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LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY OF ZWITTERIONIC LIQUIDS

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We have implemented full mass spectrometric assay of several 3-(1-alkyl-2-methylimidazolio)propanesulfonates, which are known as zwitterionic liquids (ZILs). Hydrophilic interaction liquid chromatography (HILIC) column and different mobile phases in both isocratic and gradient elution conditions were used for separation of the compounds present in these samples. The structures of the impurities have been confirmed by LC-MS/MS experiments. In addition, fragmentation pathways of ZILs were studied for different collision energies (0–50 eV) in positive electrospray ionization (ESI) mode under collision-induced dissociation conditions. Several new routes of fragmentation are briefly discussed. These include possible rearrangements and imidazolium ring expansions, which hypothetically proceed through carbocationic intermediates in the gas phase. Currently these processes are studied in a more detailed fashion.

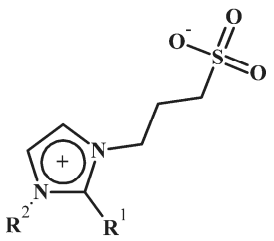
Key words: *zwitterionic liquids, hydrophilic interaction liquid chromatography, collision induced dissociation, electrospray ionization*

INTRODUCTION

Zwitterionic liquids (ZILs) are organic compounds, which contain cationic and anionic parts within one molecule. The unique characteristic of these compounds is the inability of migration of cation and anion under the influence of electric field. This structural property together with thermal stability and high conductivity explains possible use of ZILs as the electrolytes in rechargeable lithium batteries [1, 2].

There are two interesting problems associated with ZILs. The first of them is related to the presence of the impurities and synthetic by-products, which can significantly influence characteristics of ZILs in any area of technologies. The second problem is more theoretical, and is connected with the determination of mass spectrometric fragmentation pathways and gas phase chemistry of ZILs.

Inspired by these two problems, we have implemented full mass spectrometric assay of several imidazolium-based ZIL samples of a type **1**, where R^1 = Me, R^2 = *n*-Bu (**1a**), R^1 = Me, R^2 = Octyl (**1b**) and R^1 = Me, R^2 = Dodecyl (**1c**).



We have found that there is a lack of information on both chromatography and mass spectrometry of ZILs in the literature. Recently collision-induced dissociation (CID) of several imidazolium ZILs associated with the direct injection experiments have been carried out in our laboratory [3]. In addition, the fragmentation pathways of some S- and O-containig imidazolium ionic liquids were studied by positive Electrospray Ionization Fourier Transform Mass Spectrometry (ESI-FTMS) with Sustained Off-Resonance Irradiation (SORI) [4, 5]. The known capabilities of these techniques are accurate mass measurement and high precision. However, there are possibilities for suppression effects and ion – molecule reactions, which are typical for ESI-MS of crude samples, if separation is not done before the analysis. Therefore we decided to use high performance liquid chromatography (HPLC) in combination with MS in these studies. Some chromatographic data obtained in reverse-phase (RP) mode are published [6]. Although RP columns are more popular and show good reproducible retention of ZILs, there is one drawback of this type of stationary phases when HPLC-ESI-MS is performed. The mobile phases for RPLC usually contain water as a major component, which has lower volatility as well as higher viscosity than organic solvents and causes low signals of analytes. Thus we preferred to perform separations via Hydrophilic Interaction Liquid Chromatography (HILIC), which is believed to be partition of the analyte between stationary aqueous layer and organic mobile phase [7]. This separation technique gives the opportunity of using mobile phases with high organic modifier (such as acetonitrile or methanol) content.

Here we represent our primary results obtained in HILIC-ESI-MS/MS of ZILs. The studies of the problems discussed in this report are ongoing in our laboratory.

EXPERIMENTAL

Materials

All the compounds **1a**, **1b** and **1c** were synthesized in the Department of Organic Chemistry of University of Latvia by the procedures described in the literature [8]. Acetonitrile (HPLC grade), methanol (HPLC grade) and formic acid were obtained from *Aldrich*. Deionized water for mobile phases was obtained by *Milli-Q* technology. All the solvents were filtered through 0.45 μm filter, then degassed prior to analyses. The samples were also filtered through *Millipore* 0.45 or 0.2 μm filters.

HPLC-MS/MS apparatus and analyses

Separation and mass spectrometric analyses were performed using *Waters Alliance 2690* Separation Module and *Waters Micromass Quattro API* triple quadrupole mass spectrometer.

Samples were dissolved in methanol or acetonitrile (with addition of small amount of water for acetonitrile-based mobile phases to increase the solubility) to obtain approximate concentration of 1 mg/ml. For the investigation of better separation conditions in isocratic mode the mobile phases chosen were methanol/water (70–95% of methanol v/v), acetonitrile/water (70–95% of acetonitrile v/v) and acetonitrile/water+formic acid (70–95% of acetonitrile v/v, pH 2.3–6) at flow rate of 0.2 ml/min. The compounds of interest were separated using *Waters Atlantis HILIC Silica* column (150×2.5 mm); particle size 3 μm.

