BF_4 and 10 mM CH_3COONH_4 at pH 3.0 with 13% CH_3CN (v/v)	Basic fluoroquinolone antibiotics	[61]
Mixture of [BMIm]BF_4 and [EMIm]MS in CH_3CN/H_2O at pH 4.0	Antiretroviral drugs	[62]
0.4% [BMIm]Cl, 0.4% [BMMIm]BF_4 and 99.2% water (v/v)	Selenium speciation	[63]
1 mM [HMIm]Pro, 0.5 mM $Cu(Ac)_2$ in CH_3OH/H_2O (15/85, v/v) (pH 5.8)	Amino acid enantiomers	[64]
4.0 mM [BMIm]Leu and 3.0 mM $CuSO_4$ in CH_3OH/H_2O (15/85, v/v)	Ofloxacin enantiomers	[65]

More interestingly, chiral amino acid ionic liquids (AAILs) were firstly used as chiral ligands in ligand-exchange chiral separations by Yao and coworkers [64]. By using 1-hexyl-3-methylimidazolium L-proline ([HMIm]Pro) as a chiral ligand coordinated with copper (II), four pairs of underivatized amino acid enantiomers— DL-phenylalanine (DL-Phe), DL-histidine (DL-His), DL-tryptophane (DL-Trp), and DL-tyrosine (DL-Tyr)—were successfully separated as shown in Figure 5. Tremendous application potential of this new type of task-specific ILs was proved. Later, Bi et al. [65] developed a method for the separation and determination of ofloxacin enantiomers by using AAILs-based ligand-exchange HPLC.

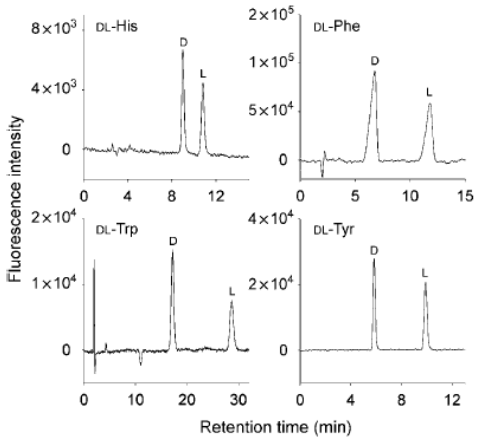


Figure 5. Enantioseparations of DL-His, DL-Phe, DL-Trp, and DL-Tyr. Mobile phase: 1 mM [HMIm]Pro, 0.5 mM $Cu(Ac)_2$, and MeOH (15% v/v) in water (pH 5.8); fluorescence detection: $\lambda_{ex}/\lambda_{em}$=280 nm/348 nm for DL-His, 215 nm/295 nm for DL-Phe, 270 nm/304 nm for DL-Trp, and 216 nm/295 nm for DL-Tyr. Adapted from the ref [64].

3. THIN LAYER CHROMATOGRAPHY

As the same in HPLC, silica or silica based stationary phases are very popular in TLC. However, a serious undesirable property of silica is its surface acidity due to the free silanols. TEA, dimethyloctylamine (DMOA) and ammonia are often used as mobile phase additives. However, these additives are not effective sometimes. In search for efficient suppressors of free silanols, ILs were used as mobile phase additives in TLC [48].

Different behavior of peptides was observed after addition of $[EMIm]BF_4$ to the eluent in comparison to the system without ILs in TLC [66]. They also optimized the separation of peptides by computer. The potential usefulness of ionic liquids for optimization of separation of peptides was demonstrated. Optimization of the separation conditions was supported by a commercially available computer program.

The separation of four ephedrines (norephedrine, ephedrine, pseudoephedrine and methylephedrine) using 1-alky1-3-methyl-imidazolium tetrafluoroborate as the elution additives in TLC was studied by He and coworkers [67]. The peak shape of ephedrines was improved greatly by the addition of ILs. The retardation factor (Rf) of the analytes increased at first, and then do not change with the increase in the concentration of ILs. These effects

demonstrated that imidazolium ILs could effectively suppress the deleterious effects of free silanols on silica-based stationary phase. Moreover, after added IL, the selectivity of four analytes were changed, which indicated there could exist simultaneous two separation mechanism to determine the retentions.

4. COUNTERCURRENT CHROMATOGRAPHY

Countercurrent chromatography (CCC) is a separation technique with mobile and stationary phases like all chromatographic techniques [68,69]. The most obvious characteristic is that there is no solid support to maintain the liquid stationary phase, and the mobile and stationary phases are both liquid phases in CCC.

Ionic liquids as new liquid phases were used in CCC by Berthod et al. [70] The partitioning of 38 aromatic derivatives with acid, base, or neutral functionalities was studied between the biphasic liquid system [BMIm]PF_6 and water. They found that pure ILs could not be used directly in CCC because of the viscosity is too high. To decrease the viscosity, a third solvent is necessary to be added. The ternary phase diagrams of [BMIm]PF_6-water and acetonitrile, methanol, ethanol, 1-propanol, and 2-propanol are presented in mass and mole percentages. The organic solvent-RTIL-water systems form two liquid phases with a viscosity low enough to allow CCC operation. The results confirmed that the polarity of [BMIm]PF_6 was high, comparable with that of ethanol. The work demonstrates that CCC is a powerful tool to estimate the liquid–liquid distribution constants of solutes in any biphasic liquid system [71].

A new aqueous two phase liquid system (ATPS) based on the water-soluble [BMIm]Cl, potassium dibasic phosphate (K_2HPO_4) and water was recently proposed when K_2HPO_4 was added to its solution [72]. Based on this foundation, Berthod and coworkers established the ternary phase diagram of the systems K_2HPO_4/[BMIm]Cl/water and Na_2CO_3/[BMIm]Cl /water and compared them to the PEG 1000 and PEG 10000/K_2HPO_4/water classical systems [73]. It was found that the [BMIm]Cl ATPS liquid phases were much easier to retain in the two CCC columns than the PEG 1000 ATPS phases. The other major difference between the two ATPSs is that the polarity of the IL-rich upper phase is significantly lower than the corresponding PEG-rich upper phase.

