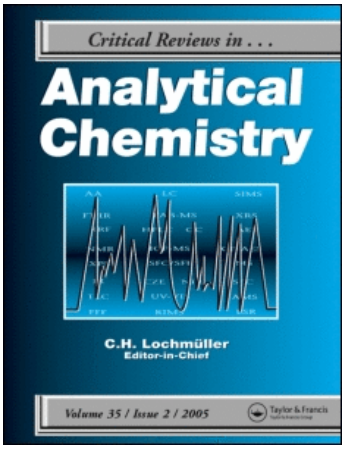

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Michał Piotr Marszałł[a]; Roman Kaliszan[a]
[a] Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland

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Application of Ionic Liquids in Liquid Chromatography

Michal Piotr Marszałł and Roman Kaliszan
Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, 80-416 Gdańsk, Poland

Interest in ionic liquids (ILs) for their potential application in analytical chemistry continues to grow. Their usefulness can be due to favourable physicochemical properties, like the lack of vapour pressure, good thermal and chemical stability as well as very good dissolution properties regarding both organic and inorganic compounds. A specific feature of ILs is that these compounds provide strong proton donor-acceptor intermolecular interactions. As a result, ILs are able to affect on the hydroxy groups of the silica supports the most popular stationary phases in liquid chromatography (LC). It is well known that the hydroxy groups, called free or isolated silanols cause serious problems in LC, especially when separating basic compounds. This review focuses on the application of ILs in LC and capillary electrophoresis (CE) and comparisons of their efficiency with standard silanol suppressing additives to mobile phases.

Keywords capillary electrophoresis, free silanols, ionic liquids, liquid chromatography, mobile phase additives

INTRODUCTION

Ionic liquids (ILs) are called by many synonyms, such as room-temperature ionic liquids, liquid organic salts, low temperature molten salts, ambient temperature molten salts or ionic fluids (1). Generally, they are organic salts formed by bulky organic cations and various anions that are liquid at room temperature. They are also called neoteric solvents, meaning new types of solvent or older materials that are finding new application as solvents.

Because of their specific properties, nowadays ILs are applied in several areas of chemistry, like organic synthesis, (bio)catalytic reactions and electrochemistry (2–6). Antimicrobial activities of ILs were also reported (7).

The number of publications presenting the original applications of ILs increases every year (Fig. 1). The growing interest in molten salts is also visible in analytical chemistry, especially in LC and CE (8–10). It is due to their non-explosive and good solvating properties, chemical stability in a wide range of temperatures and very low volatility. Thus, ILs can displace the environmentally harmful volatile organic solvents (VOCs). Therefore, ILs are considered as ideal solvents in the so called "green chemistry" (1, 11). Actually, that claim is a bit controversial. Several researchers report bioactivity of anion/cation of ILs, their carcinogenicity, cytotoxicity, genotoxicity and teratological effects (12).

In analytical chemistry, ILs were at first applied in gas-liquid chromatography as a new class of stationary phases (13–17). The study of physical properties of ILs for assessing their suitability for use as solvent in LC was by Poole et al. (18). The alkylammonium-based ILs in that study had a moderate viscosity but when mixed with commonly chromatographic solvents (acetonitrile, methanol, water), their viscosity sufficiently decreased to permit their use as mobile phases in LC.

Dialkylimidazolium ILs that contain tetrafluoroborate ($[BF_4]$) anions are water-stable compounds which dissolve in typical liquid chromatographic solvents. These agents exhibit marked ability to participate in coulombic interaction of the orientation and induction type as well as in specific solute-ion interactions, especially proton donor-acceptor interactions (19).

Effects of free silanols on retention are difficult to control and are especially deleterious as regards the chromatographic behavior of basic analytes (20, 21). The problem concerns even the most modern highly purified silica supports, which are the most popular in Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) (22). In search for efficient suppressors of free silanols, attention was turned to the imidazolium tetrafluoroborate ILs as mobile phase modifiers in LC (23). Recently Koel (9) published a review in this journal on the application of ILs in analytical chemistry in a broad sense. Chromatography was treated only marginally and

Address correspondence to Roman Kaliszan, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland. E-mail: roman.kaliszan@amg.gda.pl

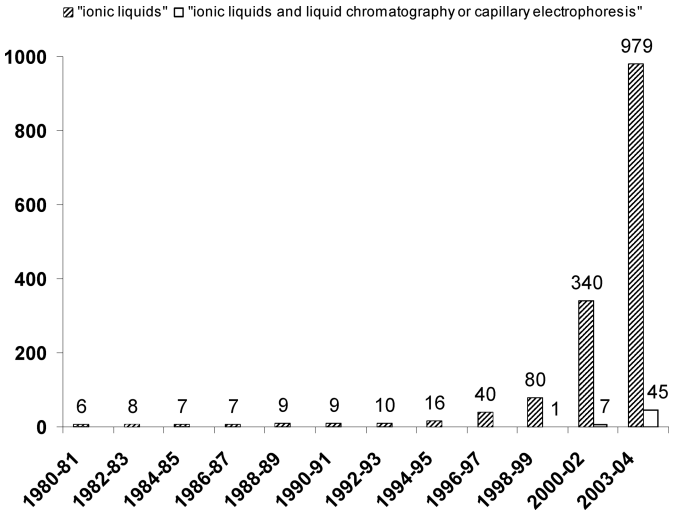


FIG. 1. The number of publication on the subject "ionic liquids" and "ionic liquids and liquid chromatography or capillary electrophoresis" identified by Scopus ⟨http://www.scopus.com/scopus/home.url⟩.

emphasis was put on electrochemistry, electrophoresis, extraction, spectroscopy and mass spectrometry.

THIN-LAYER CHROMATOGRAPHY

The first paper presented the application of ILs in TLC as the new silanol suppressing agents has been that by Kaliszan et al. (23). The evidence gathered demonstrates that imidazolium tetrafluoroborate ionic liquids are valuable, efficient suppressors of free silanols that are responsible for unwanted, irreproducible, difficult to quantify and to control, attractive interactions of chromatographic stationary phases with basic analytes. The following ILs were the subject of that study: 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-methyl-3-hexylimidazolium tetrafluroborate ([HxMIM][BF_4]) and 1-hexyl-3-heptyloxymethylimidazolium tetrafluoroborate ([Hx-HpOMIM][BF_4]). The abbreviations given are commonly accepted and represent the kind of alkyl chain at the imidazolium ring and anion of IL.

The imidazolium classes of ILs could be used as mobile phase modifiers to improve chromatographic separation of the basic compounds more effectively than the standard amine additives to eluent. Figure 2 illustrates the effect of concentration of [EMIM][BF_4] added to neat acetonitrile as the eluent, on the retardation factor of 8 basic drugs on an octadecyl bound-silica stationary phase. As evident from Figure 2, all the analytes studied were not moved from the start by 100% acetonitrile eluent. Saturation of adsorption has been observed at the imidazolium tetrafluoroborate concentration of ca. 0.5% v/v for all the three ionic liquids tested on both the silica- and the octadecylsilica-covered plates with both neat acetonitrile and with water-acetonitrile mixtures of various compositions as the mobile phases. Data in Figure 2 also demonstrate that suppressing of attractive effects of silanols with respect to basic analytes is much weaker when the standard amino quenchers are added to the eluent instead of the imidazolium tetrafluoroborates. Triethylamine (TEA), dimethyloctylamine (DMOA) and ammonia (NH_4OH) have low or negligible effect on the test drugs retention, even at the highest concentrations applied.

