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Chromatographic behaviour of ionic liquid cations in view of quantitative structure-retention relationship

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ABSTRACT

The availability of ionic liquids (ILs) in wide areas of application often results in the requirement on their determination. The attention is also often focused on the knowledge of hydrophobicity as it plays a key role in the biological effects, in the assessment of environmental risk and in the prediction of the fate of chemicals in the environment and of its influence on retention in RP HPLC. One can get information regarding hydrophobicity and retention mechanism if quantitative structure-retention relationships (QSRRs) are identified. The QSRRs were derived for logarithms of retention factors extrapolated to a pure water (or aqueous buffer) eluent, log k_w, determined for the pyridinium and imidazolium ionic liquid (IL) cations on two C8 (Supelcosil LC-8-DB, Symmetry C8) and two C18 (ACE 5 C18, Symmetry C18) stationary phases with isocratic elution by a mobile phase consisting of acetonitrile/40 mM phosphate buffer. The analyses of ILs were performed at a flow rate of 1 mL min^{-1} with UV detection at 218 nm. The QSRRs were derived based on the retention parameters determined experimentally and the structural descriptors of test analytes from molecular modeling. Separations of ILs were obtained with aqueous acetonitrile buffered at pH 3.55 mobile phases. The statistically most significant two-parameter QSRR regression equations related log k_w to the solvent accessible surface (*SAS*) of the analytes and the differences in the energies of the highest occupied and the lowest unoccupied molecular orbitals (*diffHL*). These equations were especially good in case of columns with the highest carbon loads and larger specific surface areas, i.e. Symmetry C18 and Symmetry C8. On the other hand, the column ACE 5 C18 appeared to produce the best quality separations of the ILs studied. The QSRRs derived in the research shed light on the molecular mechanism of HPLC separation of ILs and helped to predict their relative separations.

1. Introduction

The term ionic liquids (ILs) is currently used to describe a broad class of non-molecular semi-organic salts or salt mixtures consisting entirely of ionic components with a melting point below 100 °C. Many ILs are liquids at 25 °C and are called room temperature ionic liquids (RTILs). The accepted definition of RTILs is any salt that has a melting point lower than ambient temperature [1–3]. Nowadays, typical ionic liquids are composed of bulky 1,3-dialkylimidazolium, alkylamonium, alkylphosphonium or alkylpyridinium organic cations and inorganic anions, most frequently not only $AlCl_4^-$, BF_4^- or PF_6^-, but also NO_3^-, ClO_4^-, CF_3COO^-, $CF_3SO_3^-$ or CH_3COO^- and others [4]. The history, properties, application and analyses of ILs are summarized in numerous reviews, e.g. [4–8]. The unique properties of ILs such as non-volatility, non-flammability, excellent chemical and thermal stability and negligible vapor pressure result in wide use of ILs in biotechnological applications and in the pharmaceutical and chemical industry [9–11].

Hence, chemists are currently especially interested in the development of determination methods for ionic liquids. There are several situations when separation of ILs is important. First of all, at industrial production of these compounds used as pesticides, for example, also, at control of environmental contamination, at studies of their metabolism, etc. Most of the methods for separation and analysis of ILs reported so far involve high performance liquid chromatography (HPLC) with a variety of column packings and mobile phases [12–14]. The paper [12] reports the first published routine chromatographic method for gradient reversed-phase RP HPLC separation of 11 selected 1-alkyl and 1-aryl-3-methylimidazolium based RTILs with electrospray ionization mass detection and mobile phase consisting of acetonitrile and water (20 mM ammonium

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acetate/1% acetic acid). The same author [15] later describes the possible usefulness of π–π interactions in the selective separation of imidazolium and pyridinium ionic liquid cations on two polar RP phases. Very good separations of ionic liquid cations were achieved using an ether-linked phenyl phase with polar endcapping and a mobile phase consisting of methanol or acetonitrile and water (5 mM K_2HPO_4, H_3PO_4, pH 3). The method was especially useful for separation of short alkyl chain hydrophilic IL cations, known to be poorly separated on conventional RP columns.

The most popular packing materials in the RP HPLC separation of IL cations are octadecylsilica and octylsilica stationary phases and the mechanism of separation in RP HPLC is primarily based on differences in hydrophobicity: the compounds are eluted in order to increase hydrophobicity.

In studies so far published on the topic of ionic liquids in chromatography, in particular by Stepnowski et al., there were some common aspects undertaken, like the effect of lipophilicity on retention. However, no QSRRs were studied thoughtfully at the same time comprising quantum chemical descriptors of a large series of analytes and therefore our interpretation of the molecular mechanism of ILs separation is original. The more so that our interpretation of factors affecting retention seems to be comprehensive and rational from the basic physico-chemical point of view.

The prediction of retention in RP HPLC may be of use both for the optimization of chromatographic separations and for the identification of separated compounds [16]. Several approaches to the prediction of retention have been proposed, such as the model based on Hildebrand solubility parameters [17], the model based on the solvophobic theory [18], molecular statistical theory [19], the interaction indices model of Jandera [20,21], linear solvation energy relationships (LSERs) of Kamlet [22] and the quantitative structure-retention relationships (QSRRs) developed by Kaliszan [23–25].