We have performed also gradient elution analyses using acetonitrile/water 90:10 v/v as initial mobile phase composition for all samples. For compound **1a**, the initial content of acetonitrile in the mobile phase was decreased linearely to 0% during 25 min, but for the compounds **1b** and **1c** the initial mobile phase composition was changed to 61:39 during 8 min and then flow rate and mobile phase composition were nonlinearly changed from 0.2 to 0.3 ml/min and 0% of acetonitrile during next 12 minutes.

The analytes were detected in positive electrospray ionization mode. The capillary and cone voltages were set at 3 kV and 30 V respectively. The data were aquired in the range from 50 to 400 *m/z* units, scan time was 300 ms and interscan delay was 50 ms. The MS/MS experiments were performed in CID mode using argon as collision gas. For the compounds studied mass spectra were collected in the collision energy range of 0–50 eV and for the impurities at 40 eV. The data collected were averaged for every chromatographic peak, the signals were smoothed and background was subtracted using *MassLynx 4.1* software.

RESULTS

Chromatographic conditions

Initial chromatographic experiments are performed using isocratic elution conditions. Neither of methanol/water mobile phases show acceptable chromatography due to very weak resolution of impurities and compounds of interest, poor retention and overloading effects, which are not decreased even when samples are diluted up to 100 times. Very similar observations are made in case of the analysis of non-zwitterionic type ionic liquids on RP stationary phases [9]. Such effects are not observed when acetonitrile mobile phases are used for the separation. These mobile phases are found to be good for the separation, but still have a drawback of asymmetric peak shapes showing very strong retention in HILIC mode. This strong retention can be attributed to ion-exchange between acidic silanol groups on the surface of naked silica and the analytes, which (as will be show later) are basic and can exist in various forms in solution according to their pK_a values. The interactions with silanol groups are especially dominant when mobile phases with high acetonitrile content are used. Because the higher organic modifier content induces better separation in HILIC, we have decided to suppress these unwanted interactions by lowering the pH values of the mobile phases. This procedure is known to prevent the dissociation of the silanol groups by protonation [10]. The other method of minimizing these effects based on adding a competitor base like TEA to the mobile phase [11] seems poor to us, due to obvious ion suppresion in the ion source of mass spectrometer, caused by this substance.

Lowering the pH of acetonitrile/water mobile phases improves the resolution of peaks a little and greatly reduces the retention times of basic impurities. However, the problem of peak tailing still exists.

Therefore we have decided to run the samples in a gradient mode. Almost scouting gradient conditions allow to perform not only efficient separation of compounds in all the samples **1a**, **1b**, **1c**, but also to improve the peak shapes and decrease the analysis time. In addition, we are able to identify one more impurity in the sample **1a**, which is not detectable in isocratic conditions possibly due to very strong retention (Fig. 1). As can be seen from Fig. 1, the

order of retention of the analytes reflects that characteristic of the HILIC mode, i.e. the more polar and basic analytes are eluted from the column slower than less polar and basic substances. It should be noted, that the increase of the flow rate of the mobile phase in cases of samples **1b** and **1c** to 0.3 ml/min during the gradient run (see Experimental) is due to the stronger retention of basic impurities OMIM (**1b)** and DMIM (**1c**), when the flow rate value is kept to be 0.2 ml/min. Because of acceptable chromatographic performance of the gradient conditions we have chosen this mode for further investigations.