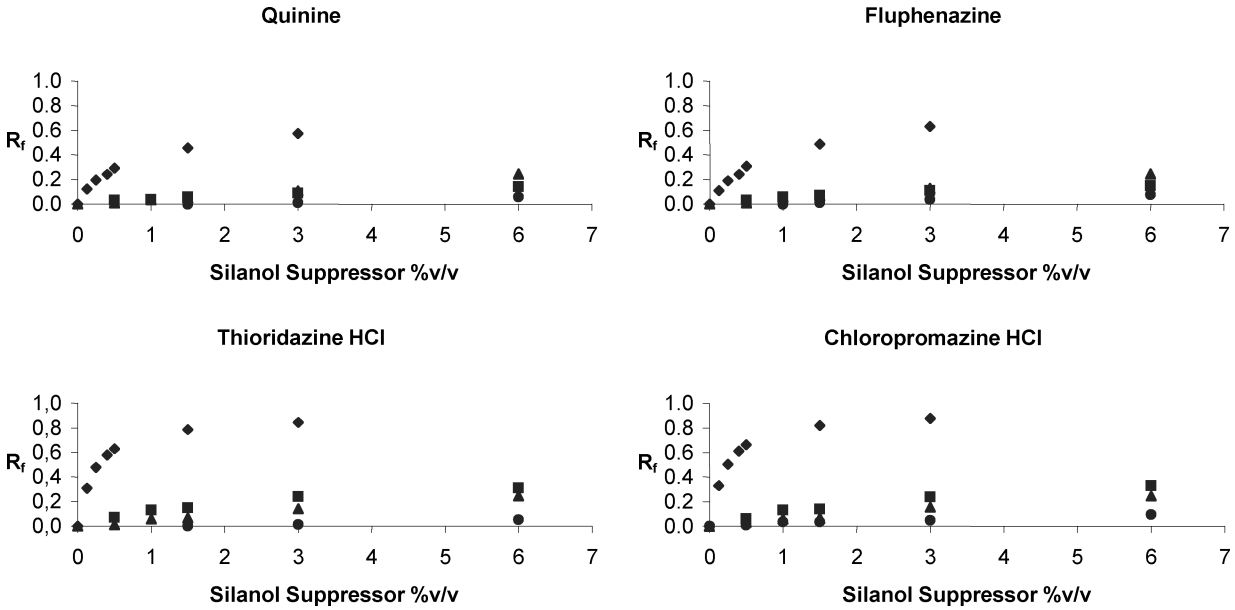


FIG. 2. Thin-layer chromatographic retardation factor, R_f, on octadecylsilica-covered plates in relation to volume percent of [EMIM][BF_4] (♦), TEA(■), DMOA(▲) and NH_4OH(●) in pure acetonitrile as the mobile phase. (Reprinted with permission from ref. 23, © 2003 Elsevier B. V.)

The silanol suppressing mechanism of action of ILs is well documented by the Langmuir plots of dependence of $1/R_f$ of an exemplary analyte (tiamenidine) on the reciprocal of the additive concentration in the eluent (Fig. 3). A typical Langmuir adsorption is observed for [EMIM][BF_4] and TEA. Both plots cross Y-axis at $1/R_f = 1$ thus indicating that the analyte would be completely unretained at infinite concentration of a silanol suppressor. The slope of the plot for [EMIM][BF_4] is much less steep than that for TEA proving a more effective adsorption of the former compound. Poor adsorption of DMOA and NH_4OH is clearly evident from Figure 3, which disqualifies those substances as the effective silanol suppressors.

The reversed-phase TLC separation systems obtained with the help of an imidazolium tetrafluoroborate additive produce the R_M parameters best fitting to the classical linear Snyder–Soczewiński dependence of R_M on organic modifier concentration in the mobile phase (Fig. 4). The improved linearity of R_M vs. % organic modifier may be used in determinations of liquid chromatographic retention parameters extrapolated to zero percent of organic modifier (R_M^0 or $log\ k_w$), which are considered the most reliable chromatographic measures of lipophilicity (hydrophobicity). That is a consequence of elimination of the uncontrollable attractive interactions of base analytes with free silanols. The elimination is much more effective than that provided by typical amine quenchers.

The unique silanol suppressor properties of imidazolium tetrafluoroborates have been exploited to improve liquid chromatographic separation of components of a basic drugs' mixture. In Figure 5 thioridazine, trifluopromazine, phenazoline, naphazoline, tiamenidine and a mixture of the drugs were spotted on octadecylsilica plates from left to right, respectively. The plates were developed with water-acetonitrile 40:60 v/v eluent, either neat or containing 1.5% v/v of various additives. The first chromatogram from the left in the upper row was obtained with a nonmodified mobile phase. Next chromatograms show a negligible effect of ammonia and DMOA on analytes' mobility. Certainly, a better separation, however by no means satisfactory, provides TEA (first plate from the left in the bottom row). Advantages of [EMIM][BF_4] are convincingly presented by the second chromatogram from the left in the bottom row (Fig. 5e). Here the analyte spots are compact, without tailing and are distributed over a wide range of plate length. The separation of the components of the mixture of the extremely badly separable by liquid chromatography drugs appears to be satisfactory. The last chromatogram in the bottom row was developed with the addition of the buffer of pH 2.87 to the eluent. That was done because 1.5% v/v solution of [EMIM][BF_4] in water was found to provide such a pH. The experiment was to check whether the separation produced by [EMIM][BF_4] had not been due to the pH change caused by the additive.

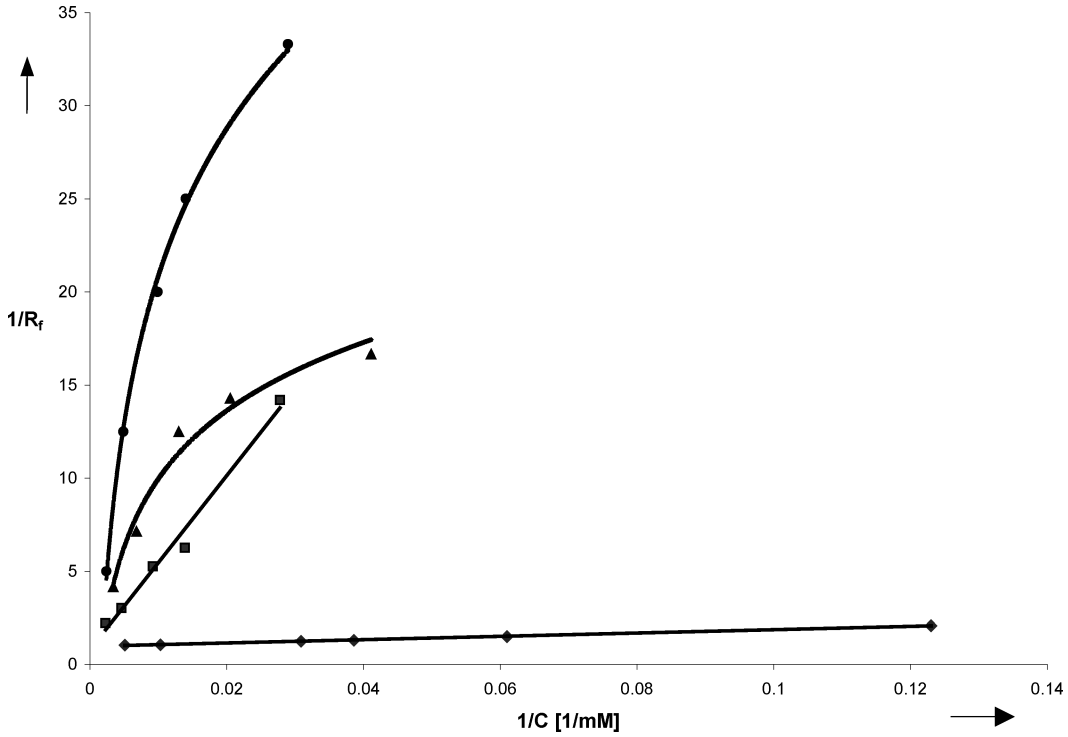


FIG. 3. Plots of reciprocal of retardation factor of tiamenidine on octadecylsilica plates with acetonitrile as eluent versus the reciprocal of concentration of additive in the mobile phase. Additives are denoted as follows: [EMIM][BF_4] (♦), TEA(■), DMOA(▲) and NH_4OH(●). (Reprinted with permission from ref. 23, © 2003 Elsevier B. V.)