QSRRs are statistically derived relationships between chromatographic parameters and the descriptors characterizing the molecular structure of the analytes. The simplest QSRR model applied for retention prediction is represented by Eq. (1):

$$\log k_w = k_1 + k_2 \log P \quad (1)$$

where $\log k_w$ is the retention factor extrapolated to a pure water (aqueous buffer) mobile phase; the coefficients k_1 and k_2 are regression coefficients, characteristic of the separation systems applied and representing differences in individual physicochemical properties between the mobile and the stationary phase; $\log P$ is the logarithm of the *n*-octanol–water partition coefficient. The $\log P$ represents a molecular hydrophobicity and its values can be calculated from structural formulas employing commercially available softwares. Hydrophobicity affects absorption, transmembrane transport, bioavailability, hydrophobic drug–receptor interactions, metabolism, pharmacological activity or toxicity of molecules as well as it represents a key parameter in the assessment of environmental risk and in the prediction of the fate of chemicals in the environment [26].

QSRRs treat chromatographic retention as a linear function of a number of mutual analyte-column-mobile phase interactions. QSRRs provide an objective tool for the investigation of the retention mechanism, retention prediction and the design of new stationary phases of specific properties, when a sufficiently large set of analytes is chromatographed to obtain reliable data. The protocol to be followed in QSRR analysis has, in general, the following steps: (1) acquisition of the experimental data set, (2) molecular structure entry, (3) structure descriptor calculation and (4) analysis of the regression relating to the experimental data and to the structural descriptors.

The aim of this research was a study of the chromatographic behaviour of 13 selected imidazolium and pyridinium based ionic liquid cations, as test analytes, on four RP HPLC columns (ACE 5 C18, Symmetry C18, Symmetry C8 and Supelcosil LC-8-DB) and the use of the thus-obtained retention parameters to derive QSRRs. The QSRRs were derived for logarithms of retention factors normalized to a hypothetical zero percent organic modifier eluent, $\log k_w$, treated as a dependent variable. Attention was focused on hydrophobicity and the theoretical structural parameters of ionic liquid cations provided by the HyperChem package. The QSRRs have been studied with the aim of predicting the hydrophobicity parameters and retention in reversed-phase systems as well as comparing in quantitative terms the properties of selected stationary phases.

As the importance of hydrophobicity is described above, an effort was undertaken to find a suitable procedure that would produce this parameter of ILs in a reliable manner. In the present work, the use of isocratic RP HPLC as the practical method for the hydrophobicity determination ($\log k_w$) and its comparison with the theoretically calculated parameter of hydrophobicity by HyperChem software ($\log P$) is described.

2. Experimental

2.1. Equipment

Chromatographic measurements were made with an HPLC system, LC Module I Plus (Waters Associates, Milford, MA, USA), consisting of a pump, a variable wavelength UV–vis detector, an autosampler and a thermostat. Data were collected using Waters Millennium 2.15 software. The pH measurements were made with a Microprocessor Hanna pH meter 211 (Hanna Instruments, Bedfordshire, UK). Water was prepared with a Milli-Q Purification system (Millipore, Bedford, MA, USA) and the mobile phases were filtered using a Macherey-Nagel 0.45 μm filter (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

2.2. Chemicals and mobile phase

The ionic liquids selected for this study were: 1-ethyl-3-methylimidazolium chloride (EMIM), 1-ethyl-3-ethylimidazolium bromide (EEIM), 1-*n*-propyl-3-methyl-imidazolium tetrafluoroborate (PMIM), 1-*n*-butyl-3-methylimidazolium tetrafluoroborate (BMIM), 1-butyl-4-methylpyridinium chloride (MBPy), 1-benzyl-3-methylimidazolium tetrafluoroborate (BzMIM), 1-*n*-amyl-3-methylimidazolium chloride (AMIM), 1-(*p*-ethylbenzyl)-3-methylimidazolium chloride (EBzMIM), 1-(*p*-methylbenzyl)-3-methylimidazolium chloride (pMBzMIM), 1-*n*-hexyl-3-methylimidazolium chloride (HMIM), 1-*n*-octyl-3-methylimidazolium tetrafluoroborate (OMIM), 1-*n*-nonyl-3-methylimidazolium tetrafluoroborate (NMIM) and 1-*n*-dodecyl-3-methylimidazolium chloride (DMIM). The structure and main properties of the ILs studied are listed in Table 1. The standards of ILs were obtained from Merck (Darmstadt, Germany), Sigma–Aldrich (St. Louis, MO, USA) or as supplied by Prof. Piotr Stepnowski (Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland). Concentrations of ionic liquids for the chromatographic procedure were about 0.1 mM in a water solution.

The mobile phase contained acetonitrile and a 40 mM phosphate buffer of pH 3.55. The buffer was prepared by dissolving dihydrogen potassium phosphate (Fluka, Buchs, Switzerland) in water and adjusting the pH with an 85% solution of *o*-phosphoric acid (Fluka, Buchs, Switzerland). The pH of the buffer was measured before mixing with the organic modifier. Isocratic grade acetonitrile was used as obtained from J.T. Baker (J.T. Baker, Deventer, The Netherlands).

2.3. Chromatographic procedure

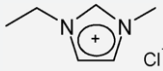
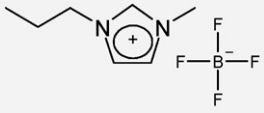
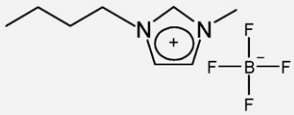
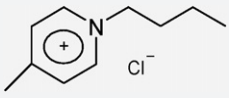
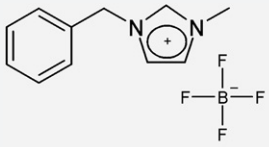
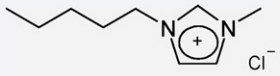
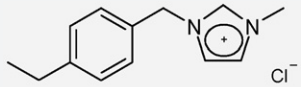
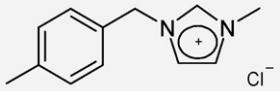
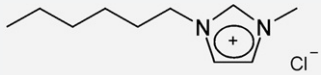
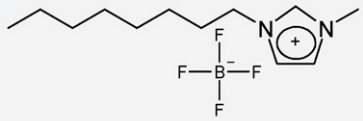
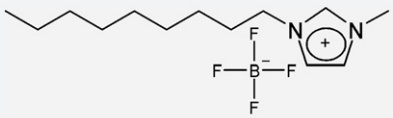
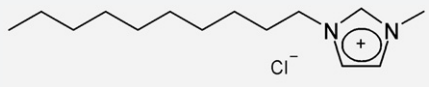
All the chromatographic measurements were performed at a flow-rate of 1 mL min^{-1} with UV detection at 218 nm. The injected