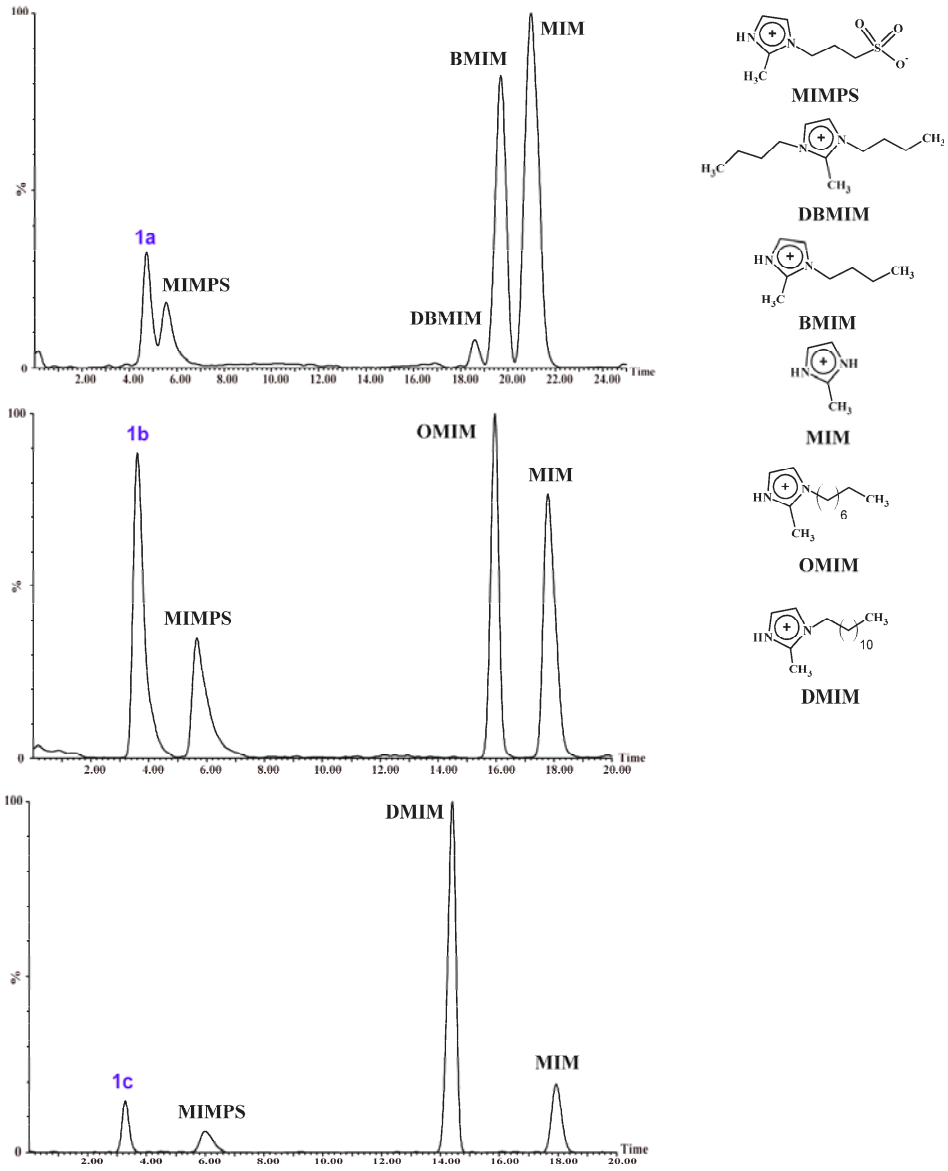
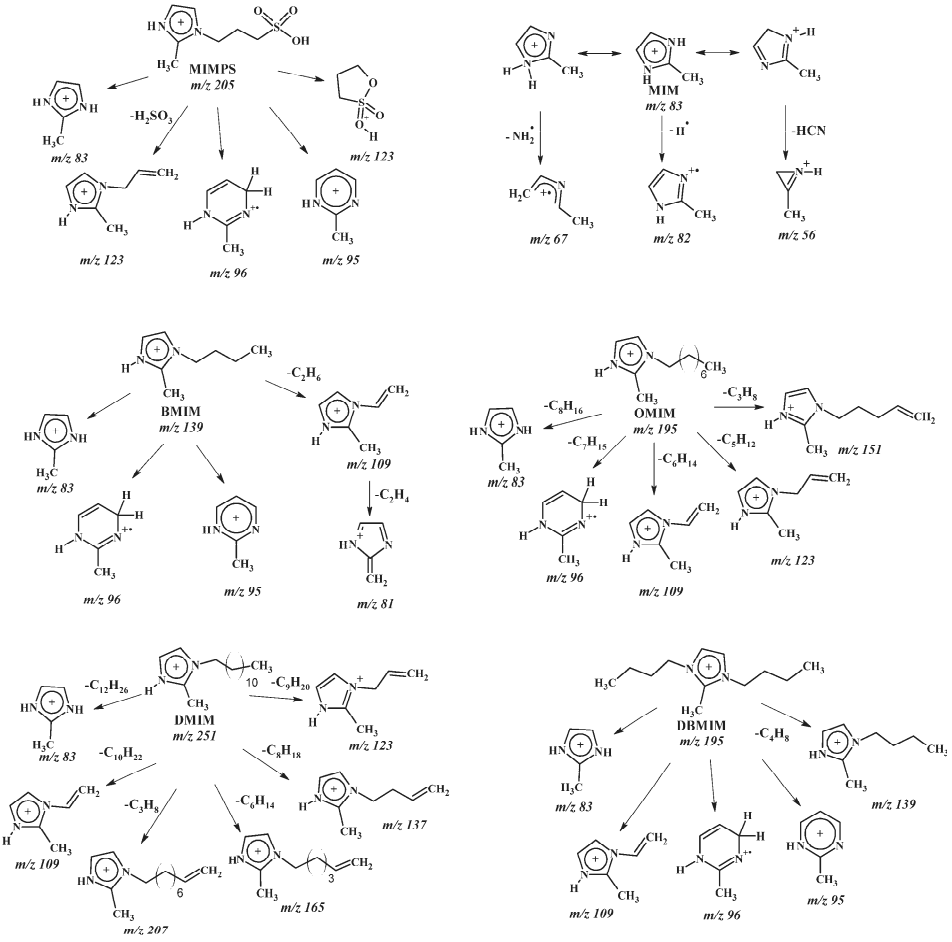


Fig. 1. TIC (total ion current) chromatograms of ZIL samples **1a**, **1b** and **1c** obtained in gradient mode using CH_3CN/H_2O as a mobile phase.