5. HPLC STATIONARY PHASES

Compared with ILs used as mobile phase additives in HPLC, the application of ILs in stationary phases is fewer and later. However, more attentions to the use of ILs in stationary phase are increasing recently. In 2004, silica particle chemically modified with 1,3-dialkylimidazolium ILs was used for the first time as the stationary phase in HPLC for the separation of alkaloids by Jiang et al. [74] The preparation of this kind of silica confined ionic liquids (SCIL) is shown in Figure 6.

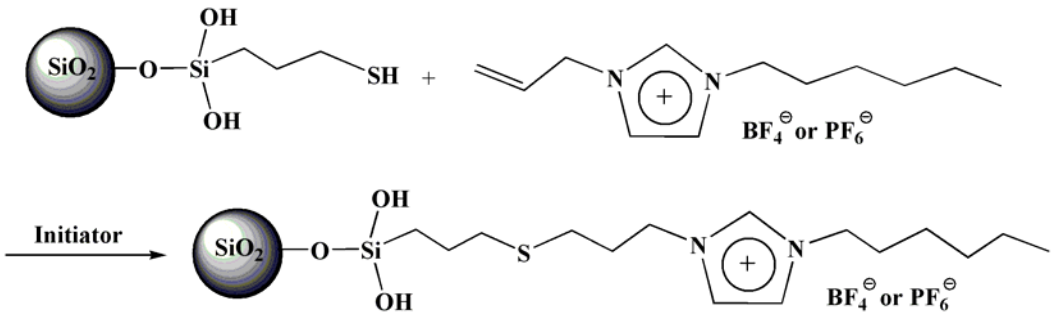


Figure 6. Preparation of ionic liquid-modified stationary phase [74].

Effective separation is ascribed to the dual mechanism of both the hydrophobicity and the ionic property of IL-modified stationary phases. They also pointed out that IL-based stationary phase has great variety and can be investigated further in HPLC because of the large family of ionic liquids with tunable substituted alkyl chains and counter anions. This point has been proved with recent increasing research of IL-based stationary phases in liquid chromatography.

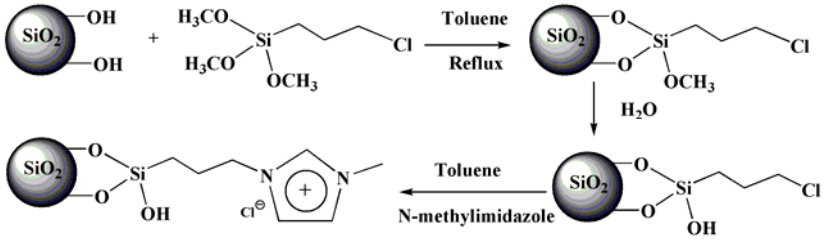


Figure 7. preparation of *N*-methylimidazolium functionalized silica [75].

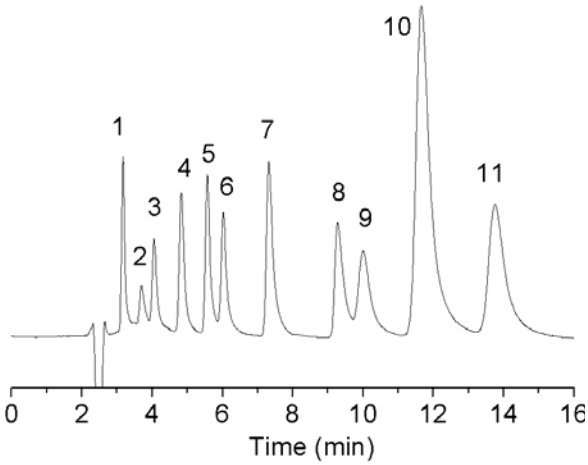


Figure 8. Separation of inorganic and organic anions and neutral organics composed of quinol (1), toluene (2), p-aminobenzoic acid (3), sodium chloride (4), potassium bromide (5), sodium nitrate (6), potassium iodide (7), and potassium thiocyanate (8), sodium p-toluene sulfonic acid (9), potassium hydrogen phthalate (10), sodium salicylate (11) with SilprIm [76].

In 2006, we prepared a new IL-modified phase named *N*-methylimidazolium immobilized on silica (SilprMIm) based on the reaction of 3-chloropropyl silica and *N*-methylimidazole [75]. The method for preparation of this IL-modified silica is the same as preparation of ILs through quaterisation reaction as shown in Figure 7. Common inorganic anions including an iodate, chloride, bromide, nitrate, iodide, and thiocyanate were separated successfully with this new anion-exchange stationary phase. Some organic anions, amines and nucleotides have also been separated respectively and the phase displayed a main strong anion-exchange mechanism and a coexistent reverse-phase interaction, etc.

In the same year, we prepared another anion-exchange stationary phase based on imidazolium- modified silica (SilprIm) with the same way [76]. Inorganic anions, organic anions and several neutral compounds were determined simultaneously and satisfactorily using this phase. The chromatogram was shown in Figure 8.

Two new silica-based long-chain alkylimidazolium stationary phases were prepared and characterized for their use in HPLC in the same method [77]. And their anion-exchange and hydrophobic interaction were compared with several different kind of solutes such as anions, bases, aromatic amines and phenols etc. *N*-Methylimidazolium was also immobilized on a core–shell support to make a *N*-mthylimidazolium functionalized ZrO_2/SiO_2-4 (Zr/SilprMim) stationary phase [78].

