# Application of Chromatography and Chemometrics to Estimate Lipophilicity of Ionic Liquid Cations

**ARTICLE** *in* QSAR & COMBINATORIAL SCIENCE · SEPTEMBER 2007

Impact Factor: 1.55 · DOI: 10.1002/qsar.200610146



**3 AUTHORS:**

Sylwia Studzińska
Nicolaus Copernicus University
**36** PUBLICATIONS **425** CITATIONS
SEE PROFILE

Piotr Stepnowski
University of Gdansk
**223** PUBLICATIONS **3,184** CITATIONS
SEE PROFILE

Bogusław Buszewski
Nicolaus Copernicus University
**444** PUBLICATIONS **6,671** CITATIONS
SEE PROFILE

QSAR & Combinatorial Science QCS

# Application of Chromatography and Chemometrics to Estimate Lipophilicity of Ionic Liquid Cations

**Sylwia Studzińska[a], Piotr Stepnowski[b] and Bogusław Buszewski[a]***

[a] Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarin Street, PL-87-100 Toruń, Poland, Phone: +48 56 6114308; Fax: +48 56 6114837; E-mail: bbusz@chem.uni.torun.pl
[b] Waste Management Laboratory, Faculty of Chemistry, University of Gdańsk, Sobieskiego 18 Street, PL 80-952 Gdańsk, Poland

**Keywords:** differential overlap, Ionic liquids, Principal component analysis, Quantitative structure–activity relationship, Stationary phase effect

Received: October 26, 2006; Accepted: December 26, 2006

DOI: 10.1002/qsar.200610146

**Abstract**

The hydrophobicity of ionic liquids, an important physical property, is currently attracting attention of scientists particularly for chemical–biological interactions. In this report, the hydrophobicity of selected ionic liquid (only imidazolium based) cations has been investigated according to some methods: chromatographic analysis, statistical, and chemometric approach. As a consequence, the lipophilicity, which is one of the important factors in biological activity of analyzed compounds, can be established. For that purpose, butyl, octyl, octadecyl, mixed, alkylamide, and cholesterolic packings were chosen and applied to the analysis of eight most commonly used ionic liquid cations. The use of different experimental and theoretical methods allow the estimation of ionic liquids properties. In the present study, chemometric and statistical methods have also been used for the comparison of hydrophobic or hydrophilic character of analyzed compounds and also to estimate their biological activity.

## 1 Introduction

Ionic liquids are now defined as salts that melt near or below 100 °C, often even lower than room temperature (Room Temperature Ionic Liquids, RTIL). These solvents consist entirely of ionic species. The most common group of RTILs contains a 1-alkyl-3-methyl imidazolium or pyridinium cation and $Cl^-$, $BF_4^-$, or $PF_6^-$ as anion. Ionic liquids are distinguished by a range of useful properties such as negligible vapor pressure, thermal stability, nonflammability, high ionic conductivity, and remarkable solubility properties [1–4]. The extremely increased volume of publications indicates that significant efforts are being made in utilizing ionic liquids in industrial applications [5]. It has been shown that they are suitable solvents for a wide variety of industrially important reactions. Those solvents became the most important substances in the field of green chemistry, which is largely a result of their negligible vapor pressure which inhibits evaporation into the air and allows simple recycling and reuse [2–4]. Even if these compounds will not evaporate and contribute to air pollution, most of them are water soluble and might enter the environment by this way [6–8]. Until now we do not know what the consequences are in these cases. That is why chemists are cautious about too much renown about ionic liquids' greenness.

Not all ionic liquids are always green, nontoxic, and environment friendly. Slender data with regard to the toxicity and ecotoxicity of ionic liquids have been available until now. The first results of toxicological effects of ionic liquids indicate that due to the structure of the compounds, similar trends might occur in test systems of different biological complexities (*e.g.*, enzymes, organisms) and with different endpoints (*e.g.*, lethality and reproduction) [9–13]. These effects might be explained with the more lipophilic properties of longer alkyl chains and the similarity of imidazolium compounds to cationic surfactants, which are known to increase membrane permeability. Hydrophobicity of ionic liquids will play the most important role in the biological membrane penetration. Despite lipophilicity being one of the many factors involved in analyte activity in human cells, it is often one of the most influential. That is why the studies of ionic liquids polarity has become so important now [14, 15]. The most common hydrophobicity measure is $\log P$ value determined for a given molecule by

**Abbreviations:** CNDO, complete neglect of differential overlap; HPLC, high-performance liquid chromatography; PCA, principal component analysis; QSAR, quantitative structure–activity relationships

several different methods. However, other methods should be developed. One of the ways of realizing this purpose is the use of chemometric approaches. The chromatographic methods can also be utilized [16]. Nevertheless, the most promising tool is the combination of those two approaches. The first works on hazardous character of ionic liquids of Jastorff *et al.* [17] were just theoretical strategy with the use of structure – activity relationships, modeling, and toxicological tests. Theoretical approach allows to optimize the first sets of compounds (initial toxicity prediction) without experimental work. However, all of those presumptions are confirmed next by the experimental studies of Ranke *et al.* [18, 19]. Studies on biological activity of imidazolium ionic liquids with different alkyl chain lengths with the use of bacteria and leukemia and glioma cells were performed [18]. More detailed study on sorption, disruption, and cytotoxicity of those compounds was done later as well [19]. It was concluded that biological activity of ionic liquids depends mainly on the alkyl chain length. Those papers are the first examples of ganging of theoretical modeling and experimental work with the use of chromatography in the field of toxicity estimation.

The main aim of our contribution was to characterize the lipophilicity of ionic liquid cations and their possible interactions with biological membranes on the basis of chromatographic analysis and with the use of a chemometric approach. Seven imidazolium ionic liquid cations were analyzed on nine different stationary phases (octadecyl, octyl, butyl, phenyl, alkylamide, cholesterolic, and mixed). Each of them takes part in different types of interactions with solute, because of various groups chemically bonded to silica. The attempt to compare the retention factors obtained on used packing materials with the standard hydrophobicity measure octanol-water partition coefficient ($\log P$) was done. On the other hand obtained results allow ionic liquid cations comparison by the use of principal component analysis (PCA). In the case of the studied compounds' polarity estimation, Complete Neglect of Differential Overlap (CNDO) method was also utilized with the use of HyperChem package. This program was also utilized for the comparison of $\log P$ determined for natural systems with hydrophobicity parameters obtained with the use of Quantitative Structure – Activity Relationships (QSAR).