Determination of impurities in ZIL samples

LC-MS/MS is a powerful technique for the both separation and structural studies of the analytes, which allows us to elucidate the structures of all the

impurities present in samples **1a**, **1b** and **1c**. According to these studies, all the impurities have the same nature (Fig. 1) and the differences in mass spectra are minimal. These compounds are basic (MIM, BMIM, OMIM, DMIM), zwitterionic (MIMPS) and non-basic (DBMIM) derivatives of imidazole which may form in the course of the synthesis of the target zwitterionic liquid (**1a**, **1b** or **1c**) when the reaction conditions are poorly controlled. The minimal differences in the mass spectra of these compounds somewhat complicate the structural elucidation, but it still can be done taking into account the molecular ions obtained in full scan aquisition and small alkyl chain fragments appeared during 40 eV CID experiments with Ar gas. The preferred 40 eV collision energy seems to be optimal for these studies due to the presence of alkyl chain fragments in structures of substances being obtained, the characteristic 2-methylimidazolium ion with *m/z* 83 and some of its fragments in the spectra, giving wider information for the interpretation. The schemes of fragmentation of impurities which correspond to the mass spectra given in Fig. 1 are shown in Scheme 1.



Scheme 1. Interpretation of the main mass spectral signals of the impurities in ZIL samples.

The isotopic composition of MIMPS molecular ion with *m/z* 205 and mass spectra obtained at 40 eV suggest that this compound contains sulfur atom. The base peak at this voltage appears at *m/z* 83, which corresponds to 2-methyl-

imidazolium ion. The difference between *m/z* 205 and *m/z* 83 is 122 Da. This indicates for possible neutral loss of sulfur-containing fragment $C_3H_6SO_3$. Similarly, other direction of fragmentation occurs, when MIMPS molecular ion loses fragment 82 Da, which corresponds to 2-methylimidazole molecule and the formation of *m/z* 123 ion, this time having composition of $C_3H_7SO_3$. These two processes can occur only, when alkyl chain containing sulfur atom is connected to the imidazole ring through nitrogen atom. The neutral loss of H_2SO_3 molecule (82 Da, isobaric with 2-methylimidazole molecule) by the hydrogen transfer from alkyl chain to sulfonate group can also occur leading to the alkenyl cation with *m/z* 123, but direct evidence of this process does not exist until labelling experiments are carried out. The difference between *m/z* 83 and 95 is 12 Da. This suggests that these fragments differ by one carbon atom. Therefore fragment with *m/z* 95 might be the product of ring expansion which leads to additional stabilization at high collision energy. This kind of ring expansion has been described already in literature in the case of functionalized imidazolium ionic liquids [5]. According to the "N-rule" the ion with *m/z* 96 is a radical-cation, which possibly originates from homolytic cleavage of the alkyl chain.

The main three fragments in MIM spectrum are at *m/z* 56, 67 and 82, however there are many fragments with very small intensities, which are hardly interpreted because of overlapping with background noise. These three main fragments can originate from different possible resonance forms of the imidazole ring. The fragment with *m/z* 82 is a radical-cation, which forms as a result of $H^{\cdot}$ loss from 2-methylimidazolium ion (*m/z* 83). The ion with *m/z* 67 possibly forms from fragment with *m/z* 83 by the loss of $NH_2^{\cdot}$. Finally, *m/z* 56 fragment corresponds to the loss of HCN molecule from one of resonance forms of fragment with *m/z* 83. This type of fragmentation is quite similar to that for 1-protonated imidazole described for ESI-CID conditions in [12].

As indicated above, the mass spectra of alkylimidazoles (BMIM, OMIM, DMIM and DBMIM) are similar and differ only in alkyl chain fragments, which all have small intensities, but can be recognized. In addition, these compounds have similar characteristic ions at *m/z* 83, 95 and 109. We suppose that the formation of alkyl chain fragments is a two stage process. The first stage is random homolytic cleavage of one of C–C bonds leading to the formation of a distonic ion. The second stage is the restoring of positive charge by the loss of $H^{\cdot}$ or subsequent loss of ethylene molecule, to form a new distonic ion and so on until stable ion is formed (*m/z* 83, 95 or 109). The formation of the last ion (*m/z* 109) will be discussed in detail in the next section.

Fragmentation of ZILs in CID conditions

The results obtained in the fragmentation studies of ZIL compounds **1a**, **1b** and **1c** can be represented by the breakdown graphs (Fig. 2) and also by common fragmentation scheme (Scheme 2). It is clearly seen from Fig. 2 that the tendency for the fragmentation decreases with increase of the alkyl chain length. Thus, the first fragments for compound **1a** bearing the N-butyl substituent appear even at 0 eV collision energy (CE), that means that the fragmentation starts in the ion source. The compounds **1b** and **1c** are less susceptible for the fragmentation. This observation could be the result of reduction of the internal energy of ion by the rapid change of possible conformations during the collisions with argon molecules. It is clear that the longer the alkyl

chain the more conformations it can have, therefore the observed order of the fragmentation becomes meaningful.