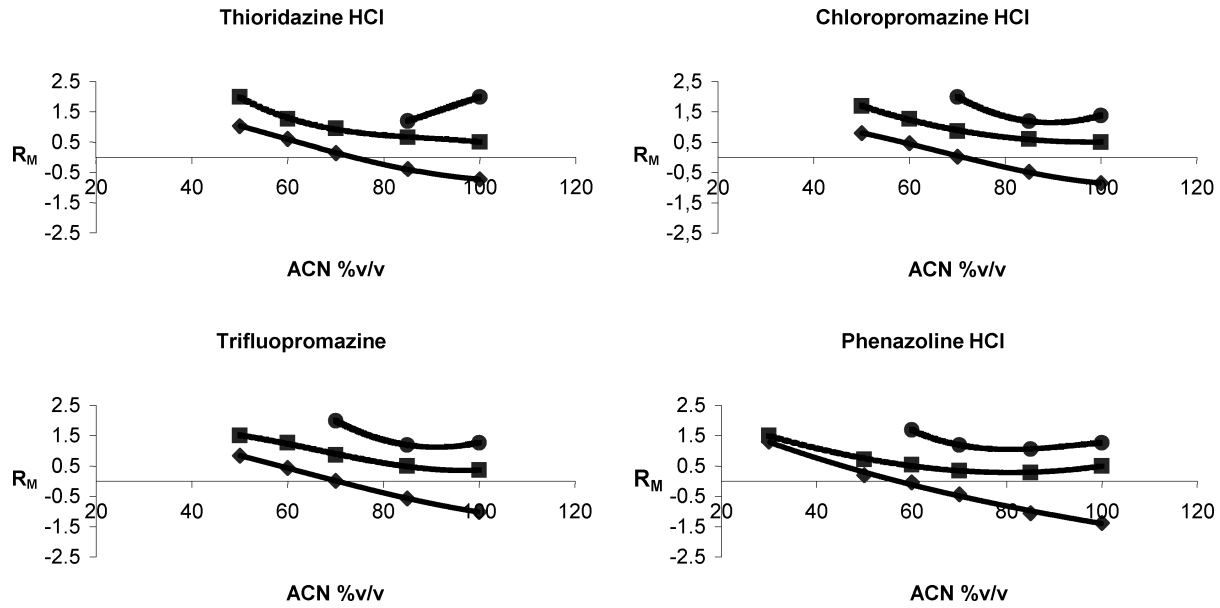


FIG. 4. Thin-layer chromatographic retention parameter, $R_M = \log(1/R_f - 1)$, of basic test drug analytes determined on octadecylsilica-covered plates in relation to the volume percent of acetonitrile in water-acetonitrile eluent. The mobile phases contained 3% v/v of [EMIM][BF_4] (♦), TEA(■) and NH_4OH(●). (Reprinted with permission from ref. 23, © 2003 Elsevier B. V.)

Summarizing the preceding observations, one can conclude that adding of imidazolium tetrafluoroborates to liquid chromatographic mobile phases produces partition chromatographic systems universally applicable for basic. Such systems should allow for a reliable prediction of retention as a function of eluent composition and hence, for a rational optimization of separation conditions.

Also the addition of an IL into the mobile phase appeared to be useful in optimization of chromatographic separation of a homologous series of peptides in normal-phase TLC on a silica support (24). Different behavior of peptides in TLC was observed after addition of [EMIM][BF_4] to the eluent in comparison to the system without the IL. Nonlinear dependence of the retention coefficient, R_M, of peptides on the volume percentage of acetonitrile in the eluent was found in normal-phase TLC with and without [EMIM][BF_4] in the mobile phase. In general, R_M increased with increasing concentration of acetonitrile. Depending on the modifier of the mobile phase, R_M can be described well with a quadratic function or with a third-degree polynomial function. On the basis of experimental data, a quadratic model was selected in the case of mobile phase without IL:

$$R_M = a + bX + cX^2 \qquad [1]$$

where $R_M = \log[(1 - R_f)/R_f]$, and R_f is the retardation coefficient in TLC; a, $b$and $c$are constants for a given analyte and a TLC system; $X$is the volume fraction of the stronger solvent in the mobile phase ($X = \%B/100$).

A third-degree polynomial model was taken on the basis of experimental data as the most appropriate for the TLC system with IL present in the mobile phase:

$$R_M = a' + b'X + c'X^2 + d'X^3 \qquad [2]$$

where $a\prime$, $b\prime$, $c\prime$ and $d\prime$ are constants for the given analyte and the TLC system.

Moreover, the preliminary experiments were undertaken to test the possibility of combining online the proposed TLC-ionic liquid separation method of peptides with matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) in the process of identification of peptides, specifically in proteomics (25, 26).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

In general, tailing problems in association with a poor separation reproducibility of basic analytes on chemically modified bonded-silica phases are often observed in both TLC and HPLC (27). Preliminary experiments were undertaken to suppress silanophilic interactions by 1,3-dimethylimidazolium methyl sulfate IL ([MMIM][$MeSO_4$]) additive to eluent in reversed-phase HPLC (28). Chromatograms illustrating the influence of [MMIM][$MeSO_4$] added to the mobile phase composed of 50:50 acetonitrile:phosphoric buffer pH 3 (v/v) on the separation of the mixture of six basic drugs are presented in Figure 6. Comparing chromatograms (a-c) in Figure 6, one will note that increasing the percentage of IL in the mobile phase decreases the retention and improves separation of all the tested analytes. Obviously, the silanol suppressing properties of alkylimidazolium-based ILs improve the shape of peaks and remove peak tailing observed in the reference system.