## 2 Experimental

### *2.1 Materials and Reagents*

Standards of 1-*n*-propyl-3-methyl-imidazolium tetrafluoroborate, 1-*n*-butyl-3-methyl-imidazolium tetrafluoroborate, 1-*n*-amyl-3-methyl-imidazolium tetrafluoroborate, 1-*n*-hexyl-3-methyl-imidazolium tetrafluoroborate, 1-*n*-ethyl-3-ethyl-imidazolium bromide, 1-*n*-butyl-3-ethyl-imidazolium tetrafluoroborate, 1-(*p*-methylbenzyl)-3-methyl-imidazolium tetrafluoroborate were obtained from E. Merck (Darmstadt, Germany) and also from Professor B. Jastorff collection (University of Bremen, Germany). Ionic liquids obtained, thanks to the kindness of Professor B. Jastorff, have been synthesized in the laboratory of Professor B. Ondruschka (University of Jena, Germany). Schematic structures of analyzed ionic liquids and their main properties are given in Table 1. Concentrations of ionic liquids were about 0.5 mM in water solution.

For the preparation of mobile phases, methanol of High-Performance Liquid Chromatography (HPLC) grade (Lab-Scan, Dublin, Ireland) was used, as well as deionized water from Milli-Q system (Millipore, El Passo, TX, USA), potassium phosphate $KH_2PO_4$ (POCh, Gliwice, Poland), and an 85% solution of HPLC-grade orthophosphoric acid (J. T. Baker, Deventer, The Netherlands).

In the current study three home-made packing materials with various interaction sites have been utilized. For the chemical modification of silica surface, the following reagents were used: γ-aminopropyltriethoxysilane and triethylamine (Fluka, Buchs, Switzerland); palmitoyl chloride (E. Merck); cholesteryl chloroformate 98%, lauric acid chloride, and 1-octadecene (Sigma-Aldrich, Gillingham, Dorset, UK); phenylpropyldimethylchlorosilane and octadecyltrichlorosilane (Wacker GmbH, Munich, Germany); 3-cyanopropyldimethylchlorosilane and octyltrimethoxysilane (Rockford, IL, USA). Toluene, hexane, methanol (POCh), tetrahydrofurane, acetonitrile, 2-propanol, petroleum ether, dichloromethane (J. T. Baker, Łódź, Poland), and morpholine (Reachim, Moscow, Russia). Main information concerning all the materials/reagents used in the study columns are listed in Table 2.

### *2.2 Bonded Phase Synthesis and Column Packing*

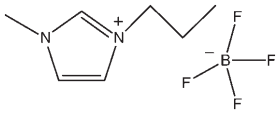
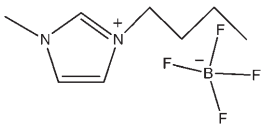
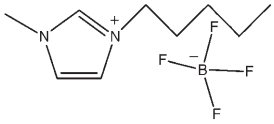
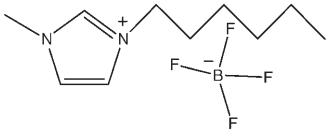
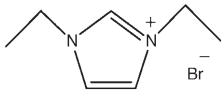
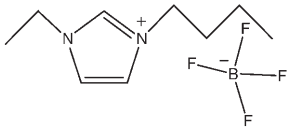
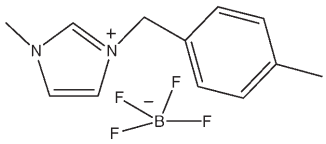
The reaction mechanism and the conditions for alkylamide (SG-AP), cholesterolic (SG-CHOL), mixed (SG-MIX), stationary phases synthesis were described elsewhere: SG-AP [20], SG-CHOL [21], and SG-MIX [22]. The stationary phases were prepared on the basis of silica gels: Kromasil® (Eka Nobel, Sweden) (SG-CHOL, SG-AP), Separon SGX (Tessek Ltd., Praque, Czech Republic) (SG-MIX). Physico-chemical characteristics of the gels were published earlier in Ref. [20 – 22]. The received packing materials were packed into 250 mm × 4.6 mm I.D. (SG-CHOL, SG-AP, SG-MIX) stainless-steel tubes using home-made apparatus equipped with Haskel packing pump (Burbank, CA, USA) under constant pressure. Methanol has been used as a packing pressurizing solvent. Received stationary phase structures and commercially available columns used in the study are presented in Figure 1 and listed in Table 2.

### *2.3 Apparatus and Chromatographic Conditions*

Chromatographic measurements were performed with the use of the LC-10Avp (Shimadzu, Kyoto, Japan) HPLC system equipped with a diode-array detector (DAD; Shimad-

**Table 1.** Structure and basic properties of ionic liquids used in the investigations (log *P* values were taken from Ref. [14] and are theoretically calculated).