sample volume was 10 μL. Columns that were employed are listed in Table 2. During chromatographic investigations these columns were thermostated at 40 °C.

The IL cations were analyzed under isocratic conditions. Depending on their retention properties, five to seven buffered mobile phases with concentrations of acetonitrile varying from 60% to 1% (v/v), were used. The retention factors of sample compounds were calculated as follows (Eq. (2)):

$$k = \frac{V_R}{V_M - 1} \quad (2)$$

Table 1
Structures and properties of ionic liquids under study.

Systematic name	Abbreviation	Structure	Molecular mass	λ (nm)
1-Ethyl-3-methyl-imidazolium chloride	[EMIM][Cl]		146	n.a.
1-Ethyl-3-ethyl-imidazolium bromide	[EEIM] [Br]		205	190
1-*n*-Propyl-3-methyl-imidazolium tetrafluoroborate	[PMIM] [BF_4]		212	212
1-*n*-Butyl-3-methyl-imidazolium tetrafluoroborate	[BMIM] [BF_4]		226	210
1-Butyl-4-methyl-pyridinium chloride	[MBPy] [Cl]		185	n.a.
1-Benzyl-3-methyl-imidazolium tetrafluoroborate	[BzMIM] [BF_4]		260	n.a.
1-*n*-Amyl-3-methyl-imidazolium chloride	[AMIM] [Cl]		188	n.a.
1-(*p*-Ethylbenzyl)-3-methyl-imidazolium chloride	[EBzMIM] [Cl]		236	203
1-(*p*-Methylbenzyl)-3-methyl-imidazolium chloride	[pMBzMIM] [Cl]		222	n.a.
1-*n*-Hexyl-3-methyl-imidazolium chloride	[HMIM] [Cl]		104	n.a.
1-*n*-Octyl-3-methyl-imidazolium tetrafluoroborate	[OMIM] [BF_4]		282	n.a.
1-*n*-Nonyl-3-methyl-imidazolium tetrafluoroborate	[NMIM] [BF_4]		296	n.a.
1-*n*-Dodecyl-3-methyl-imidazolium chloride	[DMIM] [Cl]		258	n.a.

n.a.—Not assessed.

Table 2
Basic characteristics of the columns used.

Column no.	Trade name, dimensions ($L \times$ i.d.), particle diameter	Manufacturer	V_M	Endcapping	%C	S
A	ACE 5 C18, 150 × 4.6 mm, 5 μm	ACT, Aberdeen, Scotland	2.35	Yes	15.5	300
B	Symmetry C18, 250 × 4.6, 5 μm	Waters, Milford, MA USA	3.21	Yes	19.1	335
C	Symmetry C8, 150 × 3.9 mm, 5 μm	Waters, Milford, MA USA	2.02	Yes	11.7	335
D	Supelcosil LC-8-DB, 150 × 4.6 mm, 5 μm	Sigma–Aldrich, St. Louis, MO, USA	2.43	Yes	6.0	170

L, Column length (mm); i.d., column inner diameter (mm); V_M, hold-up volume (mL; KNO_3 in acetonitrile-aqueous buffer was the marker); %C, carbon load; *S*, specific surface area ($m^2\ g^{-1}$).

Table 3
Structural descriptors of ionic liquid cations that were employed in QSRR equations.

Ionic liquid	Structural descriptor					
	log *P*	*SAS* [Å^2]	*HF* [kcal mol^{-1}]	*HE* [kcal mol^{-1}]	*HOMO* [a.u.]	*diffHL* [a.u.]
[EMIM]	−0.19	316.14	282.39	−1.13	−15.62	−8.92
[EEIM]	0.15	336.91	276.10	−0.43	−15.54	−8.91
[PMIM]	0.28	351.55	276.12	−0.55	−15.09	−8.42
[BMIM]	0.68	387.67	270.04	−0.08	−14.51	−7.85
[MBPy]	1.68	389.57	246.39	0.88	−14.73	−7.25
[BzMIM]	0.48	350.96	321.86	−2.76	−12.72	−6.17
[AMIM]	1.07	425.18	264.08	0.31	−14.03	−7.38
[EBzMIM]	1.03	411.23	310.40	−1.19	−12.42	−5.91
[pMBzMIM]	0.64	389.03	315.29	−1.62	−12.43	−5.91
[HMIM]	1.47	461.01	258.14	0.68	−13.65	−7.01
[OMIM]	2.26	532.23	246.30	1.42	−13.12	−6.48
[NMIM]	2.66	567.75	240.40	1.79	−12.93	−6.29
[DMIM]	3.05	603.28	234.49	2.15	−12.72	−6.08

For explanation and calculation of structural parameters by HyperChem see text.

where V_R is the retention volume and V_M is the chromatographic system hold-up volume measured with KNO_3 as a non-retained marker.