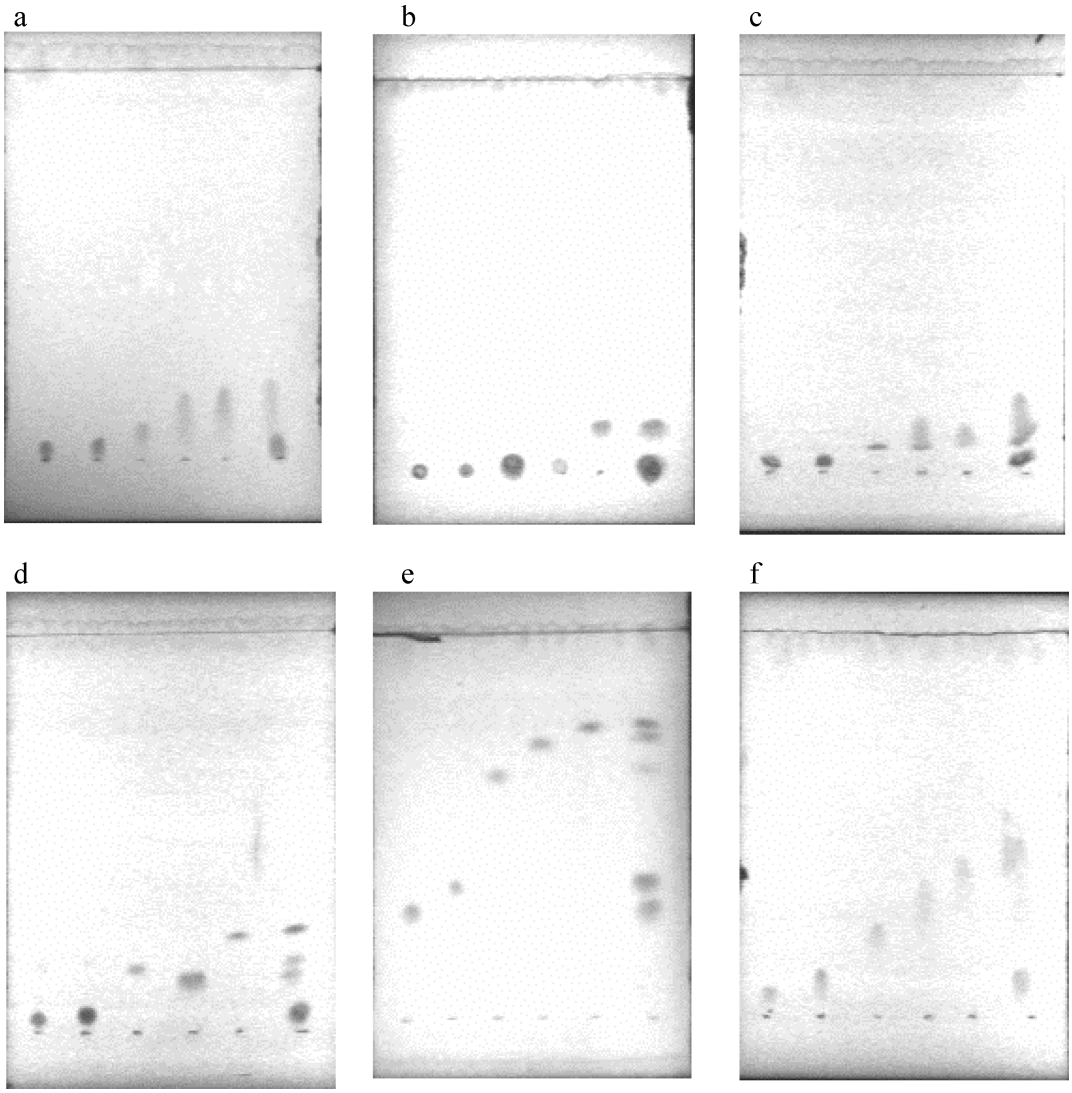


FIG. 5. Chromatograms of thioridazine, trifluoropromazine, phenazoline, naphazoline, tiamenidine and the mixture of the drugs, as spotted from left to right, on RP-18 F_{254} plates, developed with water-acetonitrile 40:60 v/v eluent either pure or containing 1.5% v/v of various additives: a–no additive; b–NH_4OH; c–DMOA; d–TEA; e–[EMIM][BF_4]; f–buffer of pH 2.87.

Influence of temperature on the retention of basic analytes was investigated (Fig. 7). The relationship between the retention parameter of analyte (log k) and the reciprocal of absolute temperature (1/T) is linear. That observation confirms that the problems with non-linearity of van't Hoff plots, assigned to temperature are marginal.

Recently, several efforts to employ ILs in the LC practice have been reported. Using imidazolium and pyridinium tetrafluoroborate ILs a successful HPLC separation of catecholoamines, nucleotides and ephedrine derivatives was achieved (29–31). The authors supposed that the separation mechanism involves the competition between imidazolium cations and polar group of analytes for free silanols of the stationary phase. Only a slight change in column efficiency and peak tailing factor after exposure of C_{18} column to ILs was noted. It means that ILs were not harmful to the silica-based column.

The effects of the length of alkyl chain and the type of counterions on different ILs as well as their concentration were determined (32, 33). The differences between ILs and tetrabutylammonium bromide (TBA) efforts on the separation of *o*-, *m*-, *p*-phthalic acids were compared. The conclusion was that ILs are ion-pair reagents in essence, although their hydrophobicity and hydrogen bonding properties also play important roles in separation mechanism. Part of the ILs coat on the surface of the octadecylsilica stationary phase. Thus, retention times of amines, including benzidine, benzylamine, *N*-ethylaniline and

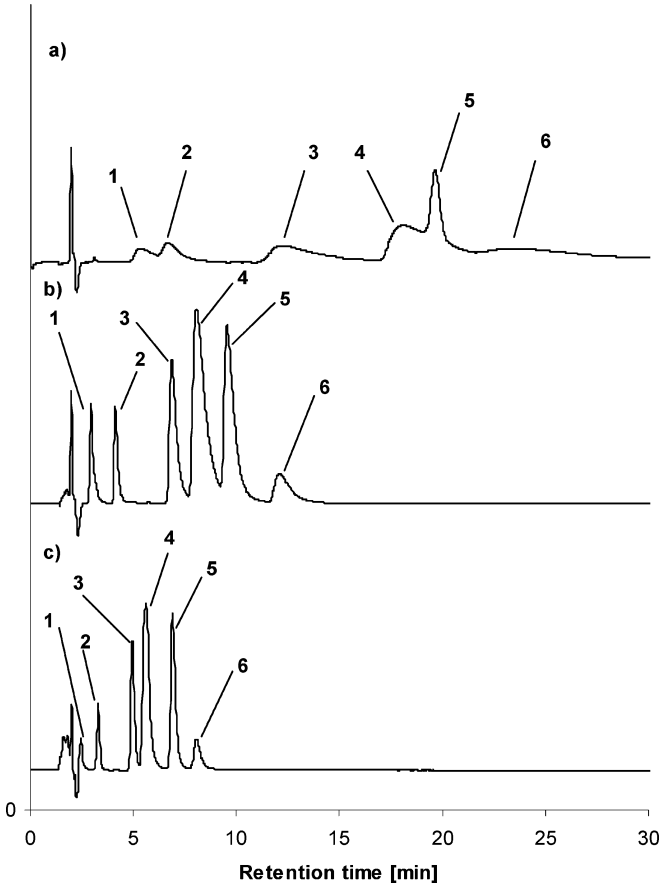


FIG. 6. Chromatograms of the separation of naphazoline (1), phenazoline hydrochloride (2), fluphenazine (3), chlorpromazine hydrochloride (4), triflupromazine (5) and thioridazine hydrochloride (6): a) acetonitrile:phosphorate buffer (pH 3) 50/50 (v/v) as the mobile phase, b) acetonitrile:phosphorate buffer (pH 3) 50/50 (v/v) with the addition of 0.05% (v/v) IL [MMIM][$MeSO_4$], c) acetonitrile:phosphorate buffer (pH 3) 50/50 (v/v) with the addition of 0.5% [MMIM][$MeSO_4$]. (Reprinted with permission from ref. 28, © 2003 Elsevier B. V.)

N,*N*'-dimethylaniline, were shortened because of the repulsion between the adsorbed imidazolium cation and the ionized amine (pH 3).