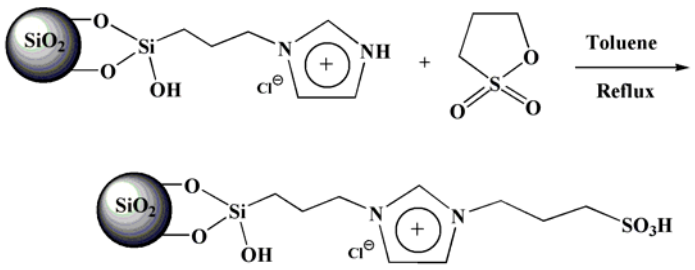


Figure 9. Synthesis steps used in the preparation of zwitterionic stationary phase (SiImPS) [79].

A new zwitterionic stationary phase (SilprPS) was synthesized through that the SilprIm was sulfopropylated in a quaternizing reaction with 1,3-propane sultone [79]. However, because this stationary phase was prepared using a step-by-step graft on silica gel as shown in Figure 9, the phase was charge-imbalanced because of not all the imidazolium groups were reacted with sultone. In order to prepare covalently bonded an acceptable charge-balanced zwitterionic separation materials, we adopted a new method: pure sulfonic ionic liquid (1-allyl-3-(butyl-4-sulfonate)imidazolium combined with $CF_3SO_3^-$) was prepared first, and then it was bonded with 3-mercaptopropyl silica *via* the surface radical chain-transfer reaction [80]. The immobilizations are confirmed with elemental analysis, Raman spectroscopy and X-ray photoelectron spectroscopy (XPS). According to the chromatographic evaluated results, the anions and their countercations combine to make "ion-pairing-like" forms and are separated by the simultaneous electrostatic attraction and repulsion interactions between inorganic anions and their countercations with this 'strong/strong' charged zwitterionic stationary phase (SiImBS). At the same time, 1-allyl-3-butylimidazolium bromide IL ([AyBIm]Br) was also prepared and covalently bonded onto the silica, and then SiImBr was obtained and used for anion-exchange phase. The multiple interactions of SiImBr including

hydrophobic, π–π, and ion–dipole interactions during the separation of polycyclic aromatic hydrocarbons (PAHs) and dipolar compounds were investigated in detail [81].

Stalcup et al. [82] also published a series of papers of SCIL stationary phases. First, butylimidazolium was covalently immobilized on a silica substrate through an n-alkyl tether and the retention characteristics of the resulting stationary phase were evaluated using 28 small aromatic test solutes with reversed-phase conditions and the linear salvation energy relationship (LSER) approach. The retention characteristics of the test solutes show remarkable similarity with phenyl stationary phases under reversed-phase conditions with methanol/water as mobile phase, despite the presence of a positive charge on the new imidazolium phase. The results obtained with acetonitrile/water mixtures are also compared with results obtained using methanol/water mixtures [83]. Later, the anion-exchange retention mechanism of this SCIL phase was demonstrated using nucleotides as solutes [84]. This SCIL phase was also used for the separation of five peptides (Gly-Tyr, Val-Tyr-Val, leucine enkephalin, methionine enkephalin, and angiotensin-II) within 5 min [85].

A novel SCIL stationary phase containing a pyridinium cation was synthesized and characterized utilizing LSER methodology in normal-phase, and it was fonud that the retention mechanism on the pyridinium bromide SCIL phase was more closely correlated to that of a cyano phase than that of a diol phase [86]. A series of SCIL stationary phases with different of cations and anions were prepared as shown in Figure 10. The synthesized phases were characterized by the LSER method to determine the effect of residual linking ligands and the role of the cation and the anion on retention [87].

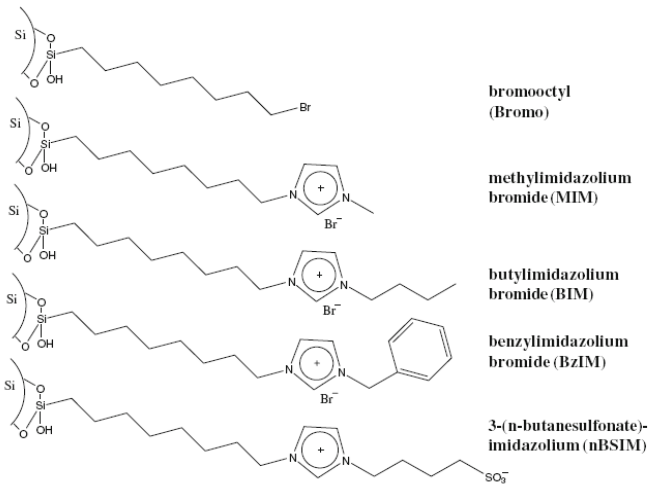
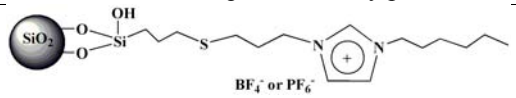
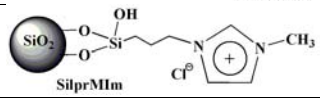
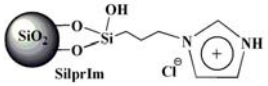
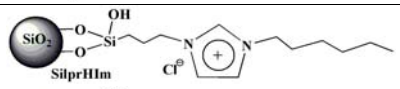
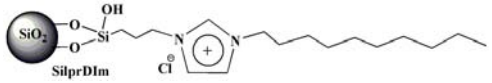
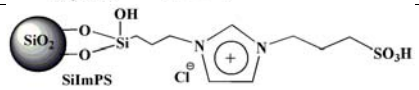
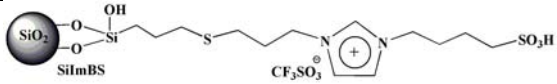
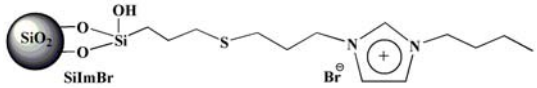
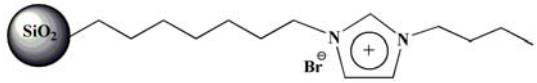
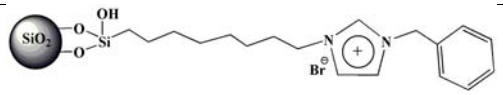
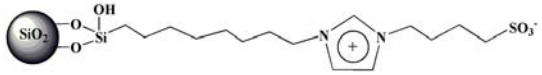
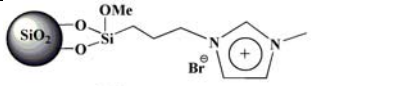
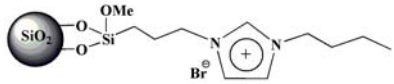
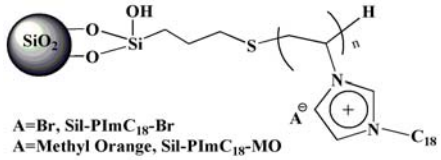


