ELSEVIER

Suppression of deleterious effects of free silanols in liquid chromatography by imidazolium tetrafluoroborate ionic liquids

Roman Kaliszan [a,*], Michał Piotr Marszałł [a], Michał Jan Markuszewski [a], Tomasz Bączek [a], Juliusz Pernak [b]

[a] *Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland*
[b] *Department of Chemistry, Poznań University of Technology, Grunwaldzka 6, 60-780 Poznań, Poland*

Abstract

Silica-based stationary phases are commonly used in liquid chromatography, but their surface acidity causes known problems, especially when separating basic compounds. Deleterious effects of free silanols are not fully removed by standard prevention procedures consisting in adding alkylamines or other amino quenchers to the eluents. We found that ionic liquids of the imidazolium tetrafluoroborate class, added to mobile phases at concentrations of 0.5–1.5% (v/v), blocked silanols and provided excellent thin-layer chromatographic separations of strongly basic drugs which were otherwise not eluted, even with neat acetonitrile as the mobile phase. The silanol suppressing potency of imidazolium tetrafluoroborates was demonstrated to markedly exceed that of the standard mobile phase additives, like triethylamine, dimethyloctylamine and ammonia. The proposed new mobile phase additives were also demonstrated to provide reliable lipophilicity parameters of base drug analytes as determined by gradient mode of high-performance liquid chromatography. By applying the readily available and environmentally friendly imidazolium tetrafluoroborate ionic liquids, simple and efficient means of improvement of liquid chromatographic analysis of organic bases were elaborated.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Silanol groups; Ionic liquids; Adsorption; Lipophilicity; Mobile phase composition; Stationary phases, LC; Imidazolium tetrafluoroborates

1. Introduction

The favorable physical characteristics of silica makes silica-based stationary phases the most popular in liquid chromatography, both in HPLC and in TLC [1]. Silica is also used to make capillaries for CE. However, a serious undesirable property of silica is its surface acidity due to the free or isolated (non-hydrogen-bonded) silanols. Effects of free silanols on HPLC and TLC retention are difficult to control and are especially deleterious as regards the chromatographic behavior of basic analytes. Retention of acids can also be affected by the free silanols due to electrostatic exclusion phenomena [2]. The problem concerns even the most modern highly purified silica supports, including those considered to be the least acidic ones [3]. The reason is that the coverage of the silanol groups in the chemically bonded phases is less than 50–60% [4] and the physical

deactivation of silica by adding adsorbable cations to the mobile phases provides coverage of only one third of all the silanol groups [5–7].

Numerous adsorbable amino quenchers have been tested to suppress free silanol effects in liquid chromatography and in capillary electrophoresis. An exhaustive list of these agents, with respective original references, has recently been provided by Righetti et al. [8]. Those authors mention: triethylamine (TEA), 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), ethylenediamine, 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 ammonium chloride and the recently introduced quenchers for dynamic coating of silica walls in capillary

* Corresponding author. Tel.: +48-58-349-3260;
fax: +48-58-349-3262.
E-mail address: roman.kaliszan@amg.gda.pl (R. Kaliszan).

electrophoresis, i.e. quaternary piperazine derivatives like *N*-(methyl-*N*-ω-iodo-butyl), *N′*-methylpiperazine [9,10].

The mobile phase additives most often applied in liquid chromatography are triethylamine and dimethyloctylamine (DMOA) [11]. In TLC, ammonia is still commonly used. These additives are often ineffective in case of strongly basic analytes. Moreover, their presence causes slow equilibration of the chromatographic system when changing mobile phases [1]. Hence, their use should be avoided, especially if the gradient elution mode is to be applied.

In search for efficient suppressors of free silanols, we turned our attention to the imidazolium tetrafluoroborate derivatives possessing properties of ionic liquids. Ionic liquids ("green solvents") call much attention as solvents for catalytic and organic reactions due to their unique interactions with the active species [12,13].

Alkylammonium nitrate and thiocyanate liquid salts have already been studied as potential solvents for reversed-phase liquid chromatography [14]. These compounds have not called wider interest, however. Whereas the alkylammonium nitrate salts were claimed to be suitable for eluent strength modification in HPLC, the thiocyanates appeared of a little use due to their corrosive action on the chromatographic system. Instead, tetralkylammonium sulfonate ionic liquids were studied as potential stationary phases in gas chromatography [15]. Later on, also 1-butyl-3-methylimidazolium hexafluorophosphate and the analogous chloride salt [16] as well as 4-methyl-*n*-butylpiridinum tetrafluoroborate [17] were employed as stationary phases in GC. On the other hand, dialkylimidazolium-based liquid organic salts were used as buffer electrolytes in non-aqueous capillary electrophoresis [18,19]. Room temperature ionic liquids were employed as running electrolytes in CE by Yanes et al. [20].

Previous attempts to exploit ionic liquids focused on the modification of interactions of analytes with the mobile phases. No actual advantages of ionic liquids over the conventional chromatographic eluents have been demonstrated, however. That does not mean that the strong proton–acceptor properties of new classes of ionic liquids cannot be utilized in chromatography, e.g. to suppress deleterious effects of free silanols on liquid chromatographic separations.

Dialkylimidazolium ionic liquids that contain such anions as $[BF_4]^-$ are water-stable compounds which dissolve in typical liquid chromatographic solvents, like acetonitrile. By attaching alkoxy group to imidazolium cations new ionic liquids have recently been obtained which display particularly strong antielectrostatic effect [21]. These properties attracted our attention.