A comparative study of peak shape, chromatographic behavior, elution strength and resolution of β-blockers using reversed-phase column and isocratic elution and aqueous-organic mobile phases containing additives such as ILs or TEA was reported (34). [BMIM][BF_4] appeared better additive compared to TEA with reference to the efficiency and improvement of asymmetry factor achieved for β-blockers, allowing to obtain higher plate numbers. The silanol screening effect is always observed for hexafluorophosphate and tetrafluoroborate ILs due to the cation. The retention factor is claimed to depend on the hydrophobic nature or chaotropic character of ILs anion.

Recently, methods for the preparation of immobilised ILs stationary phases were proposed (35). ILs can be bound to a surface either by covalent bonds between silanol groups and the anion or the cation of the IL, or without covalent bonds forming the so-called supported liquid phases (SLPs).

A new *n*-butylimidazolium stationary phase covalently immobilized on a silica support has been synthesized (36). Butylimidazolium cations with Br- counter-ions were immobilized on porous silica particles and examined as a stationary phase for LC. Retention of 28 small aromatic test solutes is appeared similar to that obtained on a conventional phenyl stationary phase. The linear solvation energy relationship (LSER)-derived system coefficients and direct comparison with a conventional phenyl phase confirmed comparable retention behavior.

Trimethoxysilane ionosilane derivatives of ILs based on alkylimidazolium bromides were synthesized for attachment to silica support material (37). The derivatives were used to modify the surface of 3 μm diameter silica particles to prepare as the stationary phase for HPLC. Preliminary evaluation of the stationary phase for HPLC was performed using aromatic carboxylic acids as model compounds. The separation mechanism appears to involve multiple interactions including ion exchange, hydrophobic interaction, and other electrostatic interactions.

An important analytical problem caused by free silanols concerns lipophilicity determinations by LC (38). The first attempts to improve correlations between reference parameter of lipohilicity, *log P,* and the reversed phase HPLC retention factors, *log k*, determined on chemically bonded alkylsilica columns consisted in reduction of free silanol sites in the reversed-phase column by the additional silylation. To further improve determination of lipophilicity of neutral and acidic compounds, Unger et al. (39) used phosphate buffer, to which N,N-dimethyloctylamine at a concentration of 4 mmol/L was added. The lipophilic N,N-dimethyloctylamine was to swamp out the binding of analytes to residual silanol sites on the stationary phase material. Recently, we proposed ionic liquids as the residual free-silanol blocking agents (23). Ionic liquid additives studied appeared especially interesting from the point of view of determination of lipophilicity of ionized forms of basic drugs. In Figure 8, the chromatographic lipophilicity parameters, log k_w, of a series of basic drugs, determined by gradient HPLC at the absence and at the presence of [EMIM][BF_4] in mobile phase, are plotted against the reference lipophilicity parameter, *log P*, from the *n*-octanol–water partition system. The correlation is evidently better if [EMIM][BF_4] is present in the buffer than if only pH is appropriately adjusted. In addition, the IL improves the shape of peaks and removes peak tailing observed in the reference system.

In chromatographic practice, the HPLC systems with normal (NP) and reversed (RP) stationary phases with masking agents, such as aliphatic amine and quaternary ammonium ions, added to the mobile phase to suppress silanol effect and corresponding

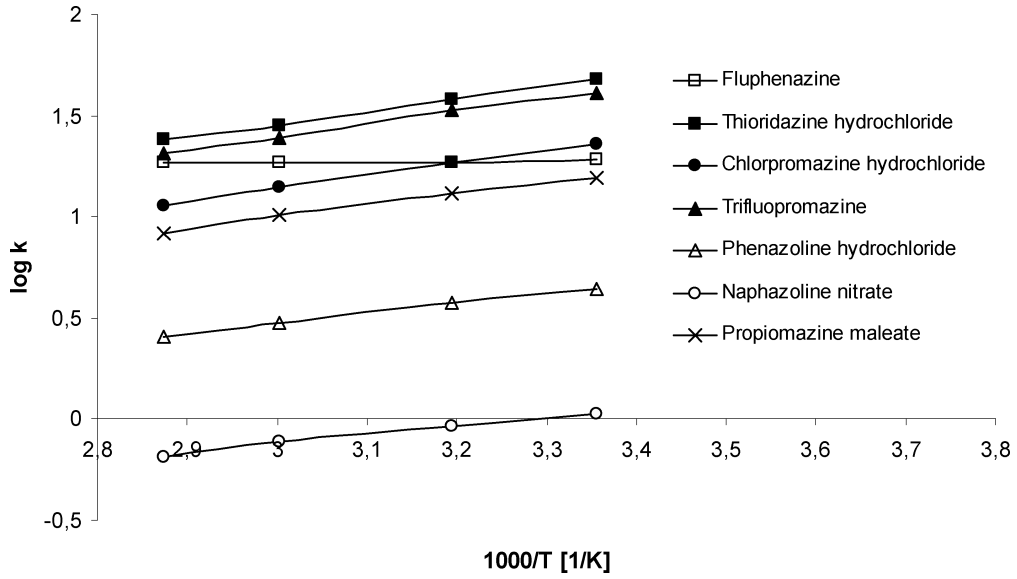


FIG. 7. van't Hoff plot of the relationship between the retention parameter of analyte *log k* and the reciprocal of absolute temperature (1/T). (Reprinted with permission from ref. (28), © 2003 Elsevier B. V.)