| Name | Abbreviation | Structure | Molecular formula | $\lambda_{max}$ (nm) | Molecular mass (g/mol) | Log P |
|---|---|---|---|---|---|---|
| 1-*n*-Propyl-3-methyl-imidazolium tetrafluoroborate | [PMIM][$BF_4$] |  | $C_7H_{13}N_2BF_4$ | 212 | 212 | −1.74 |
| 1-*n*-Butyl-3-methyl-imidazolium tetrafluoroborate | [BMIM][$BF_4$] |  | $C_8H_{15}N_2BF_4$ | 210 | 226 | −1.44 |
| 1-*n*-Amyl-3-methyl-imidazolium tetrafluoroborate | [AMIM][$BF_4$] |  | $C_9H_{17}N_2BF_4$ | [a] | 240 | −1.09 |
| 1-*n*-Hexyl-3-methyl-imidazolium tetrafluoroborate | [HMIM][$BF_4$] |  | $C_{10}H_{19}N_2BF_4$ | [a] | 254 | −0.71 |
| 1-Ethyl-3-ethyl-imidazolium bromide | [EEIM][Br] |  | $C_7H_{13}N_2BF_4$ | 190 | 212 | −1.74 |
| 1-Butyl-3-ethyl-imidazolium tetrafluoroborate | [BEIM][$BF_4$] |  | $C_9H_{17}N_2BF_4$ | 212 | 240 | −1.09 |
| 1-(*p*-Methylbenzyl)-3-methyl-imidazolium tetrafluoroborate | [pMBzMIM][$BF_4$] |  | $C_{12}H_{15}N_2BF_4$ | [a] | 274 | −0.65 |

[a] Data not available.

zu) and a Rheodyne 7125 manual injection valve (Rheodyne, Berkeley, CA, USA) with 20 µL loop. CLASS-VP program was used for the data collection.

Elution was carried out with isocratic conditions of 95% v/v 40 mM potassium phosphate buffer (adjusted with orthophosphoric acid to pH = 4) and 5% v/v methanol. The fresh buffer solution was prepared each day of work. The flow rate was 1 mL/min. The "dead time" ($t_0$) of the column was measured by injecting uracil or methanol into the system.

### *2.4 Statistical Methods*

In the interpretation of the results Statistica v. 5.1 packages for Windows (StatSoft, Tulsa, USA) and HyperChem v. 5.1 package with the ChemPlus extension (HyperCube, Waterloo, Canada) were used. HyperChem provides a possibility of molecular mechanics calculations using semi-empirical quantum mechanics. One of them is CNDO, which is used for calculating ground-state electronic properties of opened and closed-shell systems, geometry optimization, and total energy.

The Statistica v. 5.1 package allows grouping of data (objects or variables), by means of unsupervised methods [23, 24]. Those methods identify natural clustering pattern and group objects (or variables) on the basis of similarities between the samples. One of the most common methods of partitioning is PCA. According to the eigenvalue-one criterion only the principal components (PCs) with eigenvalues greater than 1 are considered as important ones.

**Table 2.** Surface characteristics of column packing materials used in the study; where $P_C$ is the carbon content and $P_N$ the nitrogen content.

| Stationary phase type | Column | Abbreviation | Column dimensions (mm) | Silica particle size (μm) | Pore diameter (Å) | Part | | Types of possible interactions | Manufacturer |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $P_C$ (%) | $P_N$ (%) | | |
| Octadecyl | Gemini 5 μ C18 110 Å | Gemini | 250 × 4.6 | 5 | 110 | 14.3 | – | Hydrogen, van der Waals | Phenomenex, Torrance, CA, USA |
| Octadecyl, end-capped | RP-18e Purospher™ Star | Star | 250 × 4.6 | 5 | 120 | 18 | – | Hydrogen, van der Waals | E. Merck |
| Octadecyl, end-capped | RP-18e Innovation Chromolith™ Performance | Chromolith | 100 × 4.6 | – | – | – | – | Hydrogen, van der Waals | E. Merck |
| Octyl | Macrosphere 300 $C_8$ 5μm | SG-$C_8$ | 250 × 4.6 | 5 | 300 | – | – | Hydrogen, van der Waals | Alltech, Deerfield, IL, USA |
| Butyl | Macrosphere 300 $C_4$ 5 μm | SG-$C_4$ | 250 × 4.6 | 5 | 300 | – | – | Hydrogen, van der Waals | Alltech |
| Octadecyl, polar groups | Synergi 4 μ FUsion-RP 80 Å | Fusion | 150 × 4.6 | 4 | 80 | – | – | Hydrogen, van der Waals | Phenomenex |
| Alkylamide | – | SG-AP | 250 × 4.6 | 5 | 100 | 9.08 | 1.02 | Hydrogen, van der Waals, donor-acceptor | Home-made[a] |
| Cholesterolic | – | SG-CHOL | 250 × 4.6 | 5 | 100 | 13.85 | 1.47 | Hydrogen, van der Waals, donor-acceptor, π-π type | Home-made[a] |
| Mixed | – | SG-MIX | 250× 4.6 | 7 | – | 14.95 | 1.42 | Hydrogen, van der Waals, donor-acceptor, π-π type | Home-made[a] |

[a]Prepared in Department of Environmental Chemistry and Ecoanalytics, Faculty of Chemistry, Nicolaus Copernicus University.

This criterion is based on the fact that the average eigenvalues of the autoscaled data are just 1.

## 3 Results and Discussion

The log*P* is a measure of the hydrophobicity and hydrophilicity of a substance. Although lipophilicity is just one of many factors involved in biological activity, it is often one of the most influential. Strictly this coefficient is only valid for neutral molecules. In practice, not only neutral molecules but also ion molecules may partition. Charged molecules have much more complex retention behavior than simple partition. Different ionization states of a molecule differ in physico-chemical and biological properties and so it is important to be able to predict which ionic form of the molecule is present at the site of action.

Latest studies of Stepnowski and Storoniak [14] are focused on ionic liquids log*P* estimation. They have reported experimentally measured and theoretically estimated lipophilicity coefficients obtained for representatives of imidazolium ionic liquid cations. In the case of imidazolium cations, the charge is distributed in the aromatic ring between the 1- and 3-nitrogen atoms and the remaining three aromatic carbons. Both "quat" nitrogen atoms have an aromatic character for which the fragmental *C*log*P* value (taken form *C*log*P*3 computer program described by A. Leo) of log*P* was estimated at −1.140 [25]. The calculation procedure proposed by Hansch and Leo [25, 26] rationalizes 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. The compounds studied generally indicate a relatively low lipophilicity and thus preferable partition to the aqueous phase [14]. The log*P* values are given in Table 1.