Figure 10. SCIL stationary phase used in reference [87].

Two trimethoxysilane derivatives (1-methyl-3-(trimethoxysilylpropyl)imidazolium and 1-butyl-3-(trimethoxysilylpropyl)imidazolium with bromide as counteranions) were synthesized and used to modify the surface of 3 μm silica particles (Figure 11) for HPLC stationary phases by Wang et al. [88] These two SCIL stationary phases were confirmed with thermogravimetric analysis (TGA), ^{13}C and ^{29}Si NMR spectroscopies, and then evaluated with aromatic carboxylic acids as model compounds. They also pointed out that the separation mechanism appears to involve multiple interactions including ion exchange, hydrophobic interaction, electrostatic interaction and others.

Table 3. Ionic liquid-modified silica stationary phases

Structures of ionic liquid-stationary phases	Solutes	References
	Alkaloids	[74]
	Anions, amines, and nucleotides	[75]
	Anions and phenols	[76]
 	Anions and organic compounds	[77]
	Anions, cations and vitamins	[79]
	Anions and cations	[80]
	Anions PAHs	[80] [81]
	Aromatic test solutes Nucleotides Peptides	[82,83] [84] [85]
 	Aromatic test solutes	[87]
 	Aromatic carboxylic acids	[88]
	PAHs and steroids	[91,92]

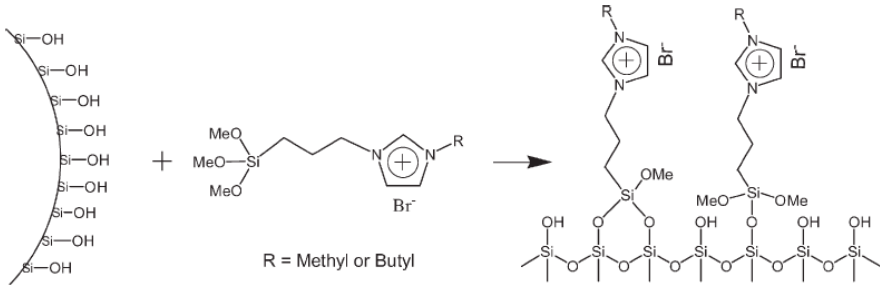


Figure 11. Scheme illustrating the modification of silica particles with the synthesized 1-alkyl-3-propylimidazolium bromide silane [88].

Because SCIL stationary phases have some special properties, they may have some potential application in the future. Some initial applications have reported recently. Three alkaloids including caffeine, theophylline and theobromine in green tea were separated by three silica-based imidazolium stationary phases by Row and coworkers [89]. Instead of using organic and toxic eluent, deionized water can be used as mobile phase for the separation of alkaloids by LC. Xylose and glucose, as the main hydrolyzed products of plant cell wall, were also separated by five synthesized SCIL stationary phases [90]. The effects of the IL cations and anions on the retention of xylose and glucose were studied and the adsorption behavior of these two monosaccharides on the stationary phases was investigated.

Because of the desirability of ionic liquids, SCILs have the same advantages to modify the materials through the change of anions and cations or its alkyl chains. The structure of ionic liquid-modified silica stationary phases and the solutes used to characterize these phases were summarized in the Table 3.

Very recently, a new chromatographic stationary phase via simple modifications of the counter anions in poly(ionic liquid)-grafted silica phase based on ionic self-assembly technology is proposed [91]. A long-chain IL monomer 1-vinyl-3-octadecylimidazolium bromide ([C_{18}VyIm]Br) was prepared first; this monomer was then polymerized on mercaptopropyl-functionalized silica through surface radical chain-transfer polymerization. The obtained PIL-grafted silica (Sil–PImC_{18}–Br) was further self-assembled with methyl orange (MO) to form Sil–PImC_{18}–MO. The synthesis scheme was shown in Figure 12. Sil–PImC_{18}–Br and Sil–PImC_{18}–MO was both packed into stainless steel columns (150×4.6 mm, id) for HPLC. The poly(ionic liquid)-grafted silica materials were characterized by elemental analysis, TGA, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy and ^{13}C NMR spectra [92].

According to Albert et al. [93], one resonance at δ=32.6 ppm is due to *trans* conformations, indicating rigid and ordered chains; another peak at δ=30.0 ppm is due to *gauche* conformations, which can be characterized as mobile and amorphous regions. As shown in Figure 13, the conformational change of the C_{18} chain is visible between the ^{13}C CP/MAS NMR spectra of Sil-PImC_{18}-Br and Sil-PImC_{18}-MO. The spectra for Sil-PImC_{18}-Br showed domination of *gauche* conformations (δ=30.3 ppm) and a low-field shoulder indicating a *trans* conformation (~32.2 ppm).