A separate analytical problem caused by free silanols concerns lipophilicity determinations by liquid chromatography [22]. The first attempts to improve correlations between reference parameter of lipophilicity, log *P*, and the reversed-phase HPLC retention factors, log *k*, determined on chemically bonded alkylsilica columns included the reduction of free silanol sites in the column by the additional silylation. To further improve determination of lipophilicity of neutral and acidic compounds, Unger and Chiang [23] used phosphate buffer with added NaCl 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. Here, we propose ionic liquids as the residual free silanol blocking agents.

The following ionic liquids were subjected to the study: 1-ethyl-3-methylimidazolium tetrafluoroborate (IL 1) from Aldrich (Milwauke, WI, USA), 1-methyl-3-hexylimidazolium tetrafluoroborate (IL 2) and 1-hexyl-3-heptyloxymethylimidazolium tetrafluoroborate (IL 3), both synthesized by Pernak et al. [21]. IL 2 is at present also available commercially (Fluka, Buchs, Switzerland). As the test analytes served eight basic drugs which were found in preliminary experiments not to be moved from the application spot on neither the silica- nor octadecylsilica-covered TLC plates by 100% acetonitrile as the eluent. Among test analytes were four phenothiazine derivatives reported previously [24] to interact strongly with silica surface. Additional test compounds were two acids (acetylsalicylic acid and salicylic acid), phenol and 2,3-dimethoxytoluene. In the study of the effects of ionic liquid additive on chromatographic parameters of lipophilicity, a series of known basic drugs was used.

2. Experimental

2.1. Materials

Ionic liquids IL 1, IL 2 and IL 3 were used as obtained, without any additional pretreatment. Test analytes were from the reference drug substance and reagent collection of the Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk. Acetonitrile and methanol of chromatographic quality were from Merck (Darmstadt, Germany). Ammonia (NH_4OH) was from POCh (Gliwice, Poland). Triethylamine and dimethyloctylamine were from Fluka (Buchs, Switzerland). Water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Methods

TLC experiments were done on aluminum-backed 5 cm × 7.5 cm × 0.2 cm plates covered with silica gel 60 F_{254} as well as on analogous plates covered with layers of RP-18 F_{254}. The ready-made TLC plates were from Merck. Chromatograms were developed to a distance of 6.0 cm in a horizontal chamber (Modin, Lublin, Poland) [25]. Acetonitrile was used as organic modifier of eluent. Spots were visualized in UV (wavelength 254 nm) with the use of a Spectroline hand lamp (Spectronics, Westburg, NY, USA). The retention data reported are means of three to six independent experiments.
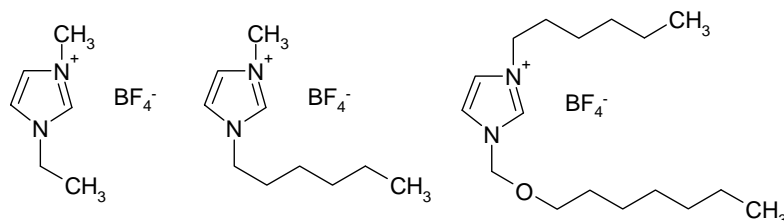
Fig. 1. Structural formulae of imidazolium tetrafluoroborate ionic liquids.

HPLC experiments were performed on a liquid chromatograph (Merck Hitachi, Frankfurt, Germany/Tokyo, Japan), consisting of a pump (L-7100), diode array detection (DAD) system (L-7455), autosampler (L-7200), thermostat (L-7350), membrane degasser (L-7612) and interface (D-7000). The experiments were performed on LiChrospher RP-18 column, 12.5 cm × 0.40 cm i.d., particle size 5 μm (Merck). Methanol–buffer eluent system

was employed. Detection was at wavelength 254 nm. It was found in preliminary experiments that the presence of IL 1 in mobile phase increased the background response of the UV detector. The increase was minor, however, and did not disturb analyte peaks. The injected sample volume was 10 μl. All the chromatographic measurements were done at 40 °C with eluent flow rate of 1.5 ml/min.