2.4. Statistical data analysis and structural descriptors of IL cations

Based on the linear regression between the logarithm of the retention factor, log *k*, and the organic modifier concentration in the eluent, φ, the values corresponding to 100% of buffered eluent, $\log k_w$, were obtained by extrapolation, following the Snyder-Soczewinski equation (Eq. (3)) of linear solvent strength (LSS) model [27]:

$$\log k = \log k_w - S\varphi \quad (3)$$

where *k* is the retention factor, k_w is the intercept corresponding to *k* in pure water or aqueous buffer as the mobile phase, *S* is a constant for a given analyte or congeneric group of analytes and φ is the volume fraction of the organic modifier in the eluent.

The IL cations were subjected to molecular modeling employing the HyperChem program with a ChemPlus extension (Hypercube Inc., Waterloo, Canada). The calculation procedure applied was the semi-empirical AM1 method. For the geometry-optimized structures, numerous structural descriptors were derived; some are listed in Table 3.

The QSRR equations derived in this study employed the following molecular structural descriptors of analytes: the logarithm of the *n*-octanol–water partition coefficient (log *P*), the solvent accessible molecular surface area (*SAS*, in Å^2), the heat of formation (*HF*, in kcal mol^{-1}), the hydration energy (*HE*, in kcal mol^{-1}), the energy of the highest occupied molecular orbital (*HOMO*, in atomic units) and the difference of energies of the highest occupied molecular orbital and the lowest unoccupied molecular orbital (*diffHL*, in atomic units). The procedure of multiple regressions was executed. Statistical calculations were performed on a personal computer employing the Statistica package (StatSoft, Tulsa, USA). Graphs were plotted using the OriginPro program (OriginLab Corporation, MA, USA).

3. Results and discussion

A reversed-phase HPLC method using C8 and C18 columns has been developed for the analysis of selected IL cations. Acetonitrile was the organic modifier of choice, because it is more suitable in comparison with methanol for chromatographic applications at very low UV detection wavelengths, which are required in the case of ILs. These salts have the absorption maxima below 210 nm [15]. The use of acetonitrile also produces sharper peaks and shorter retention times than those obtained with the use of methanol. The second component of the mobile phase was an aqueous buffer, owing to the ionic character of the analytes under study and to obtain better peak shapes [14,15,28]. After a large number of IL analyses with buffering the mobile phase with potassium phosphate, we selected this aqueous buffer containing 40 mM KH_2PO_4/H_3PO_4 with pH adjusted to 3.55 for all the analyses. All the analyzed ILs showed the reversed-phase behaviour, as they were eluted according to their hydrophobicity.

3.1. log k_w determination by isocratic method

At first, the retention parameters of IL cations obtained at the isocratic elution mode were investigated. The linear regression of the logarithm of the retention factor of the analytes, log *k*, against the volume fraction of the organic solvent in the mobile phase, φ, was used for the chromatographic determination of $\log k_w$ (Eq. (3)). $\log k_w$ well characterizes the relative hydrophobicity of ILs and can serve as the alternative to the log *P* (logarithm of the *n*-octanol–water partition coefficient), usually determined experimentally in slow-equilibrium batch experiments. The procedure of the standard experimental estimation of log *P* for ILs is more complicated than in the case of neutral, non-ionic substances due to their dissociation, formation of various ion-pairs, etc. [25].

Table 4
Slopes (S), intercepts ($\log k_w$), numbers of data points used to derive regression (n), squares of correlation coefficients (R^2) of Snyder–Soczewinski relation (Eq. (3)) determined at isocratic conditions.

Ionic liquid	ACE 5 C18				Symmetry C18				Symmetry C8				Supelcosil LC-8-DB			
	S	$\log k_w$	R^2	n	S	$\log k_w$	R^2	n	S	$\log k_w$	R^2	n	S	$\log k_w$	R^2	n
EMIM	−18.7760	−0.0714	0.9950	5	−45.3220	−0.1784	0.9190	5	−12.2440	−0.2645	0.8805	5	−4.0281	0.2641	0.8769	5
EEIM	−9.7615	0.1043	0.9553	7	−15.3560	−0.0050	0.9865	5	−9.7259	−0.0265	0.9563	6	−3.7612	0.2214	0.9037	5
PMIM	−7.6412	0.0101	0.9955	7	−11.1630	0.0064	0.9976	5	−8.7421	0.0682	0.9589	7	−3.9438	0.3443	0.8999	5
BMIM	−7.1422	0.6291	0.9369	7	−7.2234	0.4904	0.9846	7	−6.1856	0.4487	0.9595	7	−2.9567	0.4389	0.9698	5
MBPy	−7.0708	0.7881	0.9713	6	−8.0847	0.8051	0.9760	7	−7.0009	0.7247	0.9606	7	−3.4921	0.6863	0.9550	5
BzMIM	−7.3011	0.9817	0.9565	6	−7.3739	0.8777	0.9865	7	−6.0772	0.7132	0.9857	6	−3.7005	0.7328	0.9693	5
AMIM	−5.9894	0.9654	0.9927	4	−6.3998	0.9912	0.9871	6	−5.4974	0.8219	0.9779	5	−3.5053	0.8934	0.9621	6
EBzMIM	−4.9744	0.8915	0.9851	5	−7.4153	1.1537	0.9980	5	−5.9951	0.9389	0.9824	5	−3.6422	0.9005	0.9747	6
pMBzMIM	−5.4499	1.2159	0.9695	6	−6.2740	1.2404	0.9940	6	−6.3470	1.1847	0.9900	6	−4.2578	1.2123	0.9721	6
HMIM	−6.0578	1.6507	0.9929	5	−5.7020	1.3482	0.9961	6	−5.7019	1.2897	0.9947	6	−3.5143	1.1947	0.9707	6
OMIM	−2.7118	1.2378	0.9992	5	−4.8480	1.8341	0.9932	7	−4.8976	1.7856	0.9937	6	−2.5355	1.3442	0.9711	7
NMIM	−3.7583	1.9030	0.9989	5	−4.7023	2.0582	0.9896	7	−5.3525	2.2408	0.9905	6	−3.0958	1.8026	0.9766	7
DMIM	−3.9722	2.1988	0.9923	5	−4.9816	2.4632	0.9841	7	−5.4355	2.5144	0.9912	5	−3.2799	2.0741	0.9843	6