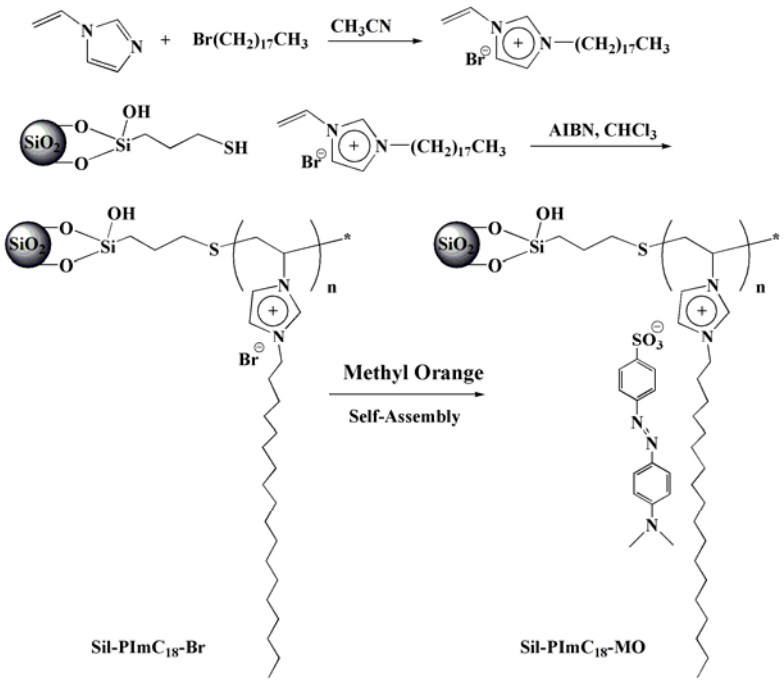


Figure 12. Poly(ionic liquid)-grafted silica stationary phases for HPLC *via* polymerization and ionic self-assembly [92].

On the other hand, Sil-PImC_{18}-MO showed predominantly *trans* conformations (δ=32.4 ppm) with an upfield shoulder indicating *gauche* conformation (δ=30.5 ppm). It is clearly to show that the C_{18} chain in Sil-PImC_{18}-MO become ordered because of the existence of rigid MO.

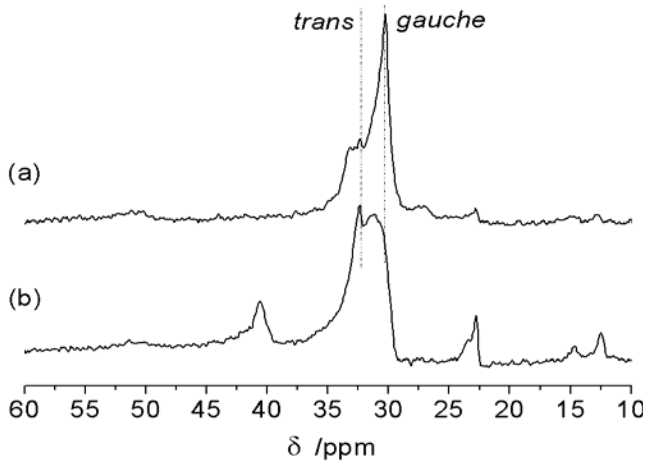


Figure 13. Partial solid-state ^{13}C CP/MAS NMR spectra of (a) Sil-PImC_{18}-Br and (b) Sil-PImC_{18}-MO at 25 °C [92].

When used in HPLC, the phase with MO as counter anions (Sil-PImC_{18}-MO) exhibits ultra-high selectivity towards shape-constrained isomers compared C_{18}, C_{30}, and Sil–PImC_{18}–

Br [92]. The shape selectivity performance could be assessed by SRM 869b, the column selectivity test mixture for liquid chromatography. This material consists of phenanthro[*3,4-c*]phenanthrene (PhPh), tetrabenzonaphthalene (TBN), and BaP with planar and non-planar shapes. In general, the late elution of BaP relative to TBN ($\alpha_{TBN/BaP} < 1$) indicates "polymeric-like" retention behavior with enhanced shape recognition abilities. Conversely, the early elution of BaP relative to TBN indicates "monomer-like" retention behavior with reduced shape selectivity.

As shown in Figure 14a, the elution order is typical of most commercial monomeric C_{18} columns ($\alpha_{TBN/BaP}$ = 1.93). In Figure 14c, Sil-PImC_{18}-Br presented some polymeric-like retention behavior, though it was not extensive; the retention of BaP was slightly stronger than that of TBN ($\alpha_{TBN/BaP}$ = 0.97).

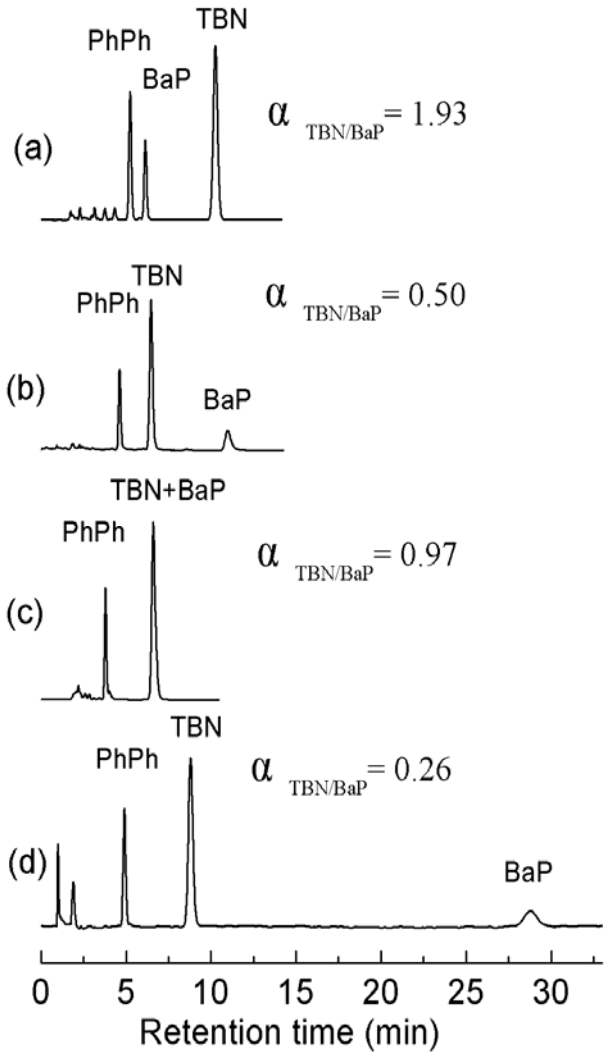


Figure 14. Separation of SRM 869b test mixture with (a) C_{18}, (b) C_{30}, (c) Sil-PImC_{18}-Br and (d) Sil-PImC_{18}-MO columns with methanol as mobile phase at 10 °C [92].