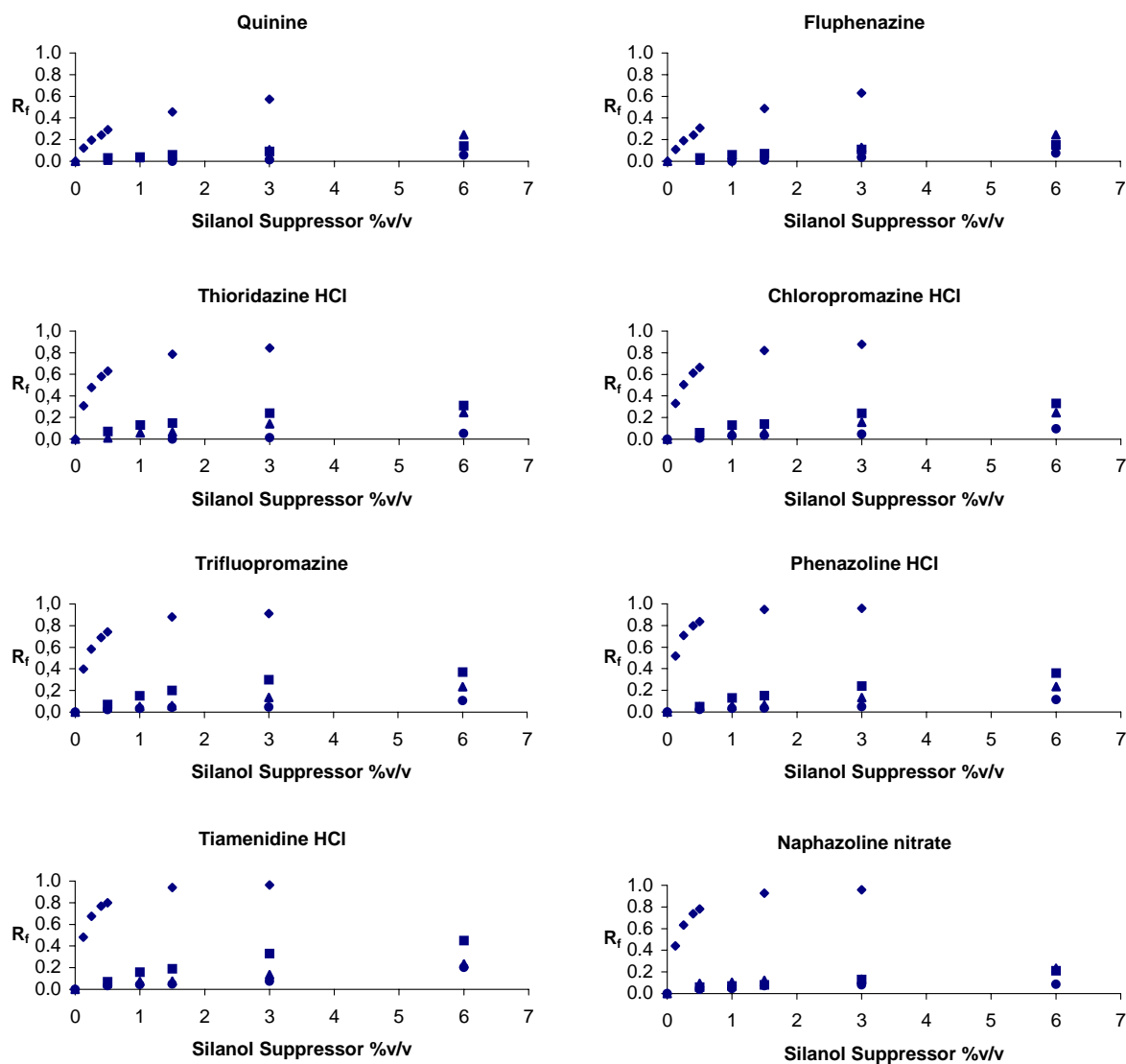Fig. 2. Thin-layer chromatographic retardation factor, R_F, on octadecylsilica-covered plates in relation to volume percent of IL 1 (◆), TEA (■), DMOA (▲) and NH_4OH (●) in pure acetonitrile as the mobile phase.

Chromatographic lipophilicity parameters, $\log k_w$, were calculated on the basis of two gradient runs by DryLab Software (LC Resources) [26]. Gradient of the range of 95–0% (v/v) of buffer in methanol–buffer eluent with both solvents either neat or containing 1.5% IL 1, was developed at gradient times of 20 and 60 min.

3. Results and discussion

In Fig. 1, the structural formulae of the three imidazoline tetrafluoroborate ionic liquids tested are given. All the agents affected the TLC retention of basic drugs on both underivatized silica and on the octadecylsilica stationary phases when added to the eluent. Fig. 2 illustrates the effect of concentration of one of the ionic liquids, IL 1, added to neat acetonitrile as the eluent, on the retardation factor of eight basic drugs on an octadecyl-bound silica stationary phase. As evident from Fig. 2, all the analytes studied were not moved from the start by 100% acetonitrile eluent. Addition of IL 1 decreased retention in a concentration dependent manner suggesting Langmuir adsorption of the silanol deactivating substance. 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 octadecylsilica-covered plates with both neat acetoni-

trile and with water–acetonitrile mixtures of various composition as the mobile phases. Data of Fig. 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. TEA, DMOA and NH$_4$OH have low or negligible effect on the test drugs retention, even at the highest concentrations applied.

Unique silanol suppressor properties of imidazolium tetrafluoroborates are well documented by the Langmuir plots of dependence of $1/R_F$ of an exemplary analyte from Fig. 1 (tiamenidine) on the reciprocal of the additive concentration in the eluent (Fig. 3). A typical Langmuir adsorption is observed for IL 1 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 IL 1 is much less steep than that for TEA proving a more effective adsorption of the former compound. Poor adsorption of DMOA and NH$_4$OH is clearly evident from Fig. 3 which disqualifies those substances as the effective silanol suppressors.