Therefore, other accurate and less tedious methods for the study of the hydrophobicity of ILs are especially needed.

One of the possibilities represents the calculation chemistry methods. Stepnowski and Storoniak [29] have reported theoretically estimated hydrophobicity for four selected imidazolium IL cations using the manual calculation procedure proposed by Hansch and Leo [30] rationalizing the means by which an N^+ cation in a quaternary alkylammonium moiety appears to become more hydrophilic as the chains are lengthened and its charge can delocalize to the maximum extent. The fragment value for a quaternary amine combines the geometric bond factor applying to a neutral solute with the negative electronic bond factor that decreases in magnitude with the square of the distance from the central nitrogen atom. In the case of imidazolium cations, the charge is distributed in the aromatic ring between the nitrogen atoms 1 and 3 and the remaining three aromatic carbons. Both "quat" nitrogen atoms have an aromatic character for which the fragmental CLOG P value (taken from CLOGP3 computer program [31]) of $\log P$ was estimated at −1.140. The $\log P$ can be also calculated from structural formulas of IL cations employing commercially available softwares, for example HyperChem used in the present study (Table 3).

The other approach is based on estimation of hydrophobicity parameter of IL cations by RP HPLC as $\log k_w$. As the alternative parameter to $\log P$, $\log k_w$ can be determined by extrapolation of the experimental chromatographic retention data to pure water as the mobile phase. The chromatographic estimation of $\log k_w$ can be found in the literature [29,32], describing the hydrophobicity of imidazolium IL cations estimated from a gradient HPLC run on C8 reversed phase or on an immobilized artificial membrane phase [29]. Also, there are reports on the hydrophobicity of imidazolium, pyrrolidinium, pyridinium, quinolinium, quaternary phosphonium and quaternary ammonium cations determined based on isocratic runs on C18 reversed phase [32]. The $\log k_w$ determined by extrapolation from the retention factors of imidazolium and pyridinium cations, determined under isocratic conditions on two C8 and two C18 reversed phases using Eq. (3), are listed in Table 4. The $\log k_w$ values of IL cations depend on the stationary phase chemistry and other column properties, e.g. carbon load and specific surface area. As shown in Table 4, most ILs show the highest values of $\log k_w$ on the Symmetry C18 column with bonded octadecyl chains and the highest values of carbon load and specific surface area, except for the $\log k_w$ values of IL cations with short alkyl chains, such as EMIM, EEIM, BMIM, which are highest on the Supelcosil LC-8-DB column with octyl moieties and the lowest carbon load and specific surface area. The correlation coefficients between the $\log k$ and φ for the most hydrophilic IL cations, such as EMIM, EEIM and BMIM, on C8 stationary phases are lower than the correlations on C18 stationary phases, probably because of weak retention on C8 stationary phases resulting in the elution of the analytes close to the column hold-up volume.

There is nothing unusual about the fact that a stationary phase providing best separation produces retention data that are poorly described by QSRRs. It is rather common. Best QSRRs are derived for systems of simplest retention mechanism. The more complex the mechanism of separation is the worse QSRRs result. Normally, better QSRRs are derived on regular hydrocarbonaceous silica stationary phases operated in reversed-phase mode, because in such systems molecular size ("bulkiness") of analyte determines retention. On polar phases QSRRs are more difficult to obtain because we do not have simple, unequivocal measures of analyte polarity. Actually, polarity is hard to define and quantify, unlike the size of molecule. Still, we sometimes succeed in getting reliable and useful (for a given system) parameters of polarity. These may be inappropriate for individual columns because of their specific functional moieties for which polarity cannot be characterized in a unique universal manner, unfortunately. On the other hand, that makes chromatography to remain a fascinating experimental science.

3.2. Molecular structure descriptors employed in QSRRs

The $\log k_w$ parameters of Eq. (3) were correlated with $\log P$ calculated by the HyperChem software. The linear regression analysis based on Eq. (1) showed rather poor correlations between $\log k_w$ and $\log P$ values when all alkylimidazolium, benzylimidazolium and pyridinium cations were included (ACE 5 C18 column, $R^2 = 0.7424$; Symmetry C18 column, $R^2 = 0.8014$; Symmetry C8 column, $R^2 = 0.8360$; and Supelcosil LC-8-DB column, $R^2 = 0.6741$).