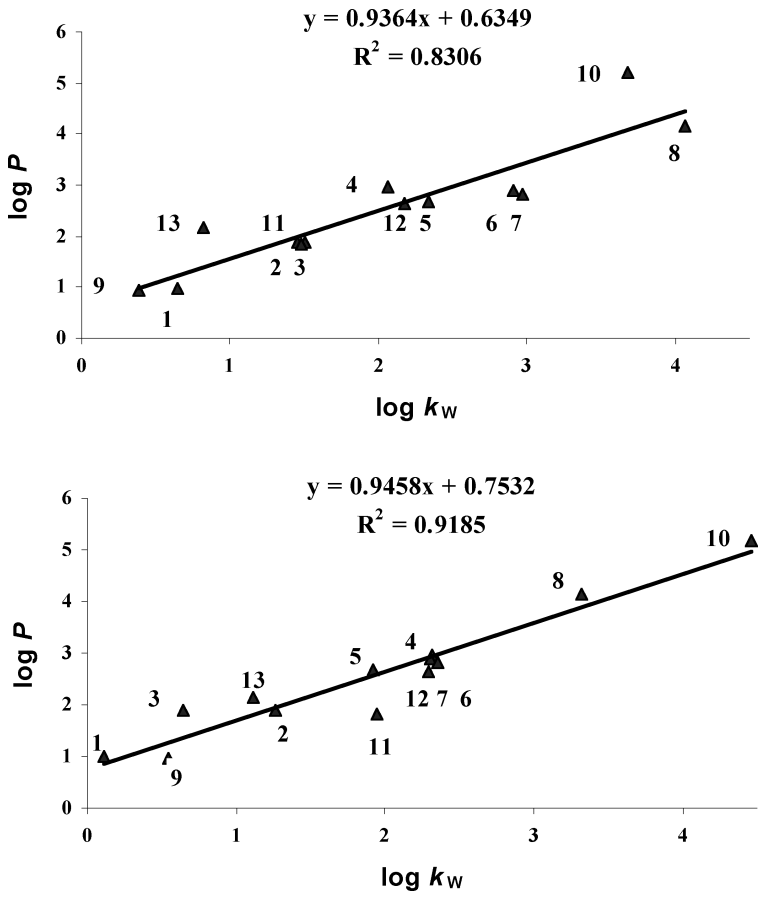


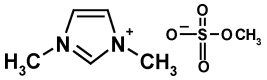
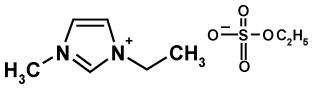
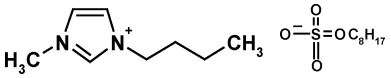
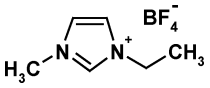
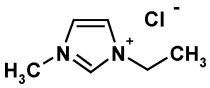
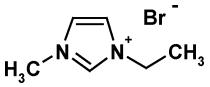
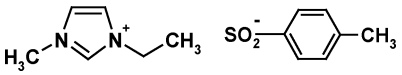
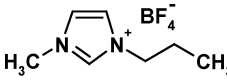
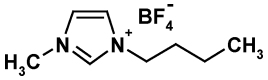
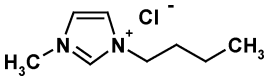
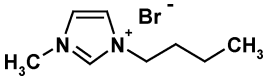
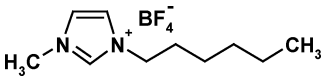
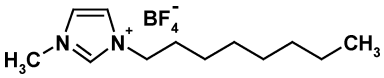
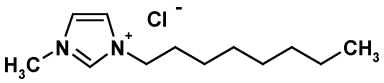
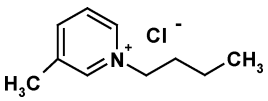
FIG. 8. Relationships between *log P* [28] and the lipophilicity parameter *log* k_w, determined by gradient HPLC with a buffered at pH = 2.87 water-methanol eluent not comprising (upper plot) and comprising (bottom plot) 1.5% v/v of [EMIM][BF_4]. Analytes: 1–aniline, 2–4-chloroaniline, 3–metoprolol, 4–propranolol, 5–3,4-dichloroaniline, 6–3,5-dichloroaniline, 7–betaxolol, 8–phenotiazine, 9–4-metoxyaniline, 10–thioridazine, 11–timolol, 12–quinine, 13–N-ethylaniline.

peak tailing are often employed (40). In basic investigations on tailing reducers, chromatographers used the two-site retention model to assess efficiency of various masking agents (41, 42). The HPLC method applying the dual retention model of Nahum and Horváth was used to the evaluate the silanol suppressing potency of imidazolium-based ionic liquids with different anonic and cationic parts (Table 1) (43). The Nahum–Horváth model has the following form:

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \qquad [3]$$

where k_0 is the retention factor obtained in the absence of silanol suppresor and k is the retention factor, obtained at the concentration $[A]$ of the silanol suppressor. According to Eq. 3,

TABLE 1
Structural formulae of ionic liquids.

Structure of ionic liquids	Formula	Name
	[MMIM][MSO_4]	1,3-dimethylimidazolium methyl sulfate
	[EMIM][ESO_4]	1-ethyl-3-methylimidazolium ethyl sulfate
	[BMIM][OSO_4]	1-buthyl-3-methylimidazolium octyl sulafate
	[EMIM][BF_4]	1-ethyl-3-methylimidazolium tetrafluoroborate
	[EMIM][Cl]	1-ethyl-3-methylimidazolium chloride
	[EMIM][Br]	1-ethyl-3-methylimidazolium bromide
	[EMIM][Ts]	1-ethyl-3-methylimidazolium tosylate
	[PMIM][BF_4]	1-propyl-3-methylimidazolium tetrafluoroborate
	[BMIM][BF_4]	1-buthyl-3-methylimidazolium tetrafluoroborate
	[BMIM][Cl]	1-buthyl-3-methylimidazolium chloride
	[BMIM][Br]	1-buthyl-3-methylimidazolium bromide
	[HxMIM][BF_4]	1-hexyl-3-methylimidazolium tetrafluoroborate
	[OMIM][BF_4]	1-octyl-3-methylimidazolium tetrafluoroborate
	[OMIM][Cl]	1-octyl-3-methylimidazolium chloride
	[BMPy][Cl]	1-butyl-4-methylpyridinium chloride

plots of $\frac{[A]}{k_0-k}$ against $[A]$ yield straight lines with the reciprocal of the silanophilic retention factor, k_2, as the slope. Knowing k_2, the value of K_A can be calculated from the intercept of Eq. 3. The comparison of silanol-masking by the 1-alkyl-3-methylimidazolium ionic liquids class and triethylamine (TEA) can be done by means of the silanophilic binding constant, K_A.

The 1-ethyl-3-methyl-, 1-butyl-3-ethyl- and 1-methyl-3-octylimidazolium cation, chlorides and bromides yield higher values of K_A than tetrafluoroborates. The differences observed can be due to hydrogen bonding between the hydrogen atom of the alkyl chain and the counter-ion (44). That causes that Coulombic forces are weakened and binding to the silanol group becomes weaker. Therefore, the higher K_A values were obtained for bromide and chloride derivatives than for tetrafluoroborate and sulfate.

For all the investigated tetrafluoroborate ILs, the K_A value increased with increasing number of carbon atoms in the molecule of ionic liquid. It means that the suppression efficiency of the effects of free silanols increased with the length of alkyl chain at C-1 position in imidazolium ring. Evidently, the highest effect of 1-octyl-3-methylimidazolium ionic liquids seems to be caused by their highest hydrophobicity. The silanol-imidazolium complex could be stabilized by hydrophobic interaction between the alkyl chain of the ionic liquid and the octadecylbonded silica phase. The silanol-masking potency of TEA appeared much poorer than ILs, as caused by the weaker Coulombic interactions between the alkylamine and the residual silanols.

Different value of K_A corresponds to the changes in analytes' retention (Fig. 9). In all the cases, the retention time of analytes decreased in accordance to the increasing K_A value. Becoming the basic drugs less retained improved the resolution on the one side, but worsened the selectivity on the other side. The best separation of six basic test drugs was achieved with the use 32 mM [MMIM][MSO_4].

CAPILLARY ELECTROPHORESIS (CE)

The CE separation mechanism is completely different than LC. Nonetheless, the most important feature of the CE-fused silica capillary is the presence of different types of silanol groups. They have influence on CE separation by affecting electroosmotic flow (EOF) and transport of ions during the analysis. Many approaches have been employed for controlling or eliminating of EOF, like changes of the composition, component concentration and pH of buffer, addition of organic solvents or additives, dynamic modification of the wall surface or chemical derivatization of surface silanols.

Numerous adsorbable amino quenchers have been tested to suppress free silanol effects in CE. A list of these agents, with respective original references, has recently been provided by Righetti et al. (45). These are: triethylamine, propylamine, morpholine, glucosamine, galactosamine, *N*,*N*-diethylethanolamine, *N*-ethyldiethanolamine, triethanolamine, ethanolamine, hydroxylamine, ethylamine, tetramethylammonium chloride, 1,3-diaminopropane, 1,4-diaminobutane (putrescine), 1,5-diaminopentane (cadaverine), ethylendiamine, *N*,*N*,*N*′,*N*′-tetramethyl-1,3-butanediamine, hexamethonium bromide, decamethonium bromide, diethylenetriamine, triethylenetetramine, *N*, *N*′-bis(3-aminopropyl)1,4-butanediamine (spermine), 1,4,7,10-tetraazocyclodecane (cyclen), chitosan, polyethylenimine, polydimethylallyl amonium chloride and the recently introduced quenchers for dynamic coating of silica walls in capillary electrophoresis, i.e., quaternary piperazine derivatives like *N*(methyl-*N*-ω-iodo-butyl), *N*′-methylpiperazine (45, 46).