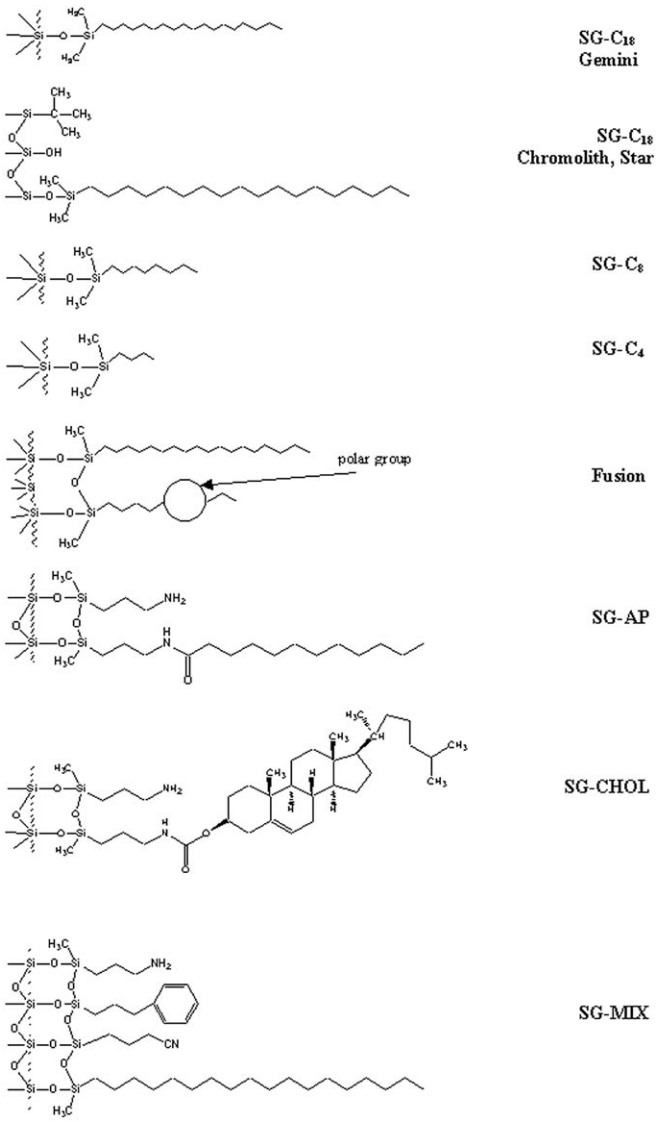



**Figure 1.** Structures of the stationary phases.

In the case of establishing the ionic liquid cations hydrophobicity, the distributions of free-electron density and potential for ionic liquids were modeled by the use of HyperChem v. 5.1 and are presented in Figure 2. CNDO method allows to plot the map of electrostatic potential field due to electronic charge distribution and nuclear charges. This is the simplest method for semi-empirical quantum mechanics calculations. These calculations solve the Schrödinger equation, with certain approximations, and allow to describe the electron properties of atoms and molecules. HyperChem calculates the electrostatic potential as the potential energy of a unit positive charge interacting with every part of the electron density cloud. Negative electrostatic potential corresponds to attraction of the proton by the concentrated electron density in the molecules, while positive value corresponds to repulsion of the proton by the atomic nuclei in regions where low electron density exists and the nuclear charge is incompletely shielded. In the present study, the ionic liquids cations structures collected in Table 1 were used as the basis for CNDO calculations, while the total charge of molecule was set as one. The polarity of a molecule can be predicted by examining the electrostatic potential map. As it can be seen from Figure 2, the typical ions used in ionic liquids show only moderate polarization charge densities. This is due to the delocalization of the molecular charge, which in the imidazolium ions is delocalized *via* conjugation and hyperconjugation. Computer modeling indicates on small lipophobic character of analyzed substances: wide electron bordering (Figure 2) within the analyzed cations increases their polarity. The spacious distribution of free electrons for PMIM and EEIM chemical entities proves their meaningful polar character in comparison with for example HMIM and pMBzMIM cations in which the delocalization of charge is much smaller. Such an observation seems to be a confirmation of Stepnowski log *P* studies, which also indicate a small hydrophobicity of the studied compounds (Table 1) [14].

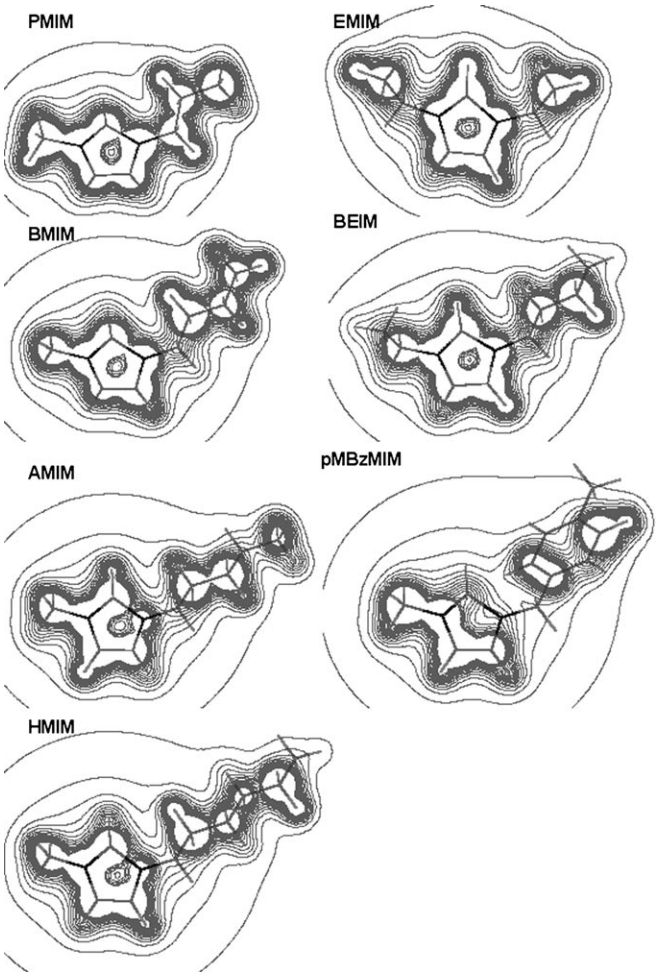
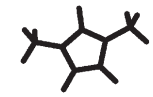
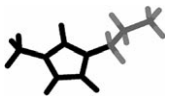
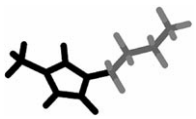
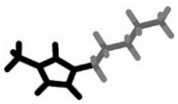
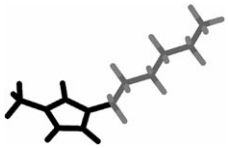
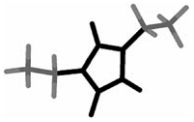
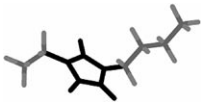
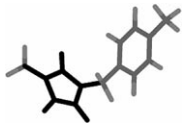