Results of comparative studies of silanol suppressing potency of the three imidazolium tetrafluoroborate derivatives, IL 1, IL 2 and IL 3, are presented in Fig. 4. Here, the respective Langmuir plots are given for the three ionic liquids added to acetonitrile eluent as the suppressors
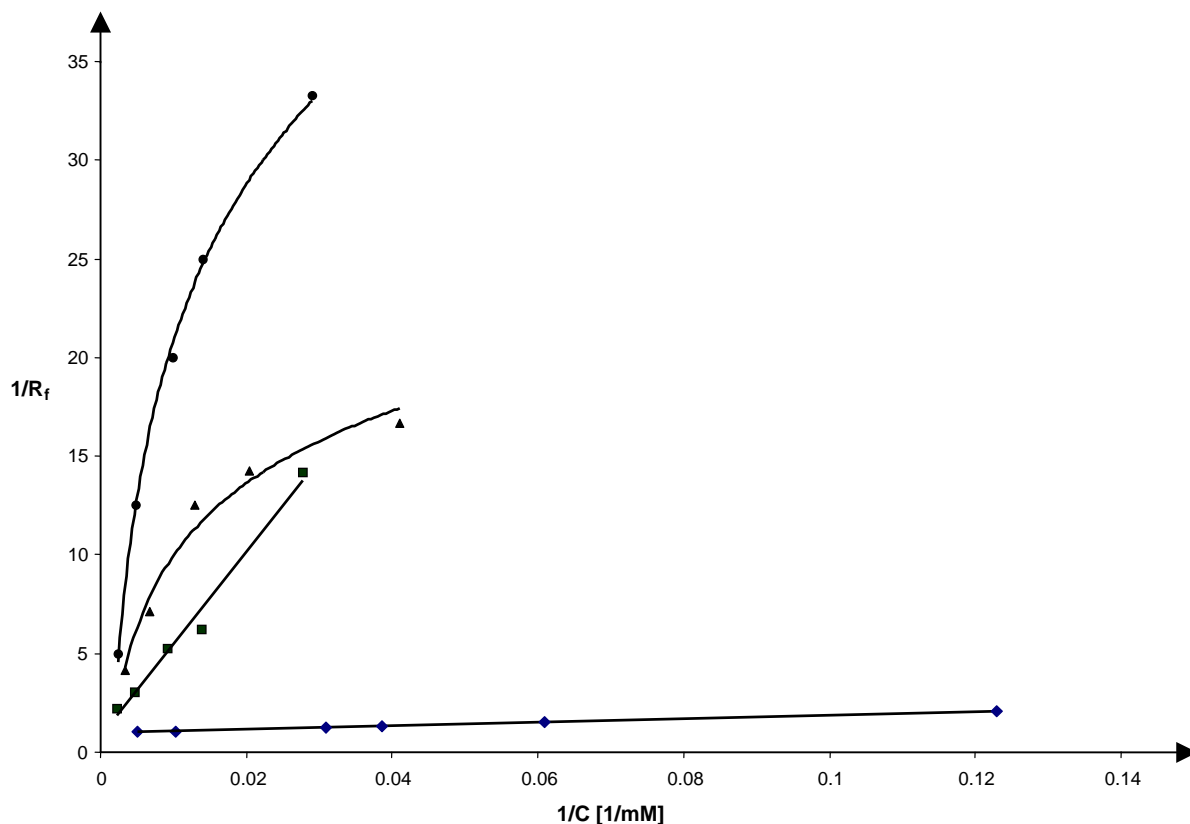

Fig. 3. Plots of reciprocal of retardation factor of tiamenidine on octadecylsilica plates with acetonitrile as eluent vs. the reciprocal of concentration of additive in the mobile phase. Additives are denoted as follows: IL 1 (◆), TEA (■), DMOA (▲) and NH$_4$OH (●).
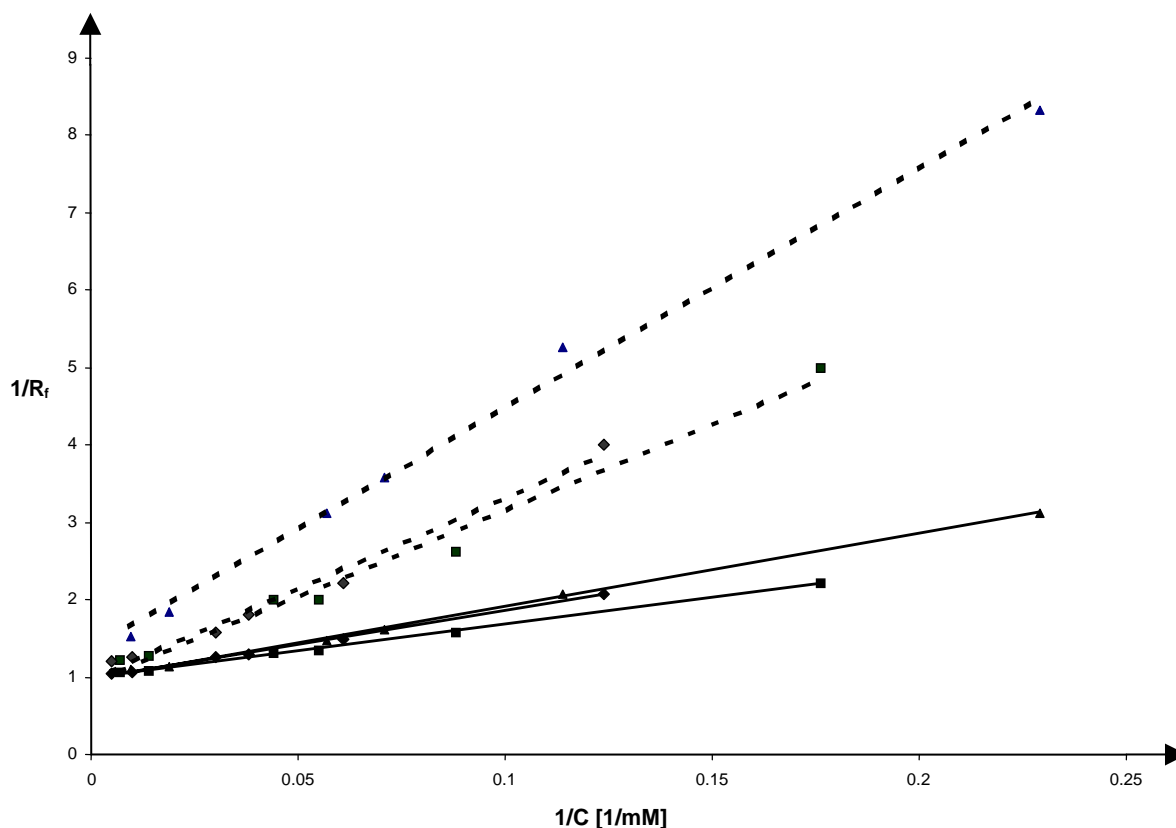
Fig. 4. Plots of reciprocal of retardation factor of tiamenidine on silica plates (broken lines) and on octadecylsilica plates (solid lines) with acetonitrile as eluent vs. the reciprocal of concentration of imidazolium tetrafluoroborate derivatives IL 1(◆), IL 2 (■) and IL 3 (▲).