Table 5
Regression coefficients (±standard deviations), numbers of data points used to derive regression (n), squares of correlation coefficients (R^2), standard errors of estimate (s) and F-test values (F) of regression equations $\log k_w = k_1 + k_2 \log P$ determined for a homologous series of alkylimidazolium cations.

	k_1	k_2	n	s	F	R^2
ACE 5 C18	0.0795 (±0.1460)	0.6922 (±0.0869)	9	0.2866	63	0.9006
Symmetry C18	−0.0622 (±0.0620)	0.8371 (±0.0369)	9	0.1216	515	0.9866
Symmetry C8	−0.1271 (±0.0324)	0.8768 (±0.0193)	9	0.0636	2068	0.9966
Supelcosil LC-8-DB	0.2109 (±0.0679)	0.5844 (±0.0404)	9	0.1333	209	0.9676

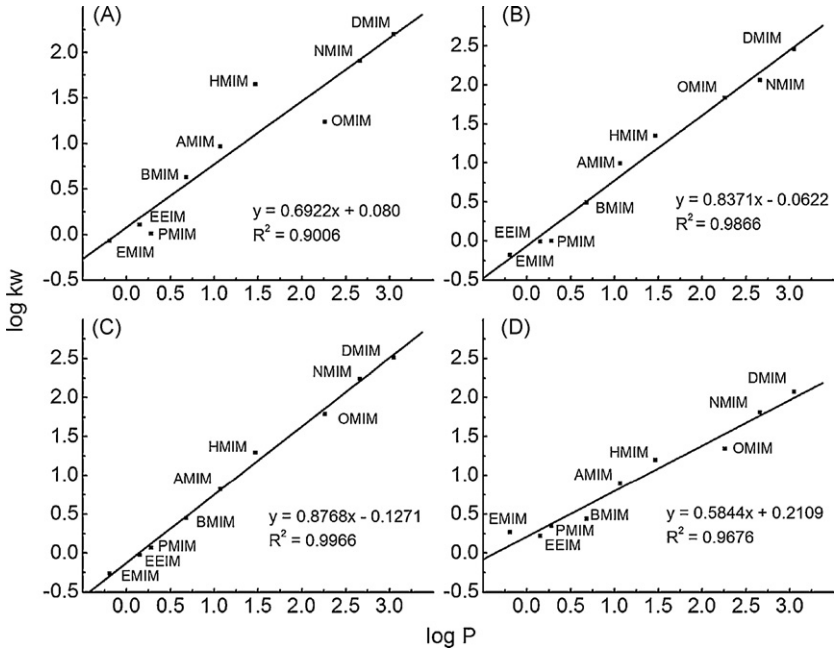


Fig. 1. Correlation of $\log k_w$ determined isocratically against $\log P$. (A) ACE 5 C18, (B) Symmetry C18, (C) Symmetry C8 and (D) Supelcosil LC-8-DB columns. Statistical descriptors of the relationships are given in Table 5.

The correlations significantly improved when only homologous *n*-alkylimidazolim cations were subjected to linear regression according to Eq. (1) (Table 5 and Fig. 1). Good correlations found for homologous alkylimidazolium cations analyzed by RP HPLC on standard octyl or octadecyl stationary phases are due to the fact that hydrophobic interactions differentiate their retention as the polar interactions remain he same for homologues. That is especially evident for cations with longer alkyl chains. Obviously, IL cations with longer alkyl chains are more strongly retained than the IL cations with shorter chains, such as EMIM, EEIM and PMIM. The longer the alkyls in the imidazolium cation, the stronger the hydrophobic interactions between the IL's alkyl chain and the hydrocarbon moiety of the stationary phase.

Table 5 demonstrates that the chromatographic parameters of hydrophobicity, $\log k_w$, correlate very well with $\log P$ values. In the case of the Symmetry C8 column, the correlation is the highest ($R^2 = 0.9966$). Regression coefficient k_2 (Table 5) reflects the degree of similarity of the lipophilic stationary phase to the reference octanol–water partition system. For a solvated hydrocarbon stationary phase, this could be less than that for liquid octanol; however, high correlations of $\log k_w$ versus $\log P$ suggest a general similarity of the processes in the RP HPLC method and in the slow-

Table 6
Regression coefficients (±standard deviations), numbers of data points used to derive regression (n), squares of correlation coefficients (R^2), standard errors of estimate (s) and F-test values (F) of multilinear relationships $\log k_w = k_1 + k_2 SAS + k_3 diffHL$ determined for all the IL cations studied.

	k_1	k_2	k_3	n	s	F	R^2
ACE 5 C18	0.7995 (±0.8983)	0.0048 (±0.0010)	0.2664 (±0.0812)	13	0.2498	42	0.8940
Symmetry C18	0.6060 (±0.3322)	0.0061 (±0.0004)	0.3065 (±0.0300)	13	0.0924	455	0.9891
Symmetry C8	-0.2536 (±0.3973)	0.0071 (±0.0004)	0.2506 (±0.0359)	13	0.1105	346	0.9857
Supelcosil LC-8-DB	0.1657 (±0.5321)	0.0047 (±0.0006)	0.1723 (±0.0481)	13	0.1480	87	0.9456

Table 7
Regression coefficients (±standard deviations), numbers of data points used to derive regression (n), squares of correlation coefficients (R^2), standard errors of estimate (s) and F-test values (F) of multilinear relationships $\log k_w = k_1 + k_2 HF + k_3 HOMO$ determined for all the IL cations studied.

	k_1	k_2	k_3	n	s	F	R^2
ACE 5 C18	11.8385 (±1.1376)	−0.0136 (±0.0024)	0.5196 (±0.0589)	13	0.2373	47	0.9044
Symmetry C18	14.1520 (±0.4920)	−0.0166 (±0.0010)	0.6244 (±0.0255)	13	0.1026	368	0.9866
Symmetry C8	14.7225 (±0.6945)	−0.0188 (±0.0015)	0.6265 (±0.0359)	13	0.1449	199	0.9755
Supelcosil LC-8-DB	10.0743 (±0.8460)	-0.0120 (±0.0018)	0.4245 (±0.0438)	13	0.1764	60	0.9226