**Figure 2.** Map of free-electron density and potential distribution in ionic liquid cations.

**Table 3.** QSAR properties of ionic liquid cations calculated by HyperChem.

| Ionic liquid cation | Structure | Mass | Volume (Å$^3$) | Solvent accessible area (Å$^2$) | $\alpha_S$ | Log $\alpha_S$ | van der Waals surface area (Å$^2$) | $\alpha_W$ | Log $\alpha_W$ |
|---|---|---|---|---|---|---|---|---|---|
| Skeleton |  | 97.14 | 393.99 | 291.90 | – | – | 279.04 | – | – |
| PMIM |  | 125.19 | 501.28 | 348.44 | 1.19 | 0.08 | 338.40 | 1.21 | 0.08 |
| BMIM |  | 139.22 | 554.86 | 384.18 | 1.32 | 0.12 | 365.64 | 1.31 | 0.12 |
| AMIM |  | 153.25 | 609.91 | 422.31 | 1.45 | 0.16 | 397.34 | 1.42 | 0.15 |
| HMIM |  | 167.27 | 663.68 | 458.61 | 1.57 | 0.20 | 430.92 | 1.54 | 0.19 |
| EEIM |  | 125.19 | 501.31 | 335.40 | 1.15 | 0.06 | 330.96 | 1.19 | 0.074 |
| BEIM |  | 153.25 | 608.97 | 408.29 | 1.40 | 0.15 | 396.41 | 1.42 | 0.15 |
| PMBzMIM |  | 187.26 | 652.08 | 381.84 | 1.31 | 0.12 | 410.09 | 1.47 | 0.17 |

On the other hand, the HyperChem also gives the possibility of lipophilicity estimation by the use of QSAR properties option. HyperChem allows the calculation and estimation of a variety of molecular descriptors commonly used in QSAR studies, *e.g.*, surface areas (solvent accessible area or van der Waals surface area) and volume. In the present work all of the mentioned values were determined on the basis of structures obtained after the CNDO method. Results are shown in Table 3.

The most interesting data are van der Waals and solvent accessible areas presented in Table 3. They can be used in conclusion of the lipophilicity of analyzed molecules. All of the studied ionic liquid cations are built on the same skeleton containing imidazolium ring with one methyl group bonded to both nitrogen atoms (Table 3). Main QSAR properties were determined for this skeleton and for the other ionic liquid cations. Next the "selectivity" parameter was estimated for analyzed substances on the basis of two equations:

$\alpha_S$ = ionic liquid solvent accessible area/skeleton solvent accessible area (1)

$\alpha_W$ = ionic liquid van der Waals surface area/skeleton van der Waals surface area (2)

Both of those correlations are based on the typical equations:

$$\alpha = k_2/k_1 \quad (3)$$

$$\ln \alpha = \ln k_2 - \ln k_1 \quad (4)$$

where $\alpha$ the selectivity, $k_{1,2}$ the retention factors for the first and second compound logarithm of obtained values were determined. The final step of those calculations was the comparison of those data with $\log P$ values. Results are presented in Figure 3. As it can be seen from the above figure, when pMBzMIM cation is taken into consideration (Figures 3A and 3C), the correlations between $\log \alpha_S$ and $\log P$ and also between $\log \alpha_W$ and $\log P$ are low. However, those dependences for only six ionic liquid cations have very high correlation coefficient (Figures 3B and D). The reason for such a situation is closely related to the analyzed compounds' structure and is a consequence of the possible interactions in which those compounds can take part. pMBzMIM poses in the side chain of skeleton benzyl ring, which can strongly interact through π-π interactions. In the case of the rest of ionic liquid cations, the structure differs only in the number and position of methyl groups and the main interactions in which they can take part are van der Waals (dispersion forces). Therefore, the correlation of $\log \alpha_S = f(\log P)$ and $\log \alpha_W = f(\log P)$ for those six ionic liquid cations are so good: the $\log P$ values also indicate the ability of compound to interact through van der Waals forces. From the presented Figure 3 and Table 3 one can conclude that with the use of chemometric approach it is possible to predict hydrophobicity of ionic liquid cations. QSAR parameters obtained with the use of HyperChem correlate well with $\log P$ values, even in vacuum conditions, which are a characteristic for this package. Therefore, dependences presented in Figure 3 can be considered as correlations of natural ($\log P$) and artificial (van der Waals and solvent accessible areas) systems. Even though this artificial system is distant from natural systems, the correlation still remains very good ($R^2 = 0.9937$ and 0.9737).

All of the above preformed chemometric calculations have one purpose: evaluation of polarity or hydrophobicity of studied ionic liquid cations. For that purpose however one may also use the chromatographic analysis results. In the investigations, ionic liquids have been analyzed on different types of stationary phases (Table 2, Figure 1), which can mimic the possibility of biological membrane interactions. All of the packing materials contain various interaction sites such as cholesterol, *n*-acylamide, aminopropyl, cyanopropyl, phenyl, octadecyl, octyl, butyl, and residual silanols localized on the silica gel surface. Such functional groups variety are similar as in natural biological barriers and can give similar solute bonding. Results obtained from HPLC analysis are listed in Table 4.