of attraction by free silanols of the selected test drug (tiamenidine) on both silica-covered (broken lines) and octadecylsilica-covered plates (solid lines). Evidently, all the three imidazolium tetrafluoroborate derivatives are more strongly adsorbed on the octadecylsilica phase. That can be due to an auxiliary effect of octadecyl moieties bound to silica surface. However, if that assumption is correct, then IL 2 should be better adsorbed than IL 1 as the molecular mass and the lipophilicity calculated [27] from structural formula, $C \log P$, are larger for IL 2 than for IL 1 ($C \log P$ equals 3.31 for IL 1 and 5.43 for IL 2). However, both molecular mass and $C \log P$ are larger for IL 3 ($C \log P = 7.94$) which is a weaker silanol suppressor on both the silica and on the octadecylsilica plates. Poorer performance of IL 3 may be assigned to its larger bulk which renders its direct contact with the active adsorption sites.

Having demonstrated similar free silanol-suppressing power of IL 1 and IL 2, we continued further research with IL 1 which is commercially available at requested quantities. In Fig. 5, the R_M values of eight basic drugs determined on octadecylsilica stationary phases are plotted against acetonitrile concentration in water–acetonitrile eluent of varying composition but of fixed concentration 3% (v/v) of IL 1, TEA and NH$_4$OH. Experiments with DMOA did not produce measurable retention data and the results are not included in Fig. 5. Also, in case of NH$_4$OH only

a limited range of acetonitrile concentration in the eluent allowed for obtaining reliable R_M data.

Fig. 5 documents that the reversed-phase TLC systems obtained with the help of an imidazolium tetrafluoroborate additive produce the R_M parameters best fitting to the classical linear Snyder–Soczewiński dependence on organic modifier concentration in the mobile phase. That is a consequence of removal of uncontrollable attractive interactions of base analytes with free silanols. The removal is much more effective than that provided by typical amine quenchers. The generally improved linearity of R_M versus percent organic modifier illustrated in Fig. 5 may be utilized 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). Linearity of the dependence of retention in liquid chromatography on eluent composition is also a prerequisite of reliable prediction and optimization of separation in both isocratic and gradient HPLC [1,28].

Actual value of imidazolium tetrafluoroborates for improving liquid chromatographic separations is illustrated in a straightforward way in Fig. 6. Here, thioridazine, trifluoropromazine, 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
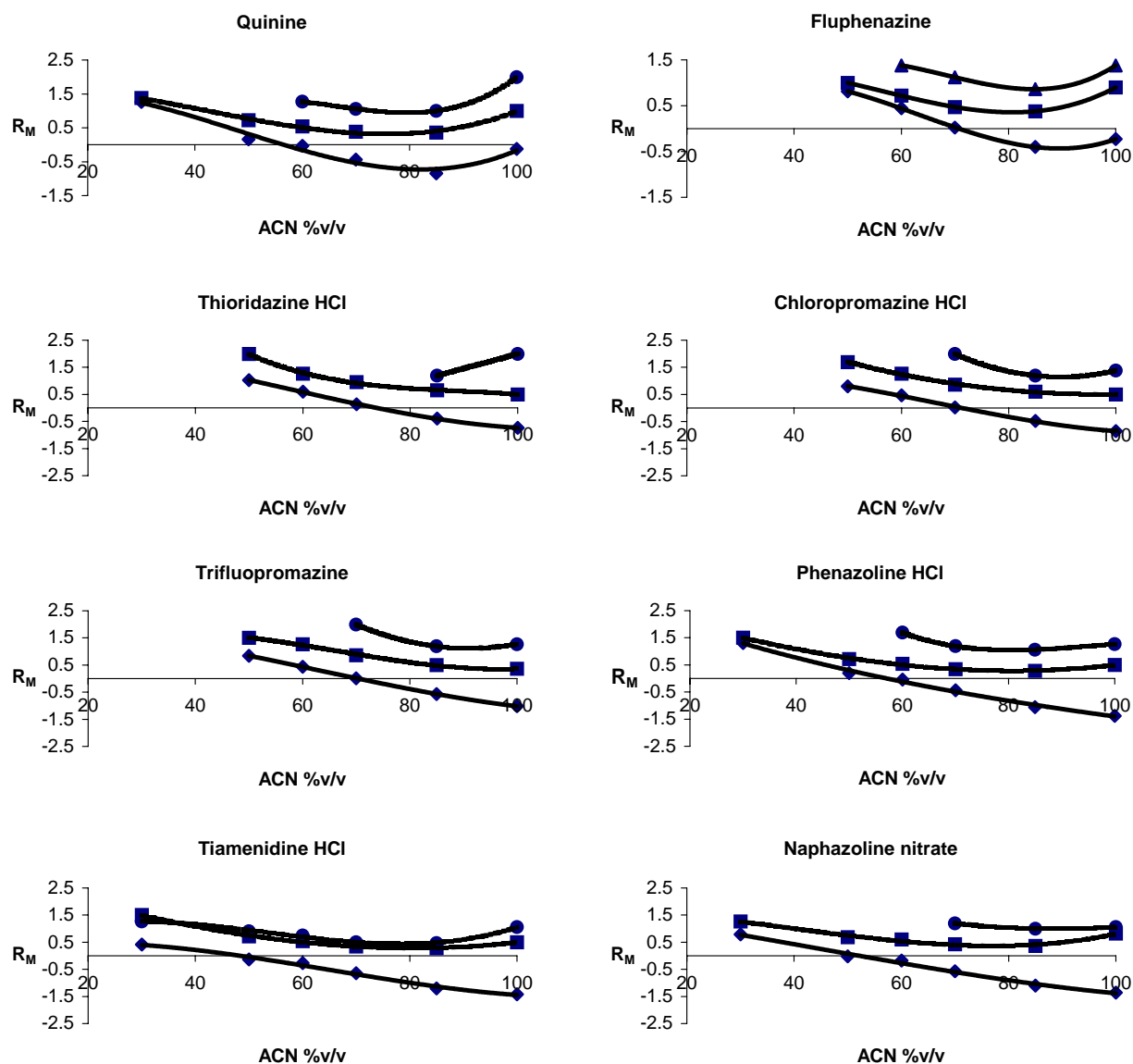
Fig. 5. Thin-layer chromatographic retention parameter, $R_M = \log(1/R_F - 1)$, of basic test drug analytes determined on octadecylsilica-covered plates in relation to the volume percent of acetonitrile in water–acetonitrile eluent. The mobile phases contained 3% (v/v) of IL 1 (◆), TEA (▲), and NH$_4$OH (●).