Table 8
Regression coefficients (±standard deviations), numbers of data points used to derive regression (n), squares of correlation coefficients (R^2), standard errors of estimate (s) and F-test values (F) of multilinear relationships $\log k_w = k_1 + k_2 HE + k_3 HOMO$ determined for all the IL cations studied.

	k_1	k_2	k_3	n	s	F	R^2
ACE 5 C18	7.1495 (±0.8771)	0.2606 (±0.0523)	0.4473 (±0.0633)	13	0.2593	39	0.8858
Symmetry C18	8.4184 (±0.4363)	0.3249 (±0.0260)	0.5358 (±0.0315)	13	0.1290	231	0.9788
Symmetry C8	8.2390 (±0.5608)	0.3681 (±0.0335)	0.5262 (±0.0405)	13	0.1658	151	0.9679
Supelcosil LC-8-DB	5.9145 (±0.6139)	0.2369 (±0.0366)	0.3601 (±0.0443)	13	0.1815	56	0.9181

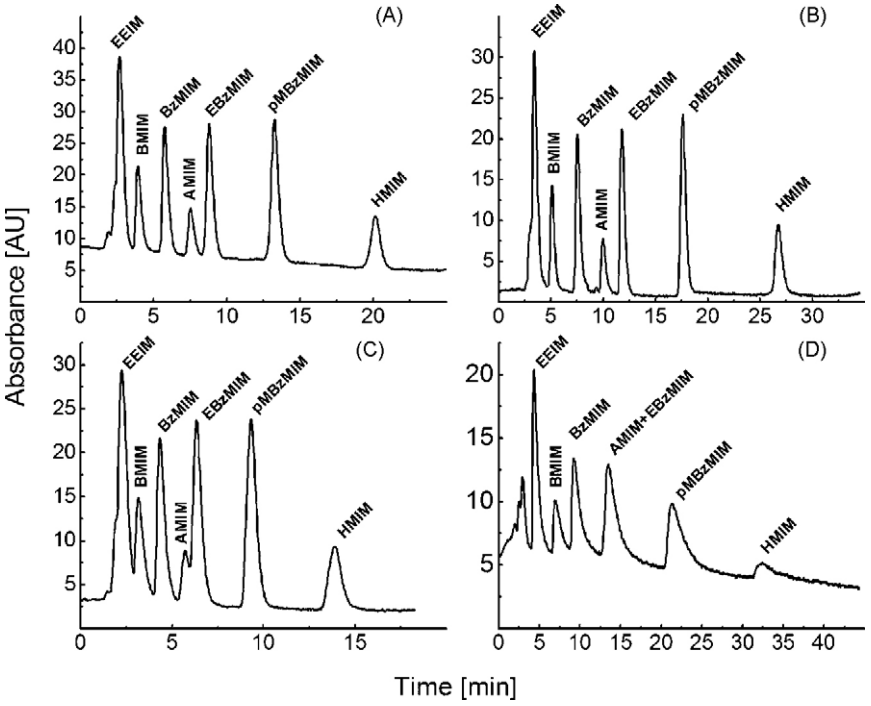


Fig. 2. Separation of selected ionic liquid cations on (A) ACE 5 C18, (B) Symmetry C18, (C) Symmetry C8 and (D) Supelcosil LC-8-DB columns. Mobile phase: 10/90% (v/v) acetonitrile/phosphate buffer (40 mM KH_2PO_4/H_3PO_4, pH 3.55), flow rate 1 mL min^{-1}, temperature 40 °C and UV detection (λ = 218 nm).

equilibrium "shake-flask" octanol–water partition measurement system. The more similar to octanol the solvated stationary phase is, the closer to one the slope k_2 is in the regression Eq. (1) [33,34]. The k_2 coefficients are the highest in case of Symmetry columns, for which the partitioning mechanism of retention is probably less distorted by specific polar intermolecular interactions than for the other two columns.

Statistically significant QSRR correlations were found between the log k_w and the following structural descriptors of IL cations from molecular modeling calculations: hydration energy (*HE*), energy of the highest occupied molecular orbital (*HOMO*), heat of formation (*HF*), solvent accessible molecular surface area (*SAS*) and difference of energies between the highest occupied molecular orbital and the lowest unoccupied molecular orbital (*diffHL*) (Tables 6–8). The results in Tables 6–8 show the significant role of the parameter *HOMO* and *diffHL*, which account for ability of analytes to participate in polar interactions of the electron pair donor–electron pair acceptor type (charge transfer interactions). These interactions are expected to be stronger between a solute and polar molecules of the eluent than between the same solute and the mostly hydrocarbonaceous (non-polar) ligand of the stationary phase. The correlations in Tables 6–8 are highest for Symmetry C18 and Symmetry C8 columns with the highest value of specific surface area in comparison to the ACE 5 C18 and the Supelcosil LC-8DB columns. In these QSRRs the analytes bulkiness terms (*SAS, HF, HE*) are more significant than the polarity-related terms: *HOMO* and *diffHL*. The correlations suggest that for the retention of ILs on the studied stationary phase materials the dispersive London type interactions are most important. These interactions are obviously stronger between the analyte and the bulky hydrocarbon ligand of the stationary

Table 9
Retention factors (k), peak weight at half height ($w_{h/2}$), factor of asymmetry (F_{as}), plate number (N), separation factor (α) and resolution (Rs) of ionic liquids separating the four columns studied under the conditions as shown in Fig. 2.