For establishing the correlation of retention parameters with standard hydrophobicity measure ($\log P$ taken from Table 1) the plots presented in Figure 4 were performed. Parameters characteristic for linear relationships are listed

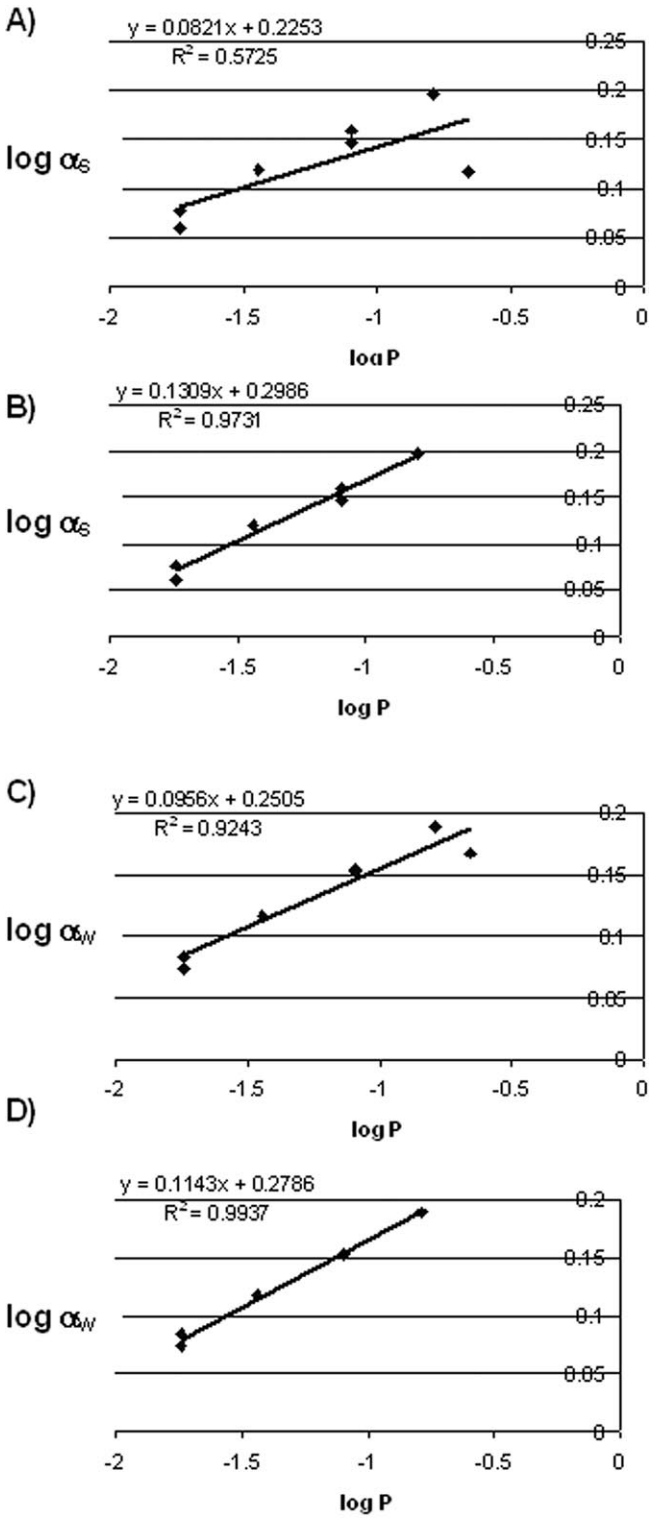



**Figure 3.** Correlation between $\log \alpha_S$ and $\log \alpha_W$ with $\log P$ values, where (A) $\log \alpha_S = f(\log P)$ for all studied ionic liquids, (B) $\log \alpha_S = f(\log P)$ for analyzed compounds except pMBzMIM, (C) $\log \alpha_W = f(\log P)$ for all the studied substances, and (D) $\log \alpha_W = f(\log P)$ for only six ionic liquids, without pMBzMIM.

**Table 4.** The retention factor ($k$) value for the chromatographic conditions: 95% v/v 40 mM $KH_2PO_4$ (pH = 4), 5% v/v MeOH.

| Ionic liquid cation | SG-CHOL | SG-MIX | SG-AP | Chromolith | Fusion | SG-$C_4$ | SG-$C_8$ | Gemini | Star |
|---|---|---|---|---|---|---|---|---|---|
| PMIM | 0.00 | 0.03 | 1.97 | 0.60 | 3.08 | 0.85 | 1.50 | 0.68 | 1.20 |
| BMIM | 0.07 | 0.23 | 3.51 | 2.14 | 6.72 | 1.33 | 3.04 | 3.04 | 4.15 |
| AMIM | 0.22 | 0.78 | 5.62 | 8.00 | 18.16 | 2.14 | 6.78 | 11.64 | 16.64 |
| HMIM | 0.56 | 2.64 | 8.68 | 31.48 | 58.88 | 3.79 | 11.89 | 28.19 | 45.15 |
| EEIM | 0.00 | 0.03 | 2.33 | 0.00 | 3.33 | 0.04 | 0.03 | 0.00 | 0.00 |
| BEIM | 0.21 | 0.55 | 4.84 | 5.18 | 10.98 | 1.90 | 3.25 | 7.50 | 10.06 |
| pMBzMIM | 0.49 | 2.32 | 5.98 | 25.31 | 50.12 | 5.83 | 20.48 | 33.86 | 47.72 |