The dialkylimidazolium-based liquid organic salts with [PF_6^-], [CH_3COO^-] and [CF_3COO^-] anions were successfully used as buffer electrolytes in non-aqueous capillary electrophoresis (NACE) (47, 48). The authors claimed that anionic part and concentration of IL change the general electrophoretic mobility. Anions of ILs adsorb on the silica surface, rendering it negative, so that the mobile imidazolium-cations create bulk flow towards the cathode. Additionally, analytes become charged in presence of ILs conditioning effective separation of hydrophobic dyes, aromatic acids and phenols.

Yanes and co-workers (49, 50) described the development of CE method for the separation of the polyphenols found in grape seed extracts. They used tetramethylammonium tetrafluoroborate and 1-alkyl-3-methylimidazolium-based ILs as the electrolyte in the background electrolyte. According to those autors ILs possess unique properties, like a strong Coulombic field around them, which promotes strong orientation and induction interactions as well as analyte-ion interactions that enhance the proton donor-acceptor interactions in solutions. The size of the uncharged polyphenols and different degrees of association with IL's cations seemed to provide effective electrophoretic mobility differences for effective separations. Proposed mechanism of separation of polyphenols relies on the association of the studied analytes with IL's cations either coating the capillary wall or in the bulk solution.

Successful CE separations of basic proteins, such as lysozyme, cytochrome *c*, trypsinogen and α-chymotrypsinogen A as well as chlorophenoxy acid herbicides and bioactive flavonoids after dynamically coating the capillary with 1-alkyl-3-methylimidazolium-based IL have been achieved (51–53). The method was proved to be simple, reliable and repeatable in the terms of migration time and peak area.

The influence of 1-alkyl-3-methylimidazolium IL in different organic solvents, like acetonitrile, acetone, dimethylformamide, dimethylsulphoxide, propylene carbonate, methanol, ethanol, *n*-propanol and their mixtures on the separation of different analytes has been investigated (54). The electrolytes of this type enabled to achieve the separation of phenolic compounds, which are soluble in acetonitrile. The mechanism of separation in organic solvent is hypothesized to involve the heteroconjugation between the background electrolyte anion and analyte. It enables

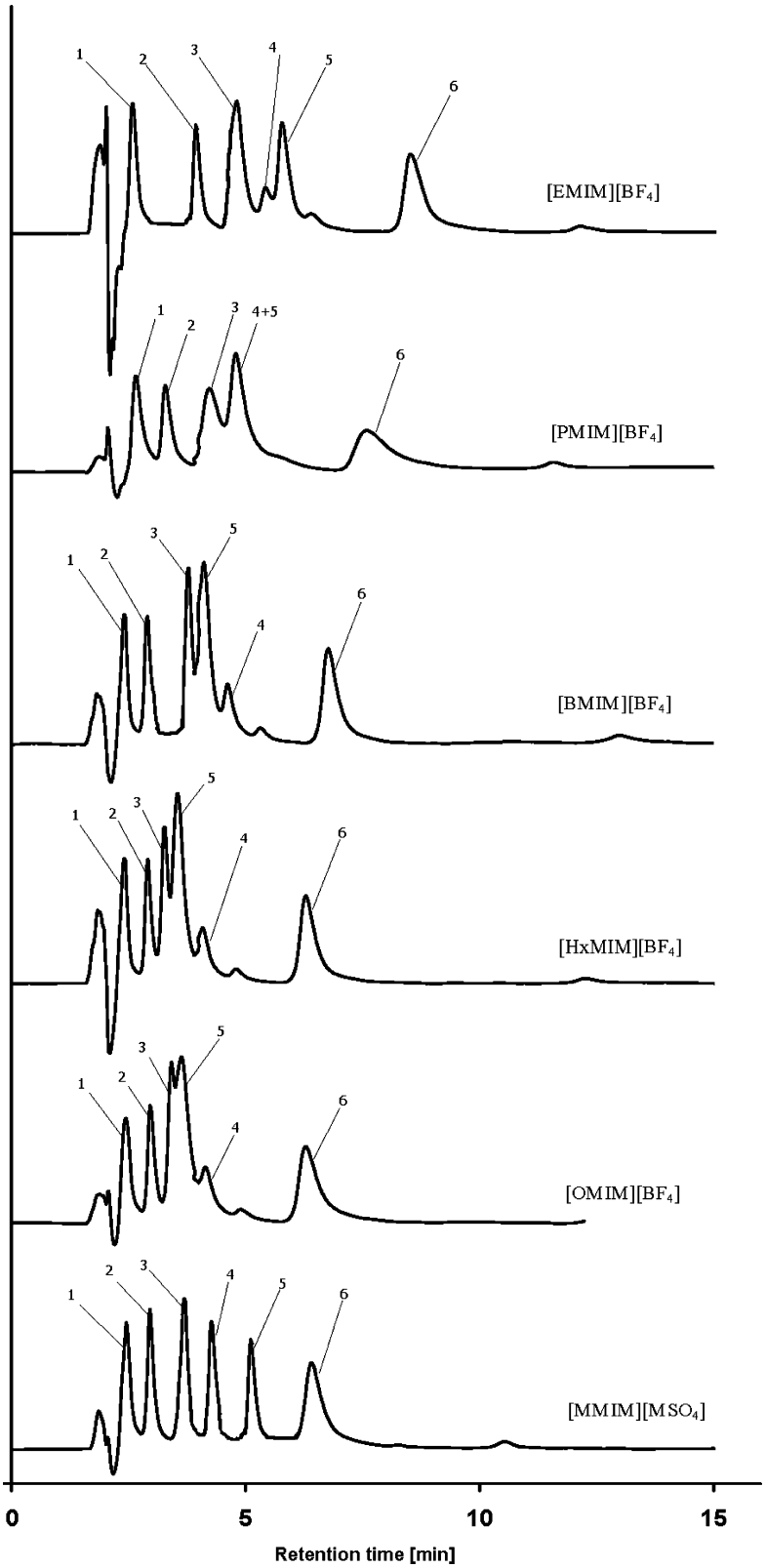


FIG. 9. Chromatograms of the test mixture of six basic drugs with the addition of different ionic liquids (32 mM) to acetonitrile-water (50/50 v/v) mobile phase: 1–naphazoline nitrate, 2–phenazoline hydrochloride, 3–chlorpromazine hydrochloride, 4–fluphenazine, 5–propiomazine maleate, 6–thioridazine hydrochloride. (Reprinted with permission from ref. (43), © 2006 Wiley-VCH Verlag GmbH & Co., KGaA.)