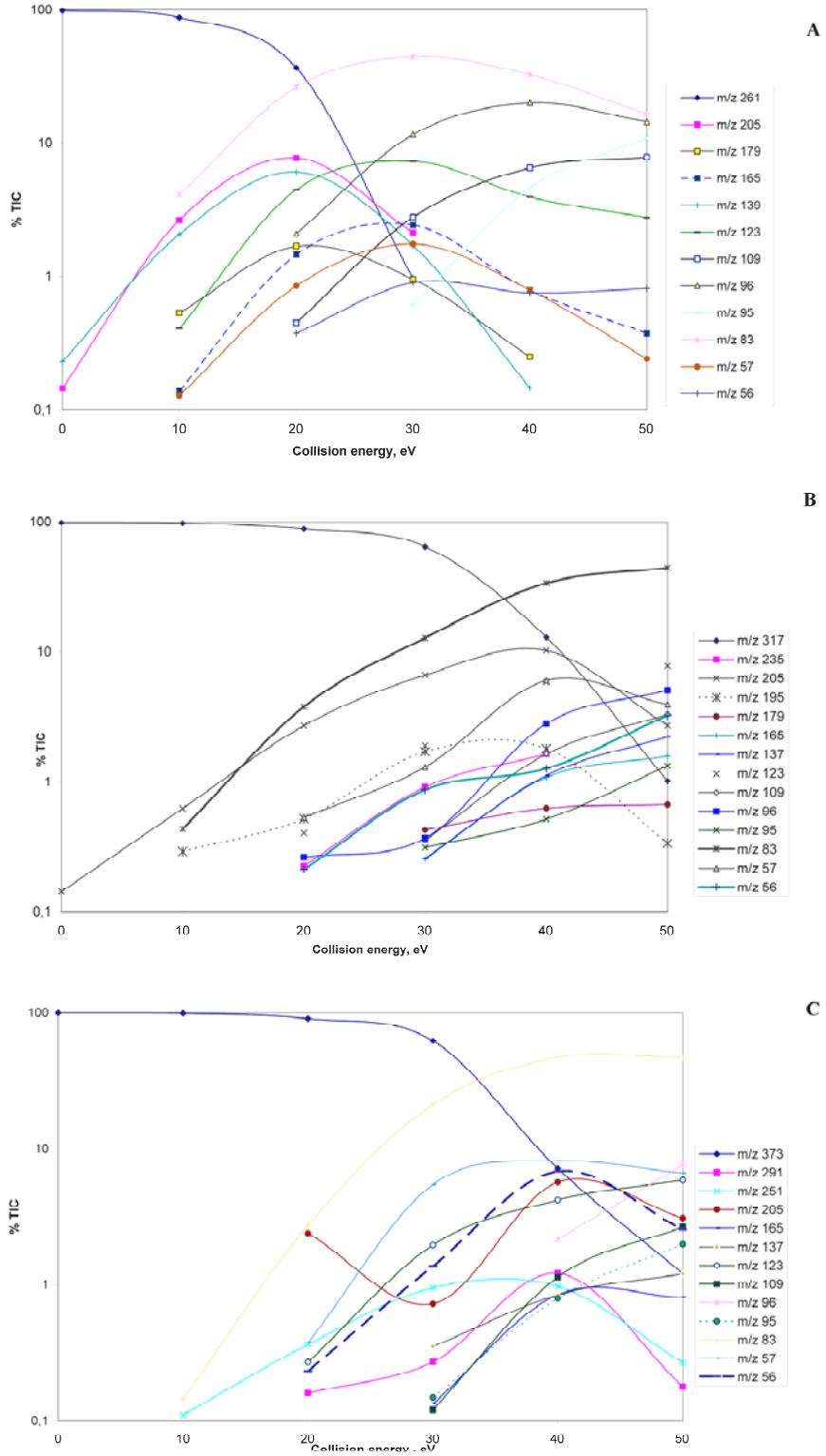
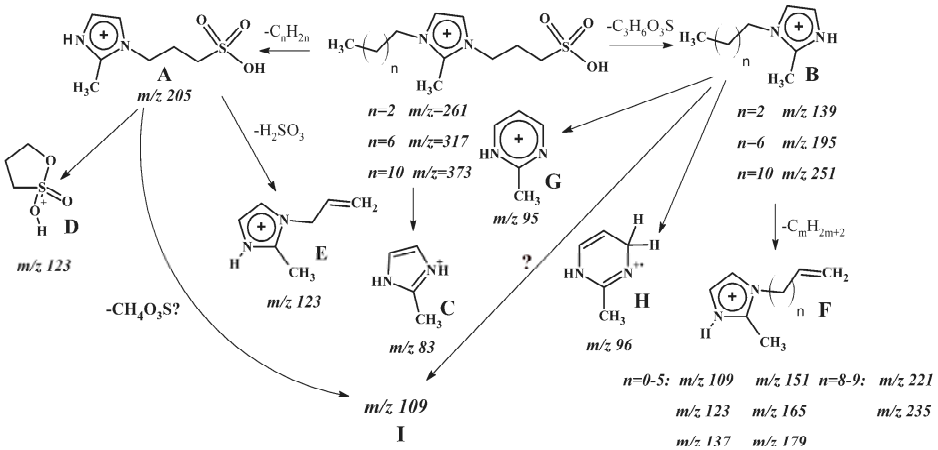


Fig. 2. Breakdown graphs of compounds **1a** (A), **1b** (B) and **1c** (C) showing % TIC of fragments at different collision energies.



Scheme 2. Fragmentation pathways proposed for the $[M+H]^+$ ions of the compounds **1a**, **1b** and **1c** (*m/z* 261, 317 and 373).