in Table 5. These dependences give the possibility of liphophilicity comparison on the basis of correlation with $\log P$ ($R^2$ values). Furthermore, a high $R^2$ value proves that permeability ($\log P$) of ionic liquid cations correlates well with their partitioning into given stationary phase ligands. The best results were observed for hydrophobic packings such as Gemini, Star, and Chromolith. For five analyzed compounds, $\log k$ interdependence with $\log P$ varies linearly with $R^2 = 0.98$. A relatively high correlation (Table 5) in the case of SG-CHOL, SG-MIX, and Fusion RP seems to be interesting. The alkyl bonded phases retain analytes – as it was concluded earlier – mainly on the basis of hydrophobicity, while cholesterolic, mixed, and Fusion types of packing may interact by some others ways. There is a possible combination of hydrophobic, donor-acceptor, π-π, hydrogen bonding interactions on those stationary phases. There is a good correlation between the chromatographic retention properties and hydrophobicity measure in the case of SG-CHOL, SG-MIX, and Fusion RP. All of the mentioned interactions are expected to be important in membrane transport of ionic liquids. Those stationary phases comprise both hydrophobic and hydrophilic interactions as well as electrostatic forces, all of which are thought to be involved in the process of solute partitioning into cell membranes. Comparison of the linear relationships slopes ($a$ values from Table 5) also provides interesting information. According to Dill [27] and Knox and Ross [28], this coefficient represents the degree of surrounding of analyte by stationary phase ligands. If the slope angle value is close to 1 then the whole system presents a better ability to mimic the $n$-octanol system and also proves the partition retention mechanism. Our results display that SG-CHOL stationary phase gives the slope angle value closest to 1.

While performing the chromatographic analysis of ionic liquid cations, some interesting similarities in chromatographic behavior between analyzed compounds were observed. Resemblances were probably connected with cations properties, especially with the earlier mentioned polarity. In the case of that conclusion, revision PCA was done by the Statistica software. Investigations were carried out on the basis of all the retention factor values collected in Table 4. The scree-plot has shown that only two factors are important. During PCA the standardization was skipped and the number of factors was reduced to two, though

**Table 5.** Parameters characteristic for linear relationships ($y = ax + b$) presented in Figure 4.

| Column | $a$ | $b$ | $R^2$ |
|---|---|---|---|
| SG-CHOL | 1.10 | 0.49 | 0.9695 |
| SG-MIX | 1.77 | 1.67 | 0.9668 |
| SG-AP | 0.49 | 1.22 | 0.9065 |
| Chromolith | 1.54 | 2.50 | 0.9814 |
| Fusion | 1.14 | 2.46 | 0.9692 |
| SG-$C_4$ | 1.28 | 1.63 | 0.6487 |
| SG-$C_8$ | 1.68 | 2.44 | 0.6528 |
| Gemini | 1.51 | 2.56 | 0.9765 |
| Star | 1.47 | 2.67 | 0.9828 |

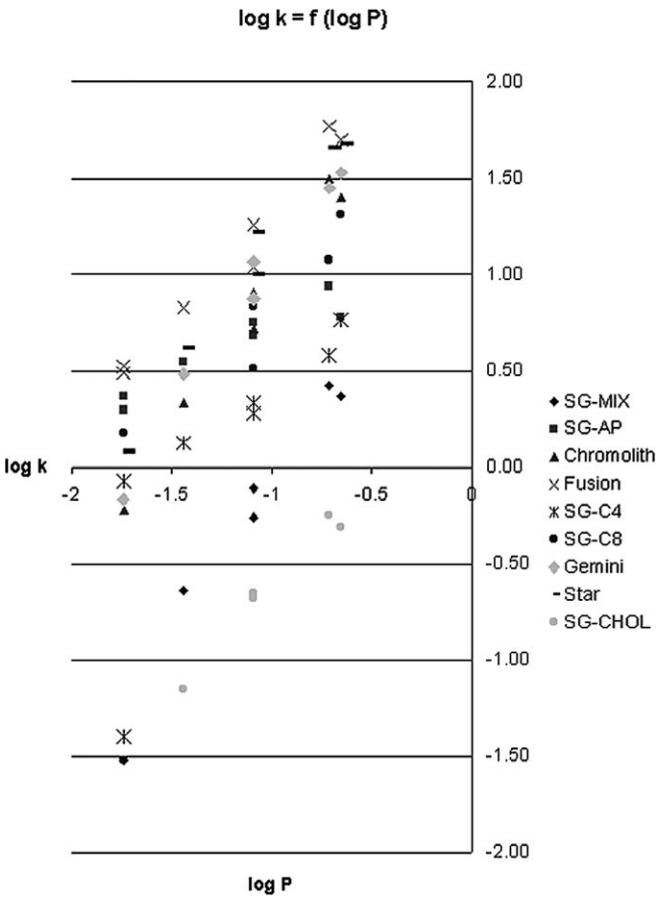



**Figure 4.** Dependences of $\log P$ values for ionic liquid cations as a function of their $\log k$ values obtained on different stationary phase types.

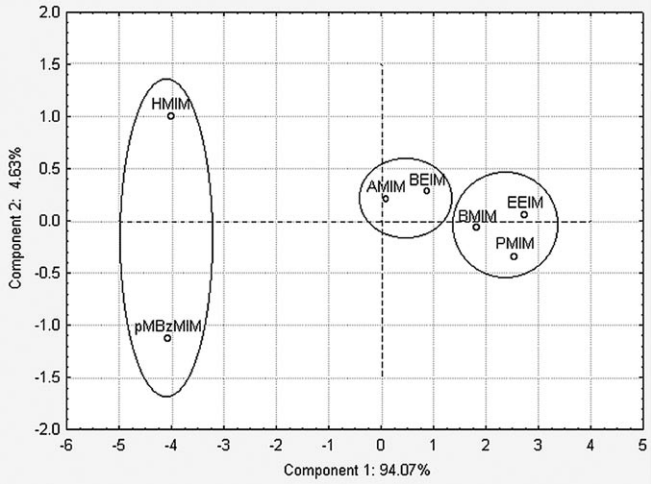



**Figure 5.** Results of statistical approach in ionic liquid cations grouping by PCA.

the cumulative explained variance for those PCs was equal to 98.1%. Figure 5 presents the score plot of all the ionic liquids in the space of first two components. Since the first component was loaded with 94.07% (represented almost all the system variations), the partition was done mainly on its basis. In the case of two components loads study, the factor analysis was also done with maximum factor number equal to 2 and minimum eigenvalue −1. Obtained data indicate that the first component is loaded with all chromatographic columns. Three ionic liquids cations groups were distinguished with regard to their similar properties: for example, PMIM and EEIM are built from analogous carbon atoms number and, as it can be concluded from $\log P$ values (Table 1), have the same hydrophobicity. The most hydrophobic pMBzMIM and HMIM create one class, while less water-repellent AMIM and BEIM are grouped into the other group. Ionic liquid cations with intermediate hydrophobicity create the third section. Such results are the confirmation of earlier conclusions made on the basis of different calculations (Table 1, Figure 2). Therefore, chromatographic analysis of ionic liquids also seems to be a useful tool in the determination of their properties.