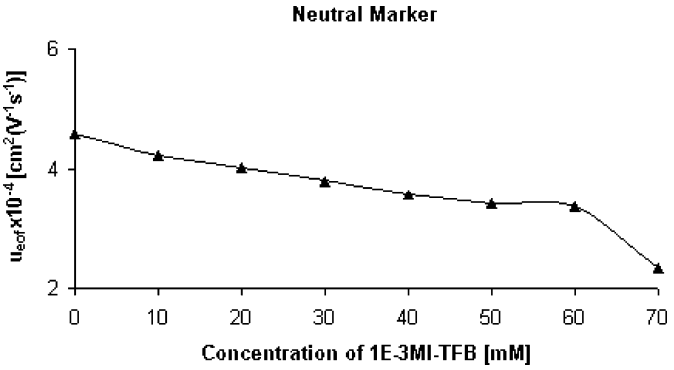


FIG. 10. Effect of concentration of 1-ethyl-3-methylimidazolium tetrafluoroborate on the EOF measured by neutral marker (benzyl alcohol). Electrophoretic condition: applied voltage +14 kV, 20 mM borate buffer. (Reprinted with permission from ref. (61), © 2005 Elsevier B. V.).

electrophoretic separation of non-dissociating analyte molecules in aprotonic solvents.

Electro-osmotic flow (EOF) in the silica capillary can be efficiently reversed by coating the surface with dialkylimidazolium cation by covalent bonding. The IL-coated capillary was used for the separation of the sildenafil citrate and its metabolite in human serum, DNA fragments as well as for determination of ammonium and metal ions (55–58).

An interesting combination was the separation of achiral and chiral analytes using ILs with polymeric surfactants poly(sodium) *N*-undecylelinicsulfate and poly(sodium) oleyl-L-leucylvalinate, as modifiers in micellar electrokinetic chromatography (MEKC) as well as with β-cyclodextrin (β-CD) as the modifier in CE (59, 60). The ILs used in that study improved the resolution of anthraquinones, ketones, phenols and binaphtyl derivatives.

Generally, all the preceding CE separation mechanisms can be explained by the simple scheme of separation of nicotinic acid and its structural isomers (61). A significant influence on migration times of acidic analytes in capillary electrophoresis could be observed when [EMIM][BF_4] was used as an additive in 20 mM sodium tetraborate buffer. Figure 10 shows the dependence of the EOF on the concentration of ILs in the background electrolyte (BGE) when the positive potential at the injector end of the bare fused-silica capillary was applied. It can be seen that increasing concentration of [EMIM][BF_4] from 0 to 70 mM results in decrease of EOF. In the concentration above 70 mM of IL in the BGE, the EOF became insignificant.

Ionic liquids added to running tetraborate buffer decrease its pH from 9.30 to 8.30. At pH 8.30 the nicotinic acid (pK_{a1} = 2.07, pK_{a2} = 4.73), isonicotinic acid (pK_{a1} = 1.70, pK_{a2} = 4.89) and picolinic acid (pK_{a1} = 1.06, pK_{a2} = 5.37) are negatively charged (62). Therefore, at a basic pH, the significant influence on the migration of carboxylic acids has electrophoretic mobility, μ_e.

Imidazolium ions are responsible for changing electrophoretic properties of capillary wall (Fig. 11). Simultaneously, due to the ionic interaction between negatively charged silica groups on inner surface of the capillary and IL's cationic molecule, imidazolium moiety becames uncharged. Such created coating on the inner surface of the capillary wall causes suppression of the EOF. At certain concentration of ILs in BGE a complete suppression of EOF could be observed. Therefore, negatively charged acids are not retained anymore by electrically neutral single layer of imidazolium ionic. Finally, charged acids' isomers migrate toward the anode and are separated by their different values of μ_e, which is described by the well-known equation:

$$\mu_e = \frac{q}{6\pi\eta r} \qquad [4]$$

where q is ion charge, η is solution viscosity and r is ion radius.

Figure 12 presents electropherograms obtained with increasing concentrations of ILs in BGE. Increased [EMIM][BF_4] concentration caused decrease of migration times of analytes, improved peaks shape and increased separation performance.

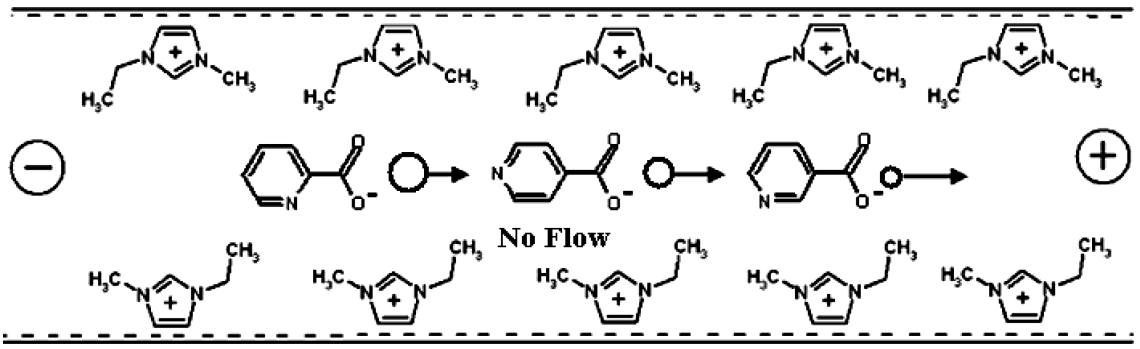


FIG. 11. Proposed mechanism of carboxylic acids' separation using 1E-3MI-TFB. Order of migration according to the charge/radius ratio. (Reprinted with permission from ref. (61), © 2005 Elsevier B. V.).

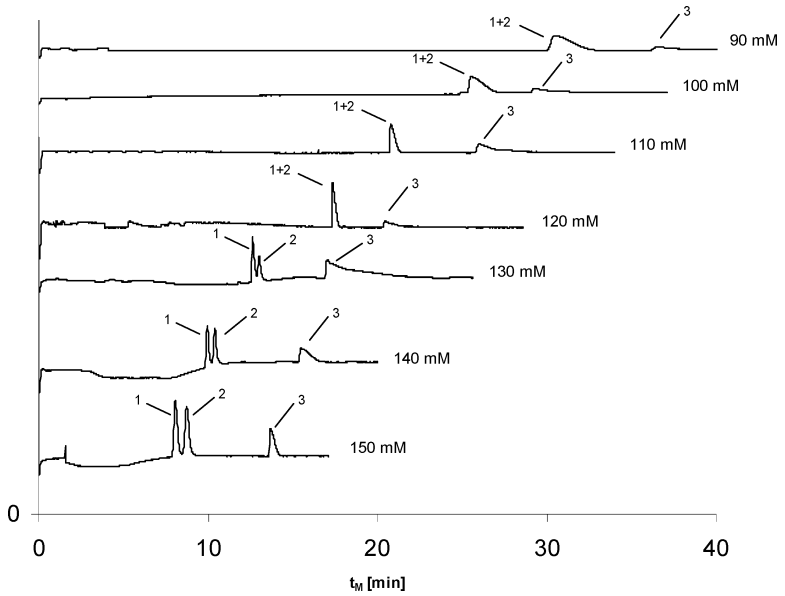


FIG. 12. Electropherograms presenting separation of nicotinic (1), isonicotinic (2) and picolinic acids (3) with increasing concentration of 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid added to the BGE. Electrophoretic condition: 20 mM borate buffer, applied voltage −14 kV. (Reprinted with permission from ref. (61), © 2005 Elsevier B. V.).

CONCLUSIONS

Rapid growth of interest in application of ILs in LC and CE is reasonable in view of their unique properties. Trend is very similar for the rest of areas of chemistry. The use of ILs opens new opportunities to solve problem of difficult separations of different analytes. Increasing number of commercially available ILs gives allows their designed application in separations sciences. The use of ILs as "green chemistry" modifiers is recommended instead of the conventional environmentally harmful agents, which are currently widely employed in analytical practice.

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