The other observation from the breakdown graph (Fig. 2) is the type of fragments obtained in a low CE region (0–10 eV). For all the compounds **1a**, **1b** and **1c** these are the first generation daughter ions *A* (*m/z* 205), *B* (*m/z* 139, 195 or 251) and *C* (*m/z* 83). This suggests that there are two directions in the fragmentation processes occurring in the low CE region. The first direction is the formation of the ion *A* by the hydrogen transfer from the alkyl chain to the nitrogen atom of the imidazole ring, which results in the alkene neutral loss. The second path is the formation of the ion *B* by the hydrogen transfer from the alkylsulfonate group to the nitrogen atom of the imidazole ring. Previous results obtained in deuterium exchange conditions show that the hydrogen transfer occurs from the protonated sulfonate group instead of migration of hydrogen atom from the alkyl chain [3]. The formation of the ion *C* is theoretically possible either from the ion *A* or the ion *B* by the same two paths described above.

Along with increasing of CE the mass spectra of ZILs become more complicated. During this CE change, the second generation daughter ions appear. One of the most stable ions at moderate CEs (10–30 eV) is the ion with *m/z* 123, for which two structures could exist (ion D or ion E). There is no clear evidence which of these structures dominates, but the formation of ions with *m/z* 179, 235 and 291 via the very similar loss of sulfurous acid from the molecular ions of the compounds **1a**, **1b** and **1c** proves the possibility of the formation of the ion E in case of the ion A at moderate CE. On the other hand, the formation of the ion D seems also quite possible, because of the fragmentation at the positively charged center (α-scission).

More complicated is the formation of ion with *m/z* 57. At the first sight it could be represented as butyl cation, which originates from direct heterolytic cleavage of C–N bond (at least for compound **1a**, which already has butyl substituent), but there is no clear explanation on the formation of butyl substituent or other precursor that could cleave to give butyl cation in cases of compounds **1b** and **1c,** simply because these substances have only octyl and dodecyl substituents. However, breakdown graphs (Fig. 2) indicate the increasing of TIC % of this ion with increasing the length of the alkyl chain. For

compound **1a**, it reaches only ≈ 1 % of TIC**,** but for compounds **1b** and **1c** it constitutes approximately 10% of TIC.

The ions *G* and *H* also may form from several precursor ions like *A* and *B*. The formation of ions of such type is quite common for the imidazolium systems and have been already described in references [5, 6]. It includes the transfer of alkyl chain from nitrogen atom of imidazolium ring to the carbon atom of the same ring and subsequent ring expansion to pyrimidinium type. In addition, this ring expansion can be either homolytic or heterolytic leading to ions *H* or *G* respectively. These ions are stable and exist in high CE region (30–60 eV) for all compounds. Besides the high CE spectra of these two ions also contain small intensive peak of ion *C* daughter ion with *m/z* 56 and the products of the homolytic cleavage of the alkyl chain, which are both discussed in the previous section. The number of such cleavage products increases with the increasing the length of the alkyl chain and CE.

The most interesting fragmentation pathway of ZILs without doubts is the formation of the ion *I* with *m/z* 109. The study of the breakdown graphs indicates that this ion is very stable at high CEs, moreover its stability can be compared to that of aromatic ions *C* and *G*. Its structure remains unclear, but if ion *A* (*m/z* 205) is considered to be a precursor, there should occur hypothetic methanesulfonic acid loss to obtain the ion *I*. One possible way of this process should be a direct homolytic β-cleavage of the C–C bond and subsequent loss of $H^{\cdot}$, but this structure could not be stable under high CEs, because of the loss of either ethynyl radical or ethylene molecule and ion with *m/z* 82 or *m/z* 81 would form. Another possibility for the formation of the ion *I* could be a rearrangement of the propanesulfonate skeleton, which leads to overall formation of sulfonate ester, which again could cleave homolytically, but for the same reasons as above, it wouldn't be stable. The same treatment can be done for the ion *B* as a precursor. However, the lack of functional groups in this structure makes this ion a poor precursor, because in this case only homolytic cleavage of the alkyl chain is possible, which leads to the same intermediate as in the above example.

For these reasons we have decided to make additional experiments with the ions *A*, *B* and molecular ion of compound **1a**. Our experiments have been directed to the isolation and subsequent fragmentation of the ion *I*, obtained from real compounds, which form the same molecular ions as the ions *A* and *B*, and deduce whether the structure of the ion *I* is the same for these compounds. The compounds chosen are MIMPS and BMIM – the impurities isolated from the sample **1a** (Fig. 3). A very important point for this experiment is to obtain a good intensity signal of the ion *I* from MIMPS and BMIM directly in the ion source, in other words, to obtain cone voltage fragmentation of these compounds. This can be achieved only if a) suitable conditions are chosen (especially voltages), b) either of the compounds can produce the ion *I*. For the optimization of these suitable conditions, initially the cone voltage is held constant while capillary voltage is constantly changed and the signal of the ion *I* is recorded for MIMPS, BMIM and compound **1a.** After optimizing the capillary voltage at which the signal of the ion *I* is maximal for every individual compound, this voltage is held constant and cone voltage is changed until suitable conditions are found. For our surprise, the both compounds MIMPS and BMIM form the ion *I* under these optimized conditions. At the next stage of