containing 1.5% (v/v) of various additives. First chromatogram from the left in the upper row was obtained with a non-modified mobile phase. Next chromatogram shows a negligible effect of ammonia on analytes' mobility. Third chromatogram from the left in the upper row illustrates weak effects of DMOA. Certainly, a better separation, however, by no means satisfactory, provides TEA (first plate from the left in the bottom row). Advantages of IL 1 are convincingly presented by the second chromatogram from the left in the bottom row. 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

IL 1 in water was found to provide such a pH. The experiment was to check whether the separation produced by IL 1 had not been due to the pH change caused by the additive.

The evidence gathered in present work documents that imidazolium tetrafluoroborate ionic liquids are valuable, efficient suppressors of free silanols which are responsible for unwanted, irreproducible, difficult to quantify and to control, attractive interactions of chromatographic stationary phases with basic analytes.

Now, it appeared that free silanols should elicit opposite effects with regards to the acidic analytes and be without effect towards the neutral compounds. A simple experiment illustrated in Fig. 7 confirmed the expectations. Chromatogram (a) in Fig. 7 shows from the left the spots of acetylosalicylic acid, salicylic acid, phenol and 2,3-dimethoxytoluene developed on the RP-18 F$_{254}$ plates
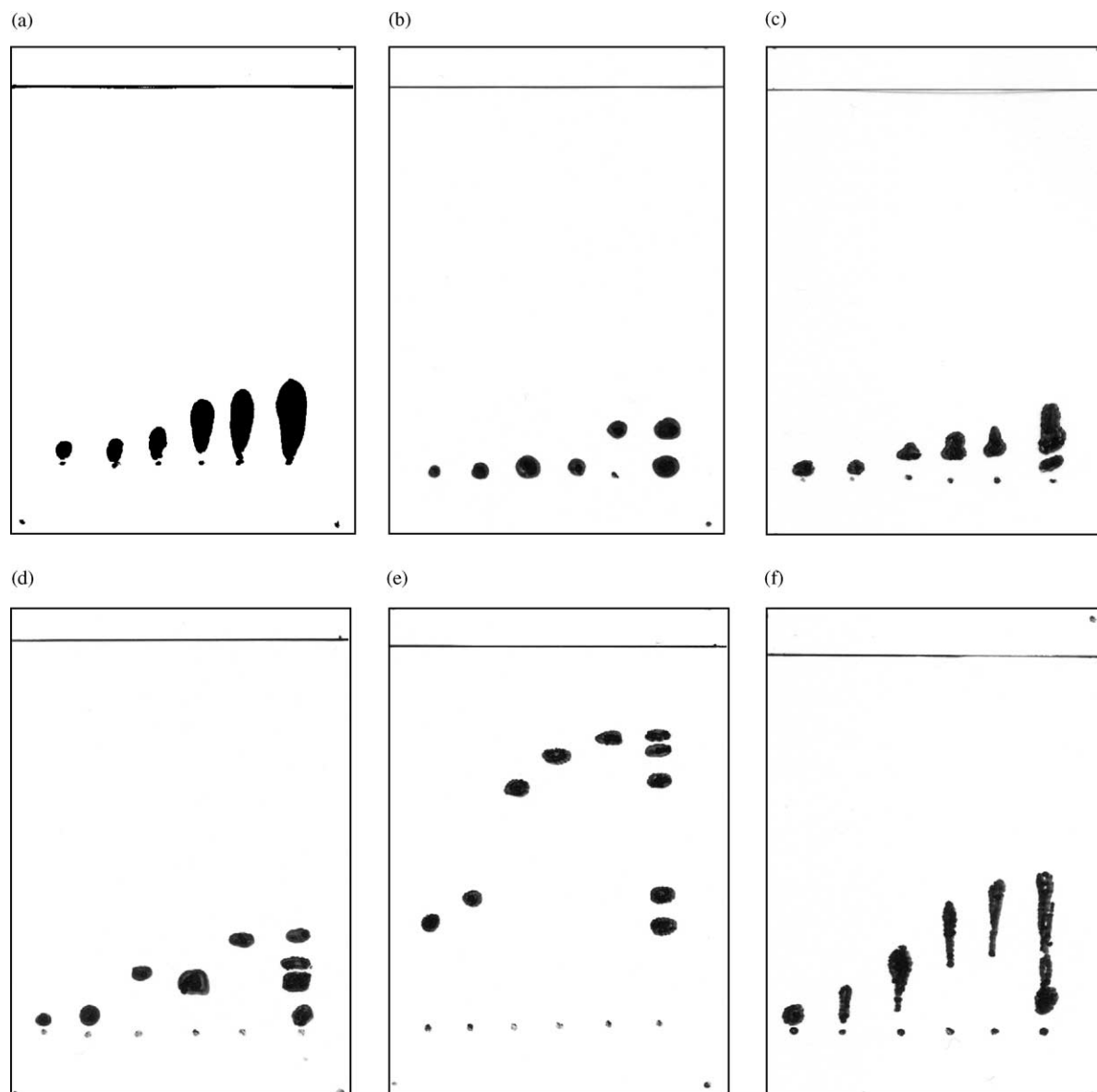
Fig. 6. Drawings of chromatograms of thioridazine, trifluoropromazine, phenazoline, naphazoline, tiamenidine and the mixture of the drugs, as spotted from left to right, on RP-18 F$_{254}$ plates, developed with water–acetonitrile (40:60, v/v) eluent either pure or containing 1.5% (v/v) of various additives: (a) no additive; (b) NH$_4$OH; (c) DMOA; (d) TEA; (e) IL 1; (f) buffer of pH 2.87.