## 4 Conclusions

The use of different experimental and theoretical methods allows the estimation of ionic liquids' properties. Such a possibility is important especially in investigations of their toxicology, while it can serve as a tentative choice to establish the relationships to their biological activities. Results obtained in this study indicate the polar character of ionic liquids. Hydrophobicity of analyzed compounds increases with the increase in side alkyl chain length. However, as it can be seen from maps of the electrostatic potential the charge distribution in studied cations is significant, which is a proof of higher polarity than it was expected. The conclusions were confirmed by the calculated QSAR properties. Statistical methods (PCA) allow for the partition of ionic liquids cations with regard to their similar chromatographic behavior on various stationary phases. Chromatographic techniques and modeling methods also allow to study ionic liquid cations' properties. Comparison of these two techniques makes possible the study of compounds of hydrophobic and hydrophilic character and tentatively permit to estimate their biological activities.

## 5 Acknowledgement

Financial support was provided by the Polish Ministry of Science and Higher Education (Warsaw, Poland) under grant 2P04G08329.

## 6 References

[1] H. Olivier-Bourbigou, L. Magna, *J. Mol. Catal. A: Chem.* **2002**, *3484*, 1–19.
[2] J. D. Holbrey, K. R. Seddon, *Clean Prod. Proc.* **1999**, *1*, 223–236.
[3] K. N. Marsh, J. A. Boxall, R. Lichtenthaler, *Fluid P. Equil.* **2004**, *219*, 93–98.
[4] M. J. Earle, J. R. Seddon, *Pure Appl. Chem.* **2000**, *72*, 1391–1398.
[5] M. Kosmulski, J. Gustafsson, J. B. Rosenholmn, *Thermochim. Acta* **2004**, *412*, 47–53.
[6] M. Koel, *Proc. Estonian Acad. Sci. Chem.* **2000**, *49*, 145–155.
[7] A. Berthod, S. Carda-Broch, *Ann. MCFA* **2004**, *III*, 1–6.
[8] P. Stepnowski, *Aust. J. Chem.* **2005**, *58*, 170–173.
[9] M. Matsumoto, K. Mochiduki, K. Kondo, *J. Biosci. Bioeng.* **2004**, *98*, 344–347.
[10] A. Latala, P. Stepnowski, M. Nędzi, W. Mrozik, *Aquat. Toxicol.* **2005**, *73*, 91–98.
[11] C. Pretti, C. Chiappe, D. Pieraccini, M. Abramo, G. Monni, L. Intorre, *Greek Chem.* **2006**, *8*, 238.
[12] R. J. Bernot, M. A. Brueseke, M. A. Evans-White, G. A. Lamberti, *Environ. Toxicol. Chem.* **2005**, *24*, 87–92.
[13] D. J. Gorman-Lewis, J. B. Fein, *Environ. Sci. Technol.* **2004**, *38*, 2491–2495.
[14] P. Stepnowski, P. Storoniak, *Environ. Sci. Poll. Res.* **2005**, *12(4)*, 199–204.
[15] L. Ropel, L. S. Belveze, S. Aki, M. A. Stadtherr, J. F. Brennecke, *Green Chem.* **2005**, *7(2)*, 83–90.
[16] R. S. Ward, J. Davies, G. Hodges, D. W. Roberts, *J. Chromatogr. A* **2003**, *1007*, 67–75.
[17] B. Jastorff, R. Störomann, J. Ranke, K. Mölter, F. Stock, B. Oberheitmann, W. Hoffmann, J. Hoffmann, M. Nüchter, B. Ondruschka, J. Filser, *Green Chem.* **2003**, *5*, 136–142.
[18] J. Ranke, K. Mölter, F. Stock, U. Bottin-Weber, J. Poczobutt, J. Hoffmann, B. Ondruschka, J. Filser, B. Jastorff, *Ecotoxicol. Environ. Safety* **2004**, *58*, 396–404.
[19] J. Ranke, M. Cox, A. Müller, C. Schmidt, D. Beyersmann, *Toxicol. Environ. Chem.* **2006**, *88(2)*, 273–285.

[20] R. Gadzała-Kopciuch, B. Buszewski, *J. Sep. Sci*. **2003**, *26*, 1273 – 1283.
[21] B. Buszewski, M. Jezierska-Świtała, R. Kaliszan, A. Wojtczak, K. Albert, S. Bochmann, M. T. Matyska, J. J. Pesek, *Chromatographia* **2001**, *53*, S204 – S212.
[22] B. Buszewski, R. M. Gadzała-Kopciuch, R. Kaliszan, M. Markuszewski, M. T. Matyska, J. J. Pesek, *Chromatographia* **1998**, *48*, 615 – 622.
[23] J. W. Einax, H. W. Zwanziger, S. Geiss, *Chemometrics in Environmental Analysis*, Wiley-VCH, Weinheim, **1997**.
[24] J. Mazerski, *Podstawy Chemmometrii*, Politechnika Gdańsk, Gdańsk (in Polish), **1997**.
[25] A. Leo, *Chem. Rev.* **1993**, *93*, 1281 – 1306.
[26] C. Hansch, A. Leo, *QSAR – Fundamentals and Applications in Chemistry and Biology*, ACS, Washington, DC, **1998**.
[27] A. Dill, *J. Phys. Chem.* **1987**, *91*, 1980 – 1988.
[28] J. H. Knox, P. Ross, *Adv. Chromatogr.* **1997**, *37*, 73 – 119.