However, in Sil-PImC_{18}-MO ($\alpha_{TBN/BaP}$ = 0.26), the retention of BaP was considerably stronger than that of TBN, indicating that Sil-PImC_{18}-MO exhibited strong polymeric-like retention behavior with ultra-high shape selectivity, higher than that exhibited for C_{30} ($\alpha_{TBN/BaP}$ = 0.50).

Between Sil-PImC_{18}-Br and Sil-PImC_{18}-MO, the most difference is different counter anions. Therefore, it was demonstrated that the counter anions perform an important role in modifying shape-selectivity of the stationary phases.

Enhanced shape selectivity was found in Sil-PImC_{18}-MO when compared to other columns as indicated by SRM 869b. As expected, Sil-PImC_{18}-MO yield more complete separations of 16 solutes of SRM 1647e as illustrated in Figure 15.

Satisfied separation for acenaphthene and fluorene ($\alpha_{3/4}$ = 1.36), and benzo[*a*]anthracene and chrysene ($\alpha_{9/10}$ = 1.42) in Sil-PImC_{18}-MO was obtained. Almost no separation was observed in other columns in the same isocratic elution conditions. Good selectivity also can be found at the separation of the set of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene ($\alpha_{12/11}$ = 1.53).

The possible interaction mechanism that Sil-PImC_{18}-MO presented ultra-high selectivity to the constrained isomers was schemed in Figure 16. It was due to that a highly ordered arrangement could be formed between the C_{18} chain and MO because of the existence of rigid azobenzene, as determined by ^{13}C CP/MAS NMR spectra. And the highly ordered arrangement would induce "molecular slots," so that long, narrow isomers are retained longer than square isomers.

With phthalate buffer solution as the mobile phases and non-suppressed conductivity detection, high column efficiencies and excellent selectivity were obtained in the separation of inorganic anions as shown in Figure 17. Chromatographic parameters are calculated and the results show that the coated column possesses significant potential for the analysis of some inorganic anions such as CH_3COO^-, IO_3^-, Cl^-, BrO_3^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, I^-, BF_4^-, and SCN^- with high column efficiency and good selectivity.

Ionic liquids can not only be covalently immobilized to the silica stationary phases; they also can be coated in the conventional column by hydrophobic interaction. Two long-chain ILs including 1-dodecyl-3-methylimidazolium bromide ([C_{12}MIm]Br) and 1-tetradecyl-3-methylimidazolium bromide ([C_{14}MIm]Br) as new cationic surfactants were coated on ODS columns, respectively, as pseudostationary phases to separate common inorganic anions for ion chromatography [94].