with water–acetonitrile (40:60, v/v). Chromatogram (b) presents analogous picture obtained after adding to the mobile phase 1.5% (v/v) of IL 1. The additive increased retention of both acids. That can be explained by the removal of the repelling interactions due to free silanols because of their suppression by IL 1. In other words, the additive eliminated the exclusion effect with respect to acidic analytes reported previously for silica-based stationary phase materials [2]. Reduction of that effect with help of imidazolium tetrafluoroborates can be recommended to get reproducible and better predictable retention parameters for anionic analytes. Chromatogram (c) (eluent buffered at pH 2.87) demonstrates that the increased retention of acids seen in the presence of IL 1 (chromatogram (b)) is not due to their

decreased ionization at the acidic pH caused by the additive. As confirmed by chromatogram (c), a low retention of acids is mostly due to exclusion effects.

Comparing chromatograms (a)–(c) in Fig. 7, one will note that neutral analyte 2,3-dimethoxytoluene retention is not much affected by addition of imidazolium tetrafluoroborate additive. The spot of phenol seems to be moved a bit up at the presence of IL 1 and at pH 2.87. That minor effect might probably be due to an increased eluting power of the water–acetonitrile eluent enriched with 1.5% (v/v) of the ionic liquid or acidified.

Summarizing the observations illustrated in Figs. 6 and 7, one can conclude that adding of imidazolium tetrafluoroborates to liquid chromatographic mobile phases
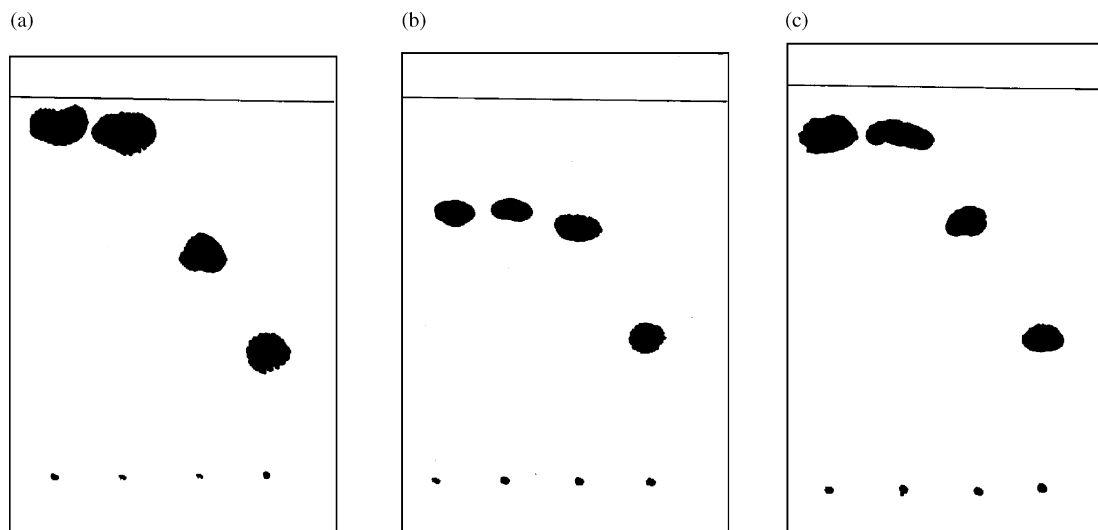
(a)   (b)   (c)

Fig. 7. Drawings of chromatograms of acetylosalicylic acid, salicylic acid, phenol and 2,3-dimethoxytoluene, as spotted from left to right, respectively, on RP-18 F_{254} plates, developed with water–acetonitrile (40:60, v/v) eluent: (a) pure; (b) containing 1.5% (v/v) of IL 1; (c) buffered to pH 2.87.

produces partition chromatographic systems universally applicable for basic, acidic and neutral analytes. 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.

Ionic liquid additives studied appeared interesting from the point of view of determination of lipophilicity of ion-

Fig. 8. Relationships between $\log P$ [29] 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 IL 1. 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.

ized forms of basic drugs by HPLC. In Fig. 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 IL 1 in mobile phase are plotted against the reference lipophilicity parameter $\log P$ from the *n*-octanol–water partition system [29]. The correlation is evidently better if IL 1 is present in the buffer than if only pH is appropriately adjusted. In addition, IL 1 strikingly improves the shape of peaks and removes peak tailing observed in the reference system.

In view of the results obtained here, the silanol suppressing properties of 1-alkyl-3-methylimidazolium based ionic liquids appear to be responsible for the effects of increased mobility of basic analytes in non-aqueous capillary electrophoresis which have recently been reported by Vaher et al. [18,19]. Evidently, those are the imidazolium cations which are adsorbed on the silica surface of the capillary wall and not the anions of the ionic liquids as postulated by the original authors.