C18 columns	ACE 5 C18						Symmetry C18					
	k	$w_{h/2}$ (mL)	F_{as}	N	α	Rs	k	$w_{h/2}$ (mL)	F_{as}	N	α	Rs
EEIM	0.110	0.444	0.633	205			0.025	0.457	1.429	308		
BMIM	0.630	0.444	1.600	443	5.745	1.680	0.509	0.429	2.333	760	20.263	2.210
BzMIM	1.425	0.444	1.143	979	2.260	2.563	1.308	0.429	2.000	1779	2.571	3.652
AMIM	2.202	0.444	1.333	1708	1.546	2.508	1.917	0.429	2.000	2841	1.466	2.782
EBzMIM	2.754	0.500	1.429	1851	1.250	1.580	2.436	0.500	1.600	2896	1.271	2.031
pMBzMIM	4.460	0.556	1.000	3170	1.619	4.397	4.404	0.571	1.333	5490	1.808	6.745
HMIM	7.289	0.666	1.100	5085	1.635	6.086	6.995	0.714	1.500	7686	1.588	7.104
C8 columns	Symmetry C8						Supelcosil LC-8-DB					
	k	$w_{h/2}$ (mL)	F_{as}	N	α	Rs	k	$w_{h/2}$ (mL)	F_{as}	N	α	Rs
EEIM	0.155	0.520	1.000	115			0.618	0.700	2.500	190		
BMIM	0.752	0.480	1.750	310	4.863	1.502	1.546	0.867	4.000	307	2.503	1.598
BzMIM	1.155	0.440	1.500	559	1.536	1.104	2.566	0.800	3.750	707	1.660	1.901
AMIM	1.602	0.520	1.750	584	1.388	1.039	4.359	0.800	3.750	1597	1.699	3.342
EBzMIM	2.077	0.520	1.168	816	1.296	1.102	4.027	1.200	3.667	624	0.924	−0.413
pMBzMIM	3.577	0.600	1.333	1356	1.722	3.017	7.267	1.480	4.286	1110	1.805	3.265
HMIM	5.776	0.800	1.333	1672	1.615	3.318	11.728	2.000	3.000	1441	1.614	3.326

phase than between the same analyte and the small molecules of the eluent. Hence, the k_2 regression coefficients in Table 6 have a positive sign.

3.3. Separation of selected IL cations on reversed-phase stationary phases

Separations of selected IL cations, including those with similar hydrophobicity, are shown in Fig. 2. Although the ACE 5 C18 C18 column provided the worst results for the prediction of the analyte's hydrophobicity with low QSRR correlations, this column and the Symmetry C18 column appear to be more suitable for the separation of IL cations as they provide better peak symmetry, improved separation selectivity and a higher plate number than the other columns (Table 9). Evidently, polar type interactions provide specificity of separations not attained due to differences in hydrophobicities themselves. Unfortunately, these specific intermolecular interactions on the ACE 5 C8 column are not fully accounted for by neither the log P nor calculation chemistry descriptors employed. Hydrophobic IL cations with long alkyl chains can be easily separated by RP HPLC, but the separation of IL cations with short alkyl chains is difficult on the alkyl-silica stationary phases due to the low hydrophobicity and hence a low retention of these compounds. It was also found that the retention of aromatic cations (BzMIM, EBzMIM, pMBzMIM) did not differ very much from the retention of cations with C_5 and C_6 alkyl chains (AMIM, HMIM). Hence, it was difficult to separate IL cations with similar hydrophobicity, such as EBzMIM and AMIM on the RP C8 columns, independent of the concentration of acetonitrile in the mobile phase and of the carbon load. Increasing the concentration of acetonitrile in the mobile phase impairs the selectivity of separation, so that these compounds cannot be separated in mobile phases containing more than 10% acetonitrile (and even less on the C8 Supelcosil LC-8-DB column with a low carbon load). The Supelcosil LC-8-DB column provides higher retention and better separation possibilities for hydrophilic IL cations, such as EEIM, in comparison to other columns tested (Fig. 2). On this column, EEIM can be separated from the non-retained peaks, probably of associated anions (Cl^-, Br^-, BF_4^-) or impurities. The two C18 columns studied are more suitable for the separation of IL cations than the C8 columns, because of stronger hydrophobic interactions between the alkyl chain of cations and longer silica bound alkyls.

4. Conclusions

In RP HPLC on C8 and C18 stationary phases, hydrophobic interactions between the stationary phases and the alkyl chains in cationic ILs seem to control the retention mechanism. However, the polar interactions, like charge transfer interactions between the cations and the stationary phase also affect the retention. Acetonitrile influences both types of interactions. Certainly, the lower is the concentration of acetonitrile, the higher is the influence of hydrophobic interactions on retention.

The four types of statistically significant QSRRs employing the empirical and the calculation chemistry structural descriptors describe the molecular mechanism of retention and may provide the classification of typical reversed-phase columns as the separation media for ionic liquid cations.

Molecular mechanism of ILs retention on the stationary phases studied involves contributions by non-polar (dispersive, bulkiness-related) and polar (charge transfer, dipole–dipole, hydrogen bonding) interactions with analytes. Specific intermolecular interactions, not fully quantified by the calculation chemistry descriptors employed, are probably responsible for good IL separation properties of the ACE 5 C18 phase. The results of this study indicate that the chromatographic method of determination of log k_w values can be recommended as a more reliable technique than the calculations by HyperChem software.

The C18 stationary phases studied are better suited for separations of ILs, even of those with similar hydrophobicity, but provide too low retention for the separation of hydrophilic ionic liquid cations with short alkyl chains, due to the weak hydrophobic interactions. To compromise the condition for separation of ILs of diverse size and hydrophobicity, stationary phases of specific separation properties might be tested.

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