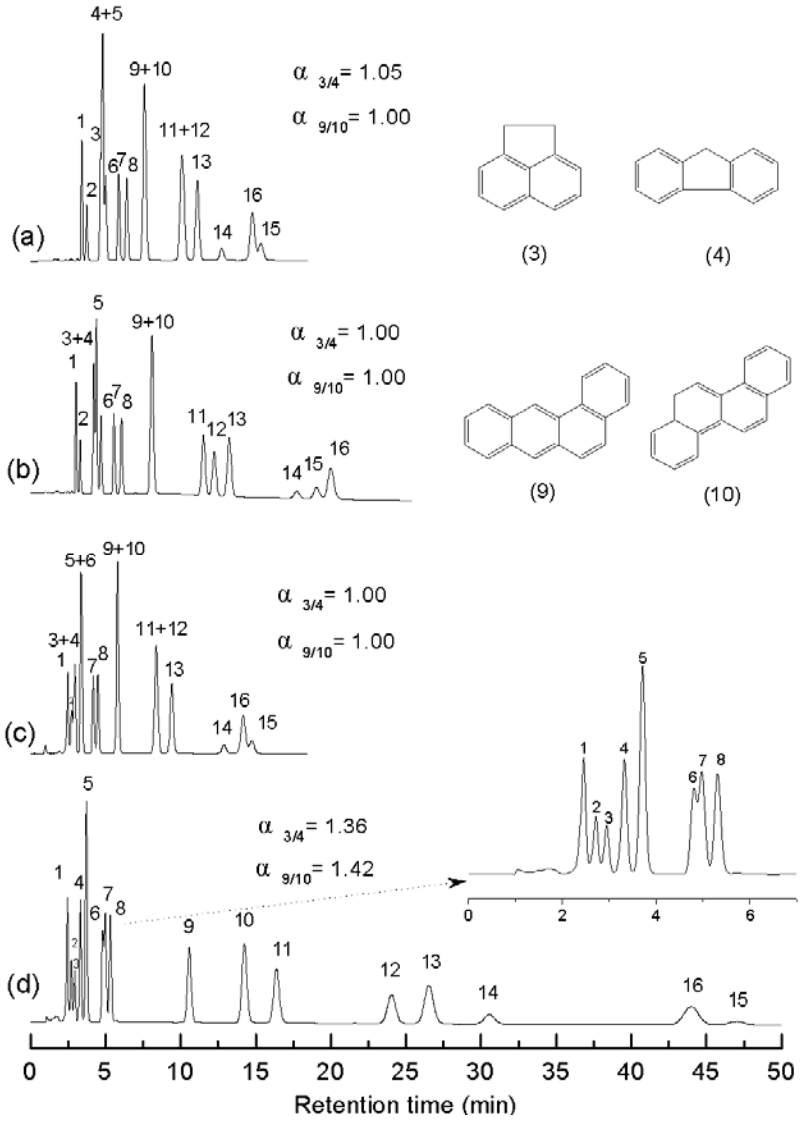


Figure 15. Separation of the 16 Priority Pollutant PAHs (SRM 1647e) with (a) C_{18}, (b) C_{30}, (c) Sil-PImC_{18}-Br and (d) Sil-PImC_{18}-MO columns with methanol/water (9:1) as mobile phase at 30 °C except (d) at 35 °C. Key: (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo[*a*]anthracene, (10) chrysene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) dibenz[*a,h*]anthracene, (15) bezo[*ghi*]perylene, (16) indeno[*1,2,3-cd*]pyrene. [92]

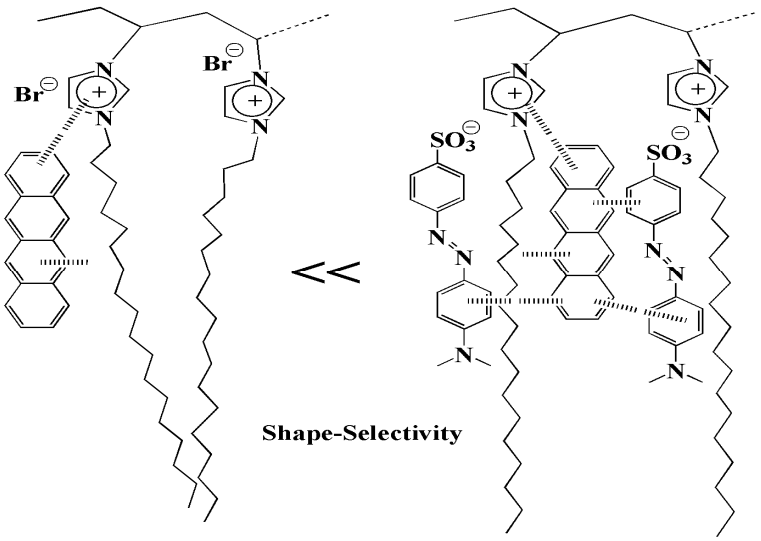


Figure 16. Possible interaction mechanism to explain why Sil-PImC_{18}-MO shows high retention towards linear PAHs [92].

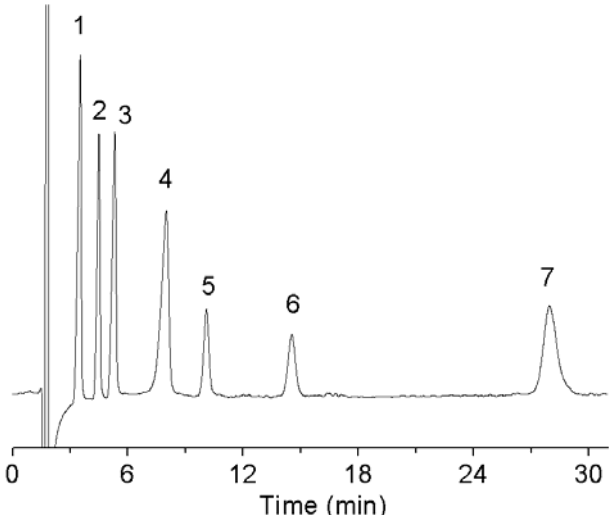


Figure 17. Separation of a mixture of anions using an ODS column (150 × 4.6 mm, i.d.) coated with [C_{12}MIm]Br. Mobile phase, 5 mM potassium hydrogen phthalate (pH 4.0). Analytes: chloride (1), bromide (2), nitrate (3), sulfate (4), iodide (5), fluoroborate (6), and thiocyanate (7) [94].

6. SUPERCRITICAL FLUID CHROMATOGRAPHY

Supercritical fluid chromatography (SFC) has attracted wide interests in the pharmaceutical industry as a useful separation technique, because supercritical CO_2 as mobile phase with its low viscosity and high diffusivity can accommodate higher flow rates with modest pressure drops [95,96].

This advantage makes it ideal for preparative chromatographic separations and requires less organic solvent than conventional preparative HPLC. Common packing columns for HPLC also can be used for SFC. Chou et al. [97] prepared an SCIL stationary phase by covalently bonding 1-octyl-3-propylimidazolium chloride IL onto a 5 μm silica and used in SFC for the first time. This stationary phase allowed the simultaneous separation of acidic, basic, and neutral compounds, including fenoprofen, ibuprofen, acetaminophen, metoprolol, naphthalene, and testosterone, under SFC conditions. They found the performance of the IL-modified column, in terms of resolution, was clearly superior to that of commercial C_{18} columns.

They also judged that electrostatic and hydrogen-bonding interactions are the dominant associative interactions for the retention of acidic and basic compounds by this SCIL stationary phase; weak hydrophobic interactions may also be involved in the low retention of neutral compounds. The same as used in HPLC, SCIL stationary phase also presented multiple-interactions to the analytes in SFC mode.

Recently, Smuts et al. [98] have investigated a class of prepared SCIL stationary phases in SFC with a two part study: a cation effect study and an anion effect study. The former study compares six SCILs with different cations including tripropylphosphonium (PPr_3), tributylphosphonium (PBu_3), methylimidazolium (Me-Im), benzylimidazolium (Bz-Im), triphenylphosphonium (PPh_3), and 4,4'-bipyridyl (4,4'-BiPy) with all the counter anions as CF_3COO^-. In the latter study, the stationary phases consisted of a newly bonded tributylphosphonium cation and different counter anions including CH_3COO^-, CF_3COO^-, Cl^-, NTf_2^-, and ClO_4^-. The general order of retentivity for the cation study was 4,4'-BiPy > PPh_3 > Bz-Im > Me-Im > PPr_3 > PBu_3. The favorable behavior of phosphonium-based stationary phases is reported for the first time in SFC and the tuning ability of SCIL stationary phases were proved again here. More SCIL stationary phases can be used in SFC in the future.