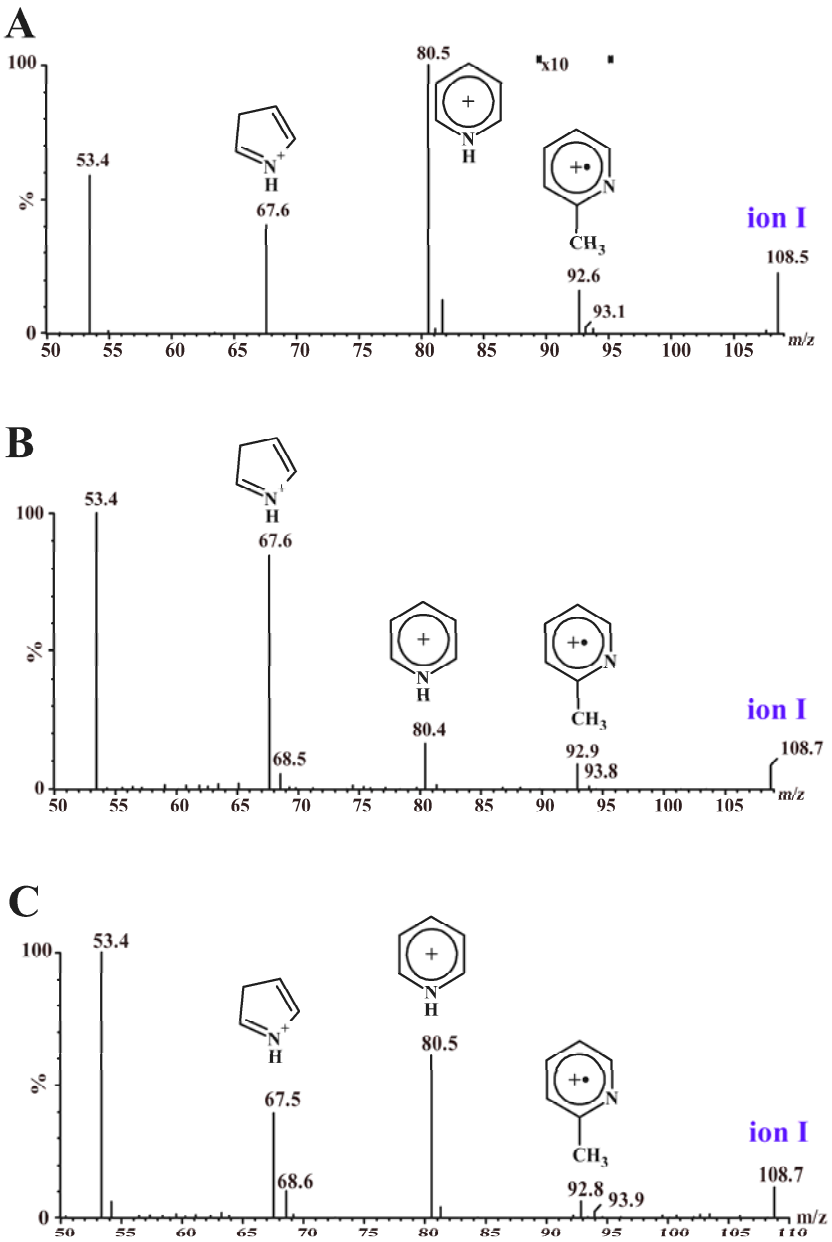
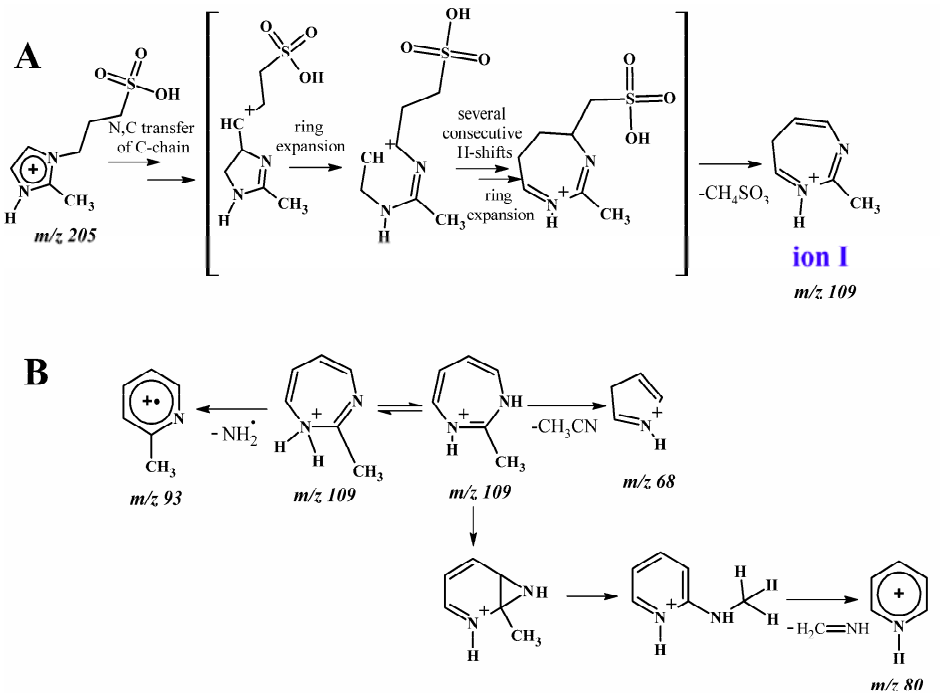


Fig. 3. CID (collision-induced dissociation) spectra of *m/z* 109 species generated from MIMPS (A), BMIM (B), and BMIMPS (C) $[M+H]^+$ ions at 35 eV collision energies. Possible structures corresponding to the fragments are given above the main peaks.

this experiment after obtaining the ion *I* in the ion source it is directed to the collision cell and fragmented at different CEs. The resulting mass spectra are compared with that obtained for compound **1a** (Fig. 3). This comparison indicates that there are no dramatic differences in all mass spectra – these consist of the same signals, which differ only in relative intensities. Thus, the structure of the ion *I*, obtained from precursor ions *A* and *B*, is the same. Therefore, it is possible that metastable intermediates for the formation of the ion *I* either from ion *A* or ion *B* are very similar in structure. It is rather complicated to postulate the structure of these intermediates until careful isotopic labelling experiments are not carried out. Interpretation of the mass spectra obtained in these expe-

riments gives three possible structures for three main peaks corresponding to the fragments of the ion *I* (Fig. 3). Two of these peaks according to R+dB rule (rings plus double bonds) may belong to pyridinium type compounds and one is pyrrole type compound. This suggests that very unusual cleavage occurs, which results in a loss of fragment containing nitrogen atom from the ion *I*.

Summarizing these observations we propose a general scheme for the formation of the ion *I* and its sequential fragmentation (Scheme 3). It can be seen from Scheme 3 that carbocations are involved in these transformations and whole process ends with mesylic acid loss (MsOH, CH_3SO_3H). It should be noted, that these hypothetical steps occur only if every of them is sufficiently fast, because the fragmentation is controlled kinetically rather then thermodynamically. Of course, at this stage the proposed scheme is only a hypothesis and the deuterium labelling experiments seem to be the next step for the investigation of gas phase chemistry of ZILs in our laboratory.



Scheme 3. Hypothetical scheme of the formation of the ion *I* (*m/z* 109) from the precursor ion A (the same as MIMPS, *m/z* 205) (A) and CID fragmentation of the ion *I*, leading to the fragments, shown in Fig. 3 (B).

N o t e : The formation of the ion *I* from the ion *B* (the same as BMIM, *m/z* 139) can be drawn in the same manner, except that the molecule of ethane is lost in the final step instead of MsOH.

CONCLUSIONS

The chromatographic results obtained in these studies show that acetonitrile-based mobile phases are superior in comparison with methanol mobile phases for the separation of basic impurities and ZILs. When operated in a gradient mode, these solvents allow shorther analysis time and improve peak shapes. HPLC in combination with tandem MS is capable to charactarize all the impurities as well as to establish the fragmentation pattern of ZILs, which is quite similar. The main routes of fragmentation involve charge-induced dis-

sociation at low CEs and homolytic cleavage of the alkyl chain at high CEs, respectively. Some unusual processes may proceed at high CEs, which possibly are based on gas phase carbocation chemistry and include several consecutive ring expansions promoted by the hydrogen transfer. However, the full fragmentation pathways can be proposed only after accurate mass measuring or isotopic labelling experiments.

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CVITERJONU ŠĶIDRUMU HROMATOGRĀFIJA–MASSPEKTROMETRIJA

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KOPSAVILKUMS

Veiktie hromatogrāfiskie pētījumi parāda, ka cviterjonu šķidrumu (CJŠ) un tajos esošo galveno piemaisījumu atdalīšanai acetonitrilu saturošās kustīgās fāzes ir piemērotākas par eluentiem, kuros ir metanols. Lietojot eluenta gradientu, ir iespējams analīzi veikt ātrāk, kā arī uzlabojas hromatogrāfisko joslu forma. AEŠH apvienojumā ar masspektrometriju ļauj noteikt piemaisījumus CJŠ, kā arī noskaidrot to fragmentēšanās maršrutus pat, ja šo piemaisījumu struktūra ir visai līdzīga. Galvenie jonu veidošanās ceļi ietver lādiņa inducēto disociāciju, ja sadursmju enerģija ir maza, un homolītisko alkilķēdes šķelšanos, ja sadursmju enerģija ir augsta. Augstas sadursmju enerģijas gadījumā novēro parādības, ko nosaka karbkatjonu īpašības gāzes fāzē; tās ietver secīgas cikla paplašināšanās stadijas, ko veicina ūdeņraža pārnese. Tomēr pilnīgai fragmentēšanās mehānismu noskaidrošanai nepieciešami jonu masu mērījumi ar augstu precizitāti vai eksperimenti ar izotopus saturošiem savienojumiem.