4. Conclusions

A simple and effective approach is proposed to reduce deleterious effects of free silanols on liquid chromatographic separation of basic and acidic analytes. Imidazolium tetrafluoroborate ionic liquids employed for that purpose are convenient to use, inexpensive, non-explosive, do not oxidize, and have no measurable vapor pressure [30]. The replacement of the commonly used alkylamine additives with the readily available imidazolium tetrafluoroborates improves chromatographic separations of the problem-causing analytes and reduces the use of environmentally harmful amines. Elimination of the irreproducible and difficult to control and quantify base-attracting and acid-repulsing effects of free silanols allows for improved prediction of
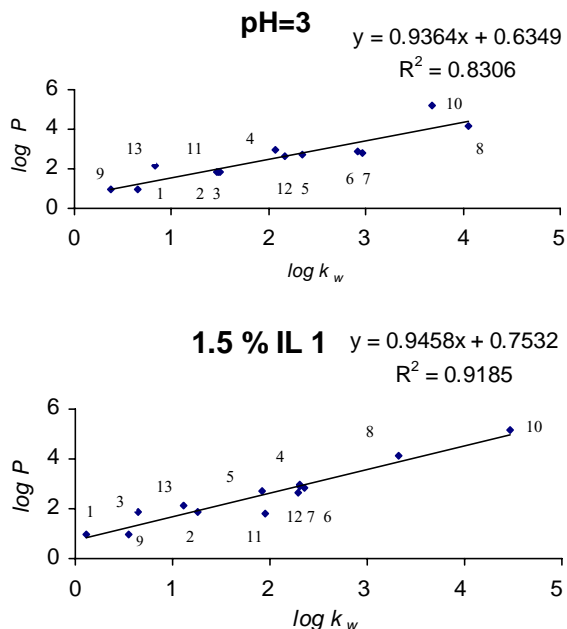
changes in retention accompanying the changes in eluent composition and thus a rational optimization of separation conditions. At the same time, a reliable liquid chromatographic systems is proposed for reproducible determination of lipophilicity of dissociated forms of organic bases.

References

[1] L.R. Snyder, J.J Kirkland, J.L. Glajch, Practical HPLC Method Development, second ed., Wiley, New York, 1977, p. 178.

[2] J.H. Knox, R. Kaliszan, G.J. Kennedy, Faraday Discuss. R. Chem. Soc. 15 (1980) 113.

[3] J.J. Gilroy, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 1000 (2003) 757.

[4] E. Bayer, A. Paulus, J. Chromatogr. 400 (1987) 1.

[5] S.M. Hansen, P. Helboe, M. Thomson, J. Chromatogr. 544 (1991) 53.

[6] J. Nawrocki, B. Buszewski, J. Chromatogr. 449 (1988) 1.

[7] L.C. Tan, P.W. Carr, J. Chromatogr. A 799 (1998) 1.

[8] P.G. Righetti, C. Gelfi, B. Verzola, L. Castelletti, Electrophoresis 22 (2001) 603.

[9] R. Sebastiano, C. Gelfi, P.G. Righetti, A. Citterio, J. Chromatogr. A 894 (2000) 53.

[10] E. Olivieri, R. Sebastiano, C. Gelfi, P.G. Righetti, A. Citterio, J. Chromatogr. A 894 (2000) 273.

[11] H.A. Claessens, Trends Anal. Chem. 20 (2001) 563.

[12] H. Olivier-Bourbigon, L. Magna, J. Mol. Catal. A 182–183 (2002) 419.

[13] C. Carda-Broch, A. Berthod, D.W. Armstrong, Anal. Bioanal. Chem. 375 (2003) 191.

[14] P.H. Shetty, P.J. Youngberg, B.R. Kersten, C.F. Poole, J. Chromatogr. 411 (1987) 61.

[15] S.K. Poole, P.H. Shetty, C.F. Poole, Anal. Chim. Acta 218 (1989) 241.

[16] D.W. Armstrong, L. He, Y.-S. Liu, Anal. Chem. 71 (1999) 3873.

[17] A. Heintz, D.V. Kulikov, S.P. Verevkin, J. Chem. Eng. Data 46 (2001) 1526.

[18] M. Vaher, M. Koel, M. Kaljurand, Chromatographia 53 (Suppl.) (2001) S302.

[19] M. Vaher, M. Koel, M. Kaljurand, J. Chromatogr. A 979 (2002) 27.

[20] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robinson, A.M. Stalcup, Anal. Chem. 73 (2001) 3838.

[21] J. Pernak, A. Czepukowicz, R. Poźniak, Ind. Eng. Chem. Res. 40 (2001) 2379.

[22] E. Forgács, T. Cserháti, Molecular Bases of Chromatographic Separations, CRC Press, Boca Raton, FL, 1997.

[23] S.H. Unger, G.H. Chiang, J. Med. Chem. 24 (1981) 262.

[24] R.W. Ross, C.A. Lau-Cam, J. Chromatogr. 370 (1986) 403.

[25] T. Dzido, E. Soczewiński, J. Chromatogr. 516 (1990) 461.

[26] L.R. Snyder, J.W. Dolan, Adv. Chromatogr. 38 (1998) 115.

[27] BioByte, BioByte Corporation, clog@biobyte.com.

[28] T. Baczek, M. Markuszewski, R. Kaliszan, M.A. van Straten, H.A. Claessens, J. High Resolut. Chromatogr. 23 (2000) 667.

[29] C. Hansch, A. Leo, D. Hoekman, Exploring QSAR, ACS Professional Society, Washington, 1995.

[30] R.L. Perry, K.J. Jones, W.D. Scott, Q. Liao, C.L. Hussey, J. Chem. Eng. Data 40 (1995) 615.