7. MONOLITHIC COLUMNS

Monolithic columns have attracted significant attention in chromatographic science because of the high porosity and large through-pore size with small-sized skeletons [99]. Compared with traditional packed columns, monolithic columns have many improvements including fast mass transfer, absence of end frits, and elimination or significant reduction of certain operation problems inherent in packed columns due to the presence of end frits, etc.

In order to combine the characteristics of *N*-methylimidazolium-based IL stationary phases and monolithic silica columns, an ionic liquid (IL) was introduced into the organic-silica hybrid monolithic column as the stationary phase for capillary electrochromatography (CEC) [100].

The monolithic silica matrix containing chloropropyl functional group was prepared *via* a sol–gel process of tetramethoxysilane and 3-chloropropyltrimethoxysilane. And then the monolithic hybrid silica was modified with *N*-methylimidazole as shown in Figure 18. The morphology of the column was characterized by scanning electron microscope. The electroosmotic flow of the IL-modified hybrid monolithic column was reversed when using acidic buffer. Four aromatic hydrocarbons were separated with 40% acetonitrile-phosphate

buffer as the mobile phase and seven inorganic ions were efficiently separated with the phosphate buffer in CEC.

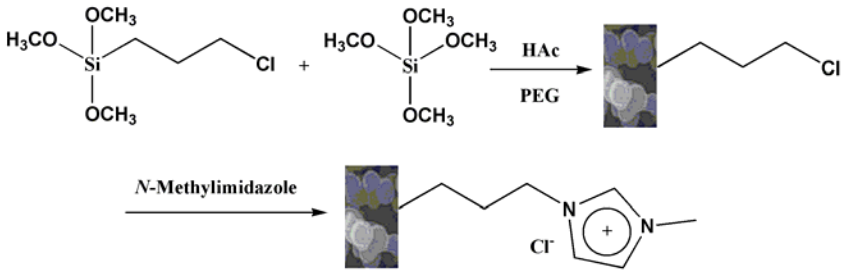


Figure 18. Preparation of *N*-methylimidazoilum-modified silica hybrid monolithic column [100].

Similar work of novel *N*-methylimidazolium-functionalized monolithic silica column was also published by Jia and coworkers [101]. In this work, the monolithic silica column was prepared first, and then the column was modified by two steps as described in our previous work [75]. The mixed-mode retention mechanism was confirmed through the separation of some compounds including inorganic anions, aromatic acids, nucleotides, phenols, alkylbenzenes and PAHs. Figure 19 shows the comparison of the separations of inorganic anions and alkyl benzenes on *N*-methylimidazolium modified column and native silica column.

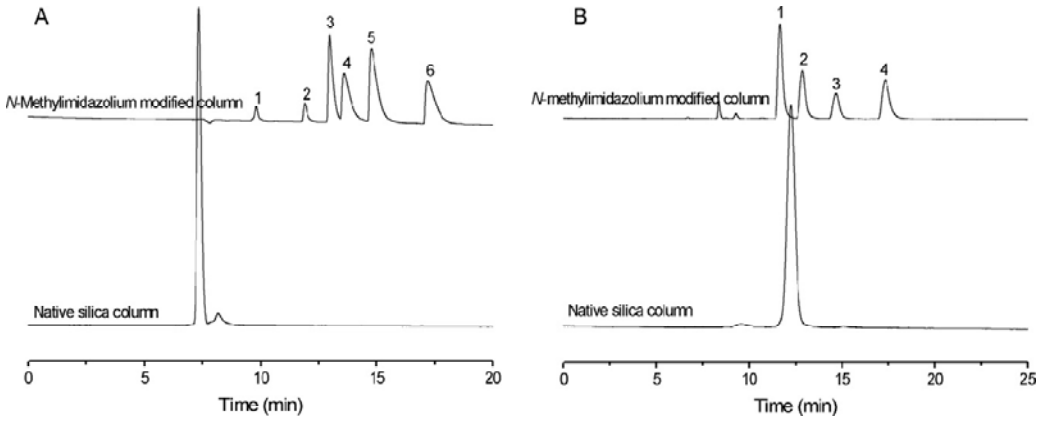


Figure 19. Chromatograms of inorganic anions and alkyl benzenes separated on N-methylimidazolium modified column and native silica column. Experimental conditions: (A): solute, inorganic anions; column, 32.5 cm × 200 μm i.d.; mobile phase, 20 mM Na_2HPO_4 (pH 5.5)-CH_3CN (40:60, v/v); linear velocity, 0.69 mm/s; detection, UV at 200nm. Peak identification: 1, iodate; 2, bromate; 3, bromide; 4, nitrite; 5, nitrate; 6, iodide. (B): solute, alkylbenzenes; column, 39 cm × 200 μm i.d.; mobile phase, methanol-water (70 : 30, v/v); linear velocity, 0.97 mm/s; detection, UV at 214 nm. Peak identification: 1, methylbenzene; 2, ethylbenzene; 3, propylbenzene; 4, butylbenzene [101].

CONCLUSION

Ionic liquids have attracted many academic and applied attentions in different fields because of their unique physical and chemical properties. In HPLC, most research about ILs was focused on the application of mobile phase additives and silica-confined ionic liquid stationary phases. Ionic liquids as additives could suppress the residual silanol so that improve the separation efficiencies, reduce the tailing of basic solutes. SCIL-stationary phases are interesting because they were found that usually have multiple interactions to the different analytes. The most importance is that SCIL-stationary phases have similar desirability as ILs so that a lot of new chromatographic materials could be desired for the using in HPLC. With the development of ILs in chromatographic science, more and more ILs will be used in HPLC.

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