The roles of 1-alkyl-3-methylimidazolium-based ionic liquids as mobile phase additives in semi-micro high-performance liquid chromatography for separation of aromatic amines

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ABSTRACT

The separation mechanism of semi-micro High-Performance Liquid Chromatography (Semi-micro HPLC) using ionic liquid-aqueous mobile phase was investigated for the first time with the retention behavior of eight aromatic amines. The increase of the [EMIm] [BF$_4$] concentration resulted in the consecutive decrease of the retention factors of eight aromatic amines. This attributes the competition between the imidazolium cations and protonated aromatic amines in forming the hydrophobic interaction with the residual silanol and octadecyl groups of octadecylsilica surface. The increase of the alkyl chain length on the imidazolium cation of the ionic liquid caused the clear decrease of the retention factors of eight aromatic amines, because this factor leads to the increase of electrostatic repulsion force. On the contrary, the increase of the mobile phase pH caused the increase of the retention factors of most aromatic amines, this is due to most aromatic amines have a decreased dissociation ability. Moreover, the different anion types of the ionic liquids exhibited a different occurrence of cation exchange and ion-pairing interactions, these two interactions affect the major electrostatic repulsion and hydrophobic interactions. The separation mechanism involves electrostatic repulsion, hydrophobic, cation exchange and ion-pairing interactions.   © 2011 Trade Science Inc. - INDIA

INTRODUCTION

Ionic liquids (ILs) are salts exhibiting a melting point below 100°C and often below room temperature so that they are liquids at room temperature[1,2]. Ionic liquids (ILs) consist of large organic cations (e.g., tetraalkylammonium, 1,3-dialkylimidazolium, *N*-alkylpyridinium, etc.) with weakly coordinating inorganic anions (e.g., BF$_4^-$, PF$_6^-$, ZnCl$_3^-$, etc.) or organic anions (e.g., [CF$_3$SO$_3$]$^-$, [(CF$_3$SO$_2$)$_2$N]$^-$, [CF$_3$CO$_2$]$^-$, etc.)[3-6]. ILs has no detectable vapor pressure and is relatively thermal stable. This will avoid environmental and safety problems in comparison with traditional organic solvents. Therefore, they are proposed as novel solvent systems to replace traditional solvents that are generally toxic, flammable, and volatile. ILs are regarded to have the potential to be alternative reaction media for "Green Chemistry"[7,8]. For this reason, ILs have
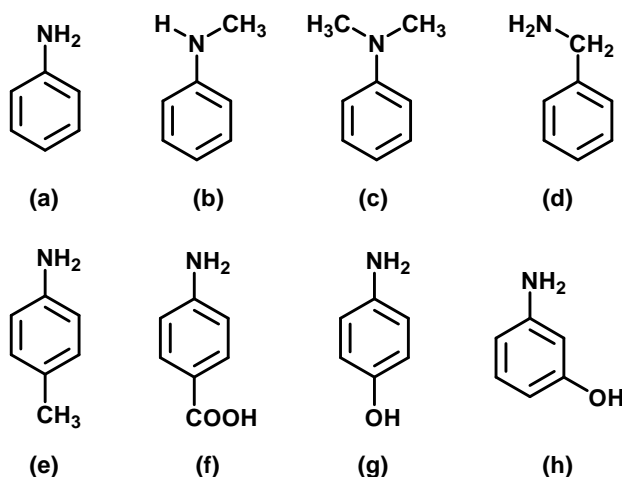
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Figure 1 : The chemical structure of analytes. (a) aniline(An); pK$_a$ = 4.70, (b) N-methylaniline(N-MA); pK$_a$ = 4.85, (c) N,N-dimethylaniline(N,N-DMA) ; pK$_a$ = 5.15, (d) benzylamine(BA); pK$_a$ = 9.33, (e) p-toluidine(p-To); pK$_a$ = 5.10 (f) p-aminobenzoic acid(p-ABA) ; pK$_{a1}$ = 4.65; pK$_{a2}$ = 4.80, (g) p-aminophenol(p-Aph); pK$_{a1}$ = 5.48; pK$_{a2}$ = 10.46, (h) m-aminophenol(m-Aph); pK$_{a1}$ = 4.37; pK$_{a2}$ = 9.815

become increasingly attractive as a green solvent for chemical applications[9-13]. The properties of ionic liquids are tunable by the choice of a cation-anion combination so that they have been described as "designer solvents"[2].

Recently, aqueous solutions of ILs have been used as the mobile phases in conventional HPLC[14-16]. However, the price of ionic liquids is still high. For this reason, semi-micro HPLC is an interesting technique because the use of miniature analytical columns reduces the cost and amount of ILs solutions; simultaneously the waste liquid pollution in the environment[17] and better separation power and sensitivity than conventional HPLC.

This research purpose was investigation of the retention behavior of eight aromatic amines in semi-micro HPLC when using the aqueous solutions of ILs ([EMIm] [BF$_4$], [BMIm] [BF$_4$], [HMIm] [BF$_4$], [OMIm] [BF$_4$] and [EMIm] [CF$_3$SO$_3$]) as the mobile phases to explain the unclear separation mechanism. These ILs are able to solvate a variety of organic and inorganic species and able to dissolve well in water[18]. The eight aromatic amines (*m*-aminophenol, benzylamine, *N,N*-dimethylaniline, *p*-aminobenzoic acid, *p*-aminophenol, aniline, *p*-toluidine and *N*-methylaniline) were selected because approximately 80% of drugs include basic functional groups. Various factors such as ionic liquid concentration, mobile phase pH, alkyl chain
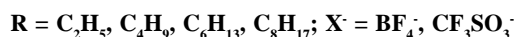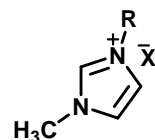

R = C$_2$H$_5$, C$_4$H$_9$, C$_6$H$_{13}$, C$_8$H$_{17}$; X$^-$ = BF$_4^-$, CF$_3$SO$_3^-$

Figure 2 : The chemical structure of ionic liquids. R = C$_2$H$_5$; X$^-$ = BF$_4^-$ =[EMIm] [BF$_4$], R = C$_2$H$_5$; X$^-$ = CF$_3$SO$_3^-$ =[EMIm] [CF$_3$SO$_3$], R = C$_4$H$_9$; X$^-$ = BF$_4^-$ =[BMIm] [BF$_4$], R = C$_6$H$_{13}$; X$^-$ = BF$_4^-$ =[HMIm] [BF$_4$], R = C$_8$H$_{17}$; X$^-$ = BF$_4^-$ =[OMIm] [BF$_4$]

length of imidazolium cation and anion type of ionic liquid were studied to understand the separation mechanism. Semi-micro HPLC was used to increase the separation efficiency and sensitivity, and in addition to save on the mobile phase of the expensive ionic liquid. This research is the first report using 1-alkyl-3-methyl-imidazolium-based ionic liquids as mobile phase additives in semi-micro HPLC.

EXPERIMENTAL

Apparatus and semi-micro HPLC analysis

The semi-micro HPLC system was composed of a LC-10AD*VP* Pump (Shimadzu, Japan), a 7520 injector with a 0.5 µL sample loop (Rheodyne, USA), a L-5025 column oven (Hitachi, Japan), and an 875-UV/Vis detector with 1 µL cell volume (Jasco, Japan). A Develosil ODS-A-3 column (no end capping; 3 µm silica particle size; 100 mm x 2 mm i.d.; trifunctional (polymeric); 19% carbon content; 3.4 µmol/m^2; Nomura Chemicals, Japan) was used. The chromatograms were recorded on a D-2500 Chromato-Integrator (Hitachi, Japan). All chromatograms were obtained by isocratic elution at 0.2 mL min^{-1} flow rate. A sample injection volume was 0.5 µL. The UV detection was operated at 254 nm in the whole study. The column equilibration time was one hour before separation of aromatic amines. The retention factor (k) was calculated using this equation.

$$k = \frac{(t_R - t_0)}{t_0} \qquad (1)$$

where t_R is the retention time of the analyte and t_0 is the retention time of the unretained compound (NH$_4$Cl).

Three replicate injections were made to determine the retention time and average values were used to calculate the retention factor. All experiments were performed at 25°C.

Reagents

The eight studied aromatic amines were: aniline (An), *N*-methylaniline (*N*-MA), *N,N*-dimethylaniline (*N,N*-DMA), benzylamine (BA), *p*-aminobenzoic acid (*p*-ABA), *m*-aminophenol (*m*-Aph), *p*-toluidine (*p*-To) and *p*-aminophenol (*p*-Aph). The chemical structure and pKa value of these aromatic amines are shown in figure 1. They were purchased from Tokyo Kasei (Japan).

The five studied 1-alkyl-3-methylimidazolium-based ionic liquids were: 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm] [BF_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm] [BF_4]), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIm] [BF_4]), 1-octyl-3-methylimidazolium tetrafluoroborate ([OMIm] [BF_4]) and 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIm] [CF_3SO_3]). The chemical structure of these ionic liquids is shown in figure 2. The examined ILs in this study has a maximum absorbance value at UV 216 nm. [EMIm][BF_4], [EMIm] [CF_3SO_3], [BMIm] [BF_4] and [OMImBF_4] were purchased from Merck (Germany). [HMIm][BF_4] was purchased from Wako Pure Chemical (Japan).

Preparation of mobile phases and standard sample solutions

(1) Mobile phases preparation

The stock solutions of specified ionic liquid concentrations (x mM) were prepared by dissolving individually the known amounts of ionic liquids with deionized (DI) water obtained from Simpli Lab-UV (Millipore). The stock solutions were filtered through 0.45 µm omnipore membrane filters (Millipore) and were stocked in a refrigerator at 4°C. The stock solutions were prepared weekly to protect the bacteria's growth.

The working mobile phases were prepared by diluting the stock solution to the required concentrations with DI water, and then they were adjusted to the required pH values with 10% (v/v) hydrochloric acid or 0.1 M sodium hydroxide. The working mobile phases were freshly prepared before use.

(2) Standard sample solutions preparation

The stock standard sample solutions of 10 mM analytes were prepared by dissolving individually the known amounts of analytes with 0.2-1 mL 1M hydrochloric acid, and then adjusting them to the required volume with pH 3.0 hydrochloric acid aqueous solutions. They were stored in a refrigerator at 4°C and were prepared monthly.

The 1 mM mixed working standard solutions were prepared by diluting the 10 mM stock standard solutions with pH 3.0 hydrochloric acid aqueous solution. Because a few of the eight samples were coeluted at the preliminary experiment, the working standard sample solutions were divided into two groups to obtain the exact retention times. The first group consisted of *m*-Aph, BA, *N,N*-DMA and *p*-ABA(1 mM mixed aromatic amine series 1 standard solution). The second group consisted of *p*-Aph, An, *p*-To and *N*-MA (1 mM mixed aromatic amine series 2 standard solution). They were freshly prepared daily to avoid potential errors from deterioration of the compounds prior to analysis with semi-micro HPLC.

RESULTS AND DISCUSSION

Effect of concentration on the retention factor (k) of eight aromatic amines with EMIm-BF_4 as mobile phase additive

The preliminary study had shown that the concentration of the ionic liquid affects the retention behavior of the aromatic amines. A series of 0-10 mM [EMIm] [BF_4] aqueous solutions at pH 3.0 was examined as the mobile phase to separate the 1mM mixed aromatic amine series 1,2 standard solutions.

Figure 3 shows the chromatograms of eight aromatic amines obtained with a no endcapping Develosil ODS-A-3 column at aqueous mobile phases (pH 3.0) containing various [EMIm] [BF_4] concentrations (a1, a 2) 0, (b1, b2) 1.25, (c1, c 2) 2.50, (d1, d2) 5.00 and (e1, e2) 10.00 mM, respectively. It should be noted that retention behaviors obtained with the addition of 1.25 mM [EMIm] [BF_4] to aqueous mobile phase. Firstly, the peak shape of all studied aromatic amines was symmetrical due to the decrease of active silanol groups because the imidazolium cations interact with the residual silanol groups on the octadecylsilica surface by a hydrophobic interaction. Moreover, the imidazoluim cations also disable the octadecyl groups of the stationary phase, which leads to the decrease in
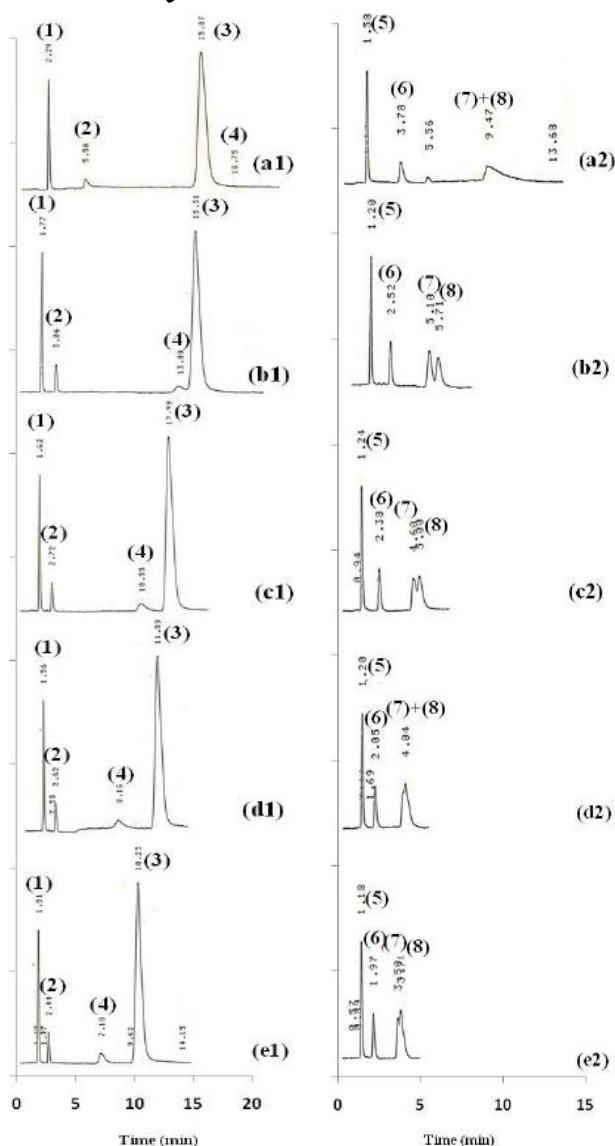
Figure 3 : Chromatograms of aromatic amines with aqueous mobile phases containing various [EMIm] [BF$_4$] concentrations at pH 3.0. (a1,a 2) 0, (b1,b2) 1.25 mM, (c1,c 2) 2.50 mM, (d1, d2) 5.00 and (e1,e2) 10.00 mM. Chromatographic conditions: column: ODS-A-3(no endcapping; 3 μm; 2.0 mm i.d. x 100 mm); flow rate: 0.2 mLmin^{-1}; UV detection : 254 nm; sample injection volume: 0.5 μL. Peaks: (1) m-Aph, (2) BA, (3) p-ABA, (4) N,N-DMA, (5) p-Aph, (6) An, (7) p-To and (8) N-MA

the possibility of hydrophobic interaction between the aromatic amines and octadecyl groups of the stationary phase. Secondly, the retention time of *N,N*-DMA was shorter than of *p*-ABA and the peak shape of *N,N*-DMA was better with using ILs as the mobile phase additive. This indicates that the octadecylsilica stationary phase surface has been changed to a partly positive stationary phase surface. Thirdly, the resolutions of *p*-ABA and *N,N*-DMA and that of *p*-To and *N*-MA were

improved using ILs as the mobile phase additive instead of using only water as the mobile phase.

Figure 4A and 4B show the effect of [EMIm] [BF$_4$] concentration on the retention factor(k) of eight aromatic amines. We found that the increase of the [EMIm] [BF$_4$] concentration affected the consecutive decrease of the retention factors of eight aromatic amines. The noticeable decrease in the retention factors of aromatic amines when using 1.25 mM [EMIm] [BF$_4$] as mobile phase was observed. However the retention factors slightly decreased with above 2.5 mM concentration. We can explain that an addition of 1.25 mM [EMIm] [BF$_4$] to an aqueous mobile phase, which causes the competition between the ethyl groups of imidazolium cations and protonated aromatic amines in forming the hydrophobic interaction with the residual silanol groups on octadecylsilica surface, results in a drastic decrease in the retention factors (k) of more hydrophobic aromatic amines. Afterwards, when the concentration of [EMIm] [BF$_4$] was increased up to 5.0 mM, the alkyl groups of imidazolium cations interact with the octadecyl groups of octadecylsilica surface by hydrophobic interaction, the retention factors of all studied aromatic amines were constant because of the formation of the adsorbed imidazolium cations layer (pseudo-positive stationary phase) is accomplished. Moreover, when the concentration of [EMIm] [BF$_4$] was further increased to 10 mM, it resulted in the slight decrease of the retention factors of all aromatic amines. This is due to the completed formation of the adsorbed imidazolium cations layer.

Figure 5 shows the relationship between the log k and the pK_a value of four aromatic amines with using the 5 mM [EMIm] [BF$_4$] aqueous solution at pH 3.0 as the mobile phase. As described above, the stationary phase surface is the absorbed imidazolium cation layer (Pseudo-positive stationary phase). The retention behavior mechanism is mainly dominated by the electrostatic repulsion because studied aromatic amines were protonated at pH3 mobile phase. If considering the pK_a value order of aromatic amine, the elution order of aromatic amine should be as follows: *N,N*-DMA>*p*-To>*N*-MA>An. As shown in Figure 5, the obtained retention factor order was An<*p*-To, *N*-MA<*N,N*-DMA. The elution order of aromatic amines was reverse against the order of pKa. The obtained retention factor of the aromatic amine was not in accordance with the pK_a
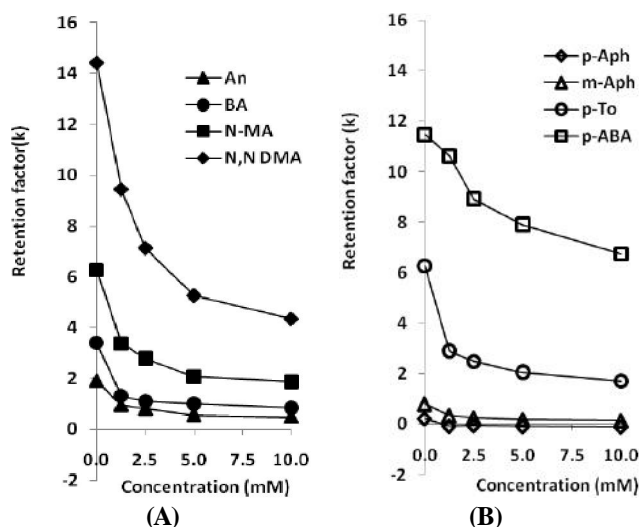
(A)   (B)

Figure 4 : Effect of [EMIm] [BF₄] concentration in aqueous mobile phase at pH 3.0 on the retention factor (k) of eight aromatic amines. Chromatographic condition was as in figure 3
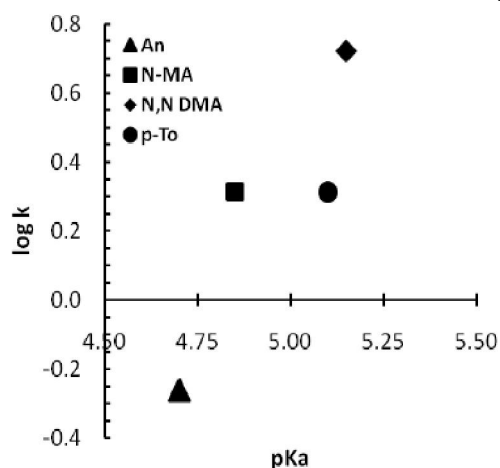

Figure 5 : The relationship between the log k and the pKa value of four aromatic amines; chromatographic condition: ODS-A-3 column (no end capping; 3 μm; 2.0 mm i.d. x 100 mm); mobile phase : 5 mM [EMIm] [BF₄] aqueous solution at pH 3.0 ; flow rate: 0.2 mL min⁻¹; UV detection : 254 nm

value order. We can expect that alkyl groups of the imidazolium cations which interacted with the octadecyl and residual silanol groups on the stationary phase surface will also interact with aromatic samples such as *N*-alkyl aniline by hydrophobic interaction. However the retention factors of *N*-MA, *N,N*-DMA and *p*-To more decreased with increasing of [EMIm] [BF₄] concentration in aqueous solution at pH 3.0 as the mobile phase than that of BA.

Effect of mobile phase pH on the retention factor (k) of eight aromatic amines with EMIm-BF₄ as mobile phase additive

The retention of ionizable compounds on the reversed phase column has been effected by the pH of the aqueous portion in the mobile phase. For this reason, the aqueous mobile phases containing 5 mM [EMIm] [BF₄] at various pH values (3.0, 3.5 and 4.5) were examined to obtain the retention behavior of aromatic amines. Figure 6A and 6B show the effect of the mobile phase pH on the retention factor of eight aromatic amines.

When the mobile phase pH was increased from 3.0 to 3.5, the retention factor of An, *N*-MA, *N,N*-DMA, *m*-Aph, *p*-To and *p*-ABA increased, except BA and *p*-Aph. This behavior can be explained as follows. These seven aromatic amines are weak base, with the exception of BA ($pK_a = 9.33$). In acidic aqueous mobile phase (pH 3.0), these aromatic amines exist in the protanated forms. When the pH of the aqueous mobile phase was increased to pH 3.5, they can dissociate

less to a protanated form; however, most of the aromatic amines are still in protonated form. It causes a decrease of electrostatic repulsion force between the protonated aromatic amines and the positive stationary phase surface. Afterwards, when the mobile phase pH was further increased to 4.5, the retention factor of An, *N*-MA, *N,N*-DMA, *m*-Aph and *p*-To increased, except that of *p*-ABA. Aromatic amines will be the molecular form, which causes a decrease of electrostatic repulsion force with larger pH value. The retention factor of *p*-ABA decreased because it is a zwitterion. It was observed that the mobile phase pH did not affect the retention factor of BA and *p*-Aph because both aromatic amines are always a protonated species under the examined pH value.

Effect of alkyl chain length on the imidazolium cation of ionic liquid on the retention factor (k) of eight aromatic amines

Though the [OMIm] [BF₄] has low solubility in water, but it could completely dissolve in water at a below concentrations of 20 mM. In this experiment, the aqueous mobile phases were carried out containing 5 mM various [RMIm] [BF₄] at pH 3.0. Figure 7A and 7B show the effect of alkyl chain length on the imidazolium cation of ionic liquid on the retention factor (k) of eight aromatic amines. It is apparent that with the increase of the alkyl chain length on the imidazolium cation of ionic liquid from ethyl, butyl, hexyl to octyl, all retention factors (k) of eight aromatic amines decreased
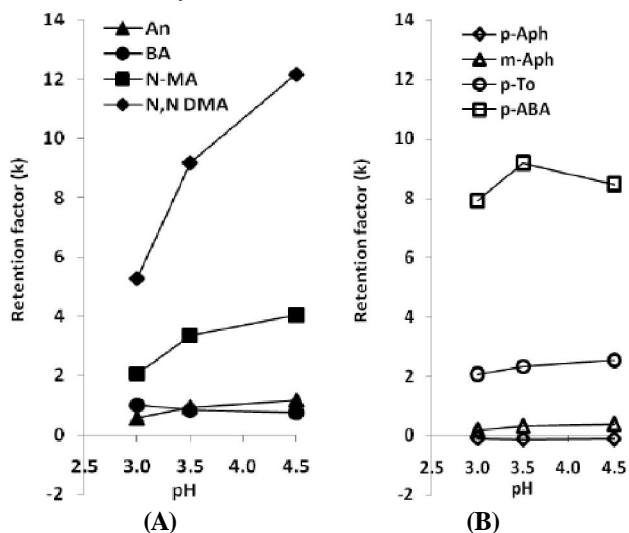
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Figure 6 : Effect of mobile phase pH on the retention factor (k) of eight aromatic amines. Chromatographic condition was as in figure 3
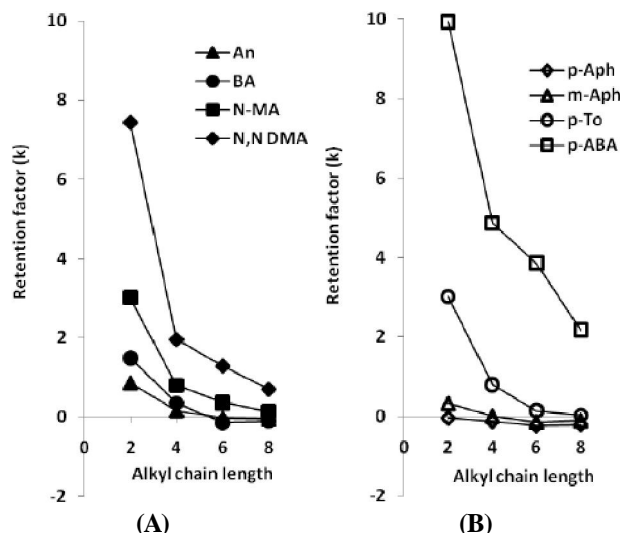

Figure 7 : Effect of alkyl chain length of imidazolium cation of ionic liquid on the retention factor (k) of eight aromatic amines. Chromatographic condition was as in figure 3

greatly. The longer alkyl chain can interact with the octadecyl more than the shorter alkyl chain; it makes the stationary phase surface become the positive charge more. It is the cause that the repulsive force of the same positive charge between the stationary phase surface and the protonated aromatic amines increase. *N,N*-DMA($pK_a = 5.15$) is a stronger base than *N*-MA ($pK_a = 4.85$). If a separation mechanism of aromatic amines is controlled by electrostatic repulsion, *N,N*-DMA will be eluted before *N*-MA. However, *N,N*-DMA was a larger retention factor than *N*-MA, this separation mechanism has an hydrophobic effect. Accordingly these indicated that the increase of the alkyl chain length on the imidazolium cation of ionic liquid affects the increase of electrostatic repulsion more than that of hydrophobic interaction.

Effect of anion type of [EMIm] cation on the retention factor (k) of eight aromatic amines

The experiment was performed to separate the 1 mM mixed aromatic amines series1,2 standard solutions by using both 5 mM of the [EMIm] [BF_4] and [EMIm] [CF_3SO_3] aqueous solutions at pH 3.0 as the mobile phases. The result is shown in the TABLE 1. It was found that using 5 mM [EMIm] [BF_4] aqueous solution at pH 3.0 as the mobile phase gave a shorter retention factor of all aromatic amines than that of [EMIm] [CF_3SO_3]. There are two possible reasons as follows: (1) the strong hydrogen bonding between the

hydrogen atom on C-2 carbon of the imidazolium cation and the counterion. (2) the anion adsorption on the stationary phase. The first reason is confirmed by following researchers. Jiang shengxiang[15] reported that there is strong hydrogen bonding between the hydrogen atom on C-2 carbon of the imidazolium cation and the counterion. Wilkes and Zaworotko[19] reported that because of the cation–anion coulombic attraction with minimal hydrogen bonding, the inter ionic interaction in[EMIm][BF4] predominates. Suarez et al.[20] reported that [BMIm] [BF_4] exhibits hydrogen bonding effect in the liquid phase. Cabovska et al.[21] proved the hydrogen bonding and hydrophobic stacking interactions between halophenols and imidazolium based ionic liquids by proton and fluorine one-dimensional NMR. In counter anion for imidazolium cation, both BF_4^- and $CF_3SO_3^-$ can form hydrogen bond with hydrogen atom on C-2 carbon of the imidazolium cation. On the other hand, because the $CF_3SO_3^-$ is stronger base than BF_4^-, it attracts with imidazolium cation more strongly than BF_4^-. Moreover, $CF_3SO_3^-$ has a larger structure, these lead to a decrease of electrostatic repulsion force on the positive stationary phase for the protonated aromatic amines. (Figure 8A and 8B).

Accordingly, the obtained retention factor of all studied aromatic amines from using 5 mM [EMIm] [BF_4] as the mobile phase was shorter than using 5 mM [EMIm] [CF_3SO_3](TABLE 1).

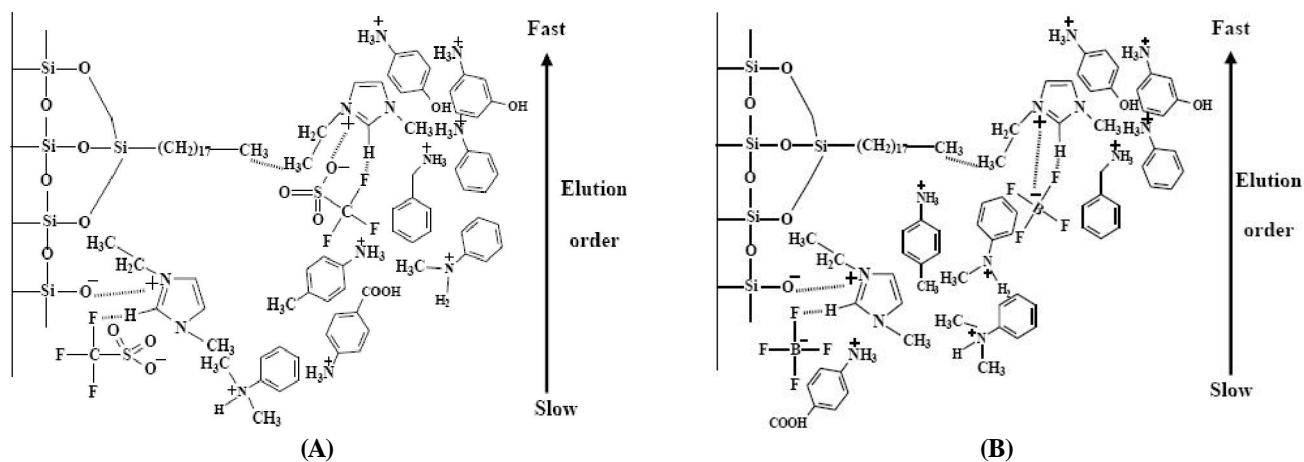For the second reason, Li Pan et al.[22] and Fabrice

Figure 8 : The aspect of hydrogen bonding of various anions (A) $CF_3SO_3^-$ (B) BF_4^-
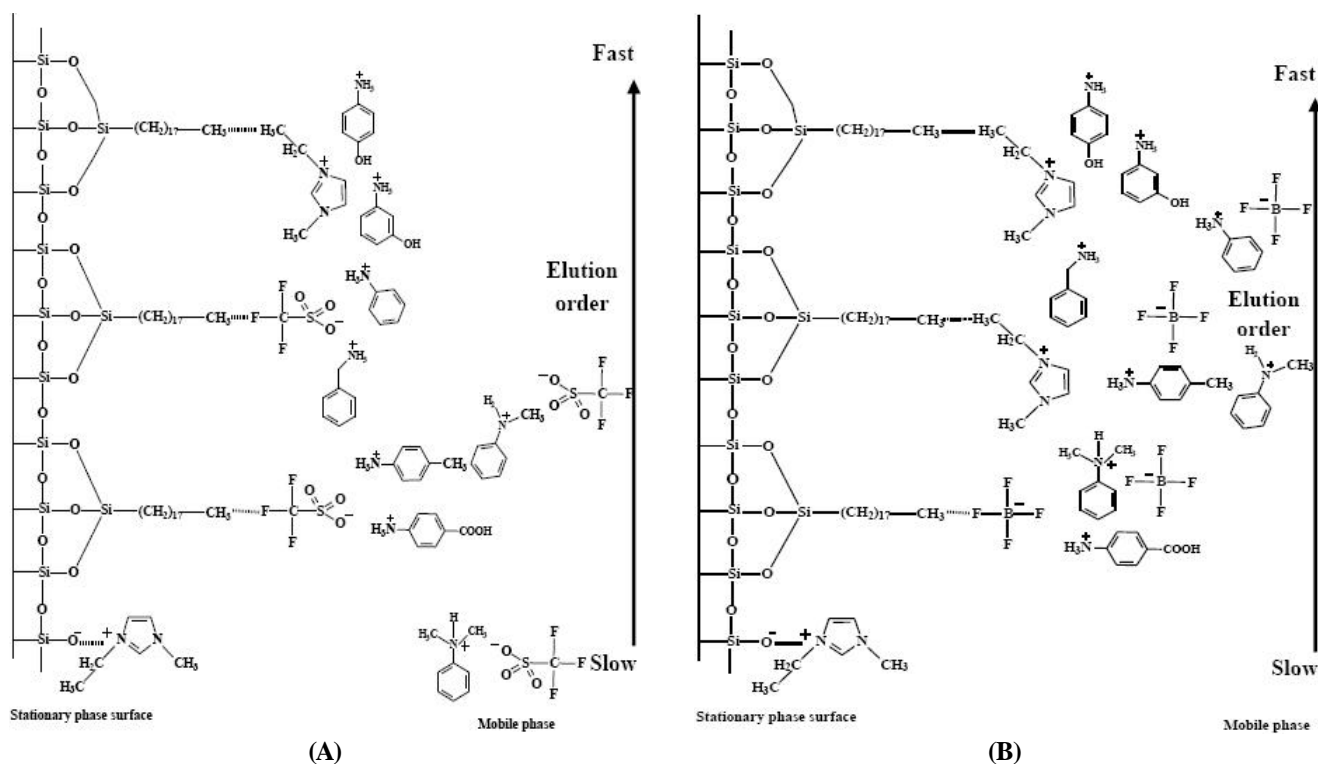

Figure 9 : The aspect of various anions adsorption (A) $CF_3SO_3^-$ (B) BF_4^-

Gritti et al.[23] demonstrated that anions could adsorb on the hydrophobic stationary phase in amounts depending on the Hofmeister anion series:

$PF_6^- > SCN^- > ClO_4^- \cong BF_4^- > NO_3^- > I^- > Br^- > Cl^- > F^- > H_2PO_4^- > SO_4^- > citrate$ (3-)

Chaotrope anions kosmotrope anions

Furthermore, Alain Berthod et al.[24] also reported the anion adsorption on the hydrophobic stationary phase and the interaction possibility of IL anions in the case of basic compounds separation as follows: (1) an interaction in a mobile phase; the IL anion forms ion-pairing with positive charge solutes, which decreases the electrostatic interaction but increases the hydrophobic interaction. (2) An interaction in a stationary phase; the anion adsorption increases the cation-exchange interaction with positive charge solutes. However, there are no current reports yet about a $CF_3SO_3^-$ adsorption. Moreover[24], reported that the major part of the 40µmol adsorbed [EMIm] [PF_6] is due to PF_6^- anion adsorption.

TABLE 1 has demonstrated that the $CF_3SO_3^-$ is more adsorbed on the octadecyl stationary phase than
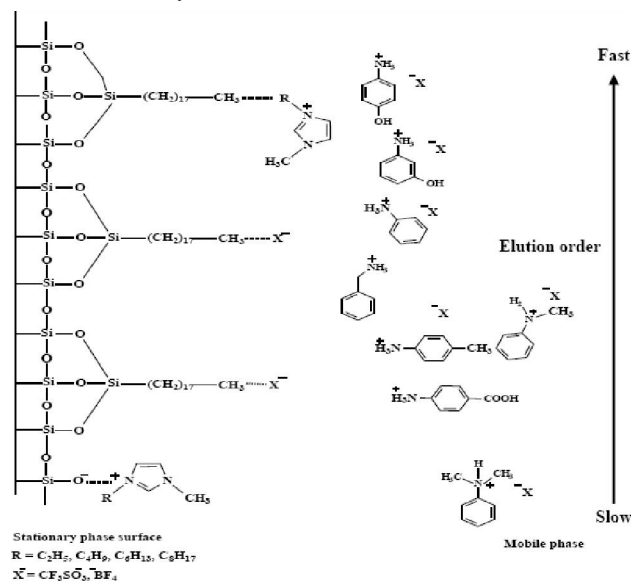
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Figure 10 : The separation mechanism with 1-alkyl-3-methylimidazolium-based ionic liquids as mobile phase additives in semi-micro high-performance liquid chromatography for separation of aromatic amines

TABLE 1 : Effect of anion type of [EMIm] cation on the retention factor (k) of eight aromatic amines

Anion type	Retention factor (k)							
	An	BA	N-MA	N,N-DMA	p-Aph	m-Aph	p-To	p-ABA
CF_3SO_3	2.19	4.70	7.43	14.19	0.24	0.86	8.36	10.66
BF_4	0.56	1.01	2.08	5.31	-0.09	0.19	2.08	7.97

Mobile phases: 5 mM [EMIm] [X] aqueous solutions at pH 3.0 (X = CF_3SO_3, BF_4)

decrease of electrostatic repulsion and the increase of the hydrophobic and cation exchange interactions.

The separation mechanism with 1-alkyl-3-methylimidazolium-based ionic liquids as mobile phase additives in semi-micro high-performance liquid chromatography for separation of aromatic amines.

According to the previously obtained results of retention behavior, we suppose that the separation mechanism with some ionic liquids as mobile phase additives for studied eight aromatic amines depends on complex interactions. The alkyl chain of imidazolium cation of ILs will interact with the residual silanol and octadecyl groups of the octadecylsilica stationary phase by hydrophobic interactions. The extent of the positive layer in stationary phase surface affected by the ILs as the mobile phase additives and theirs concentration, mobile phase pH and alkyl chain length of imidazolium cation of ionic liquid. The protonated aromatic amine samples are primarily repulsed by ionic interaction (electrostatic repulsion). Moreover, because of another part of imidazolium cation molecule, the protonated An, p-To, BA, N-MA and N,N-DMA are also retained by hydrophobic interaction. Furthermore, the anions of ionic liquids form the hydrogen bond with C-2 of imidazolium cations and also attract with N^+ atom of imidazolium cation. The anions adsorb on the octadecyl stationary phase, the stationary phase surface becomes the partly negative charge. BA is the strongest base in group of p-Aph, m-Aph and An. If the separation mechanism of aromatic amines is controlled by electrostatic repulsion only, *BA* will be eluted before them. However, BA had a longer retention factor than those; this separation mechanism has been affected by cation exchange mechanism. Besides, the anions in the mobile phase also pair with the protonated aromatic amine, N,N-DMA was eluted more slowly than p-ABA. Consequently, the separation mechanism involves electrostatic repulsion, hydrophobic, cation exchange and ion-

the BF_4^-, and the increase of the cation-exchange interaction between the partly negative stationary phase surface and the protanated aromatic amines. The anion adsorption on the stationary phase surface makes the partly negative stationary phase surface; consequently, the stationary phase surface consists of the hydrophobic, positive and negative charge parts. Besides, the $CF_3SO_3^-$ in the mobile phase also has the ion-pairing with the protanated aromatic amines, which occurs the neutral species, this leads to the decrease of the electrostatic interaction but the increase of the hydrophobic interaction at the stationary phase surface. These two interactions made the longer retention factors of all studied aromatic amines were obtained for using 5 mM [EMIm] [CF_3SO_3] at pH 3.0 as the mobile phase. And the obtained elution order of the aromatic amine was not in accordance with the pK_a value order. The ratio of retention factor using 5 mM [EMIm] [CF_3SO_3] at pH 3.0 as the mobile phase, when compared to 5 mM [EMIm] [BF_4], was 465% for BA, 402% for p-To, 391% for An, 357% for N-MA and 267% for N,N-DMA. As BA is the highest pK_a value in all studied aromatic amines, it most strongly attracts with the negative stationary phase surface (see Figure 9A and 9B). This is due to the fact that [CF_3SO_3] species more adsorb on the stationary phase surface and also it can pair with the protonated aromatic amines, these lead to the

pairing interactions. The separation mechanism with some ionic liquids as mobile phase additives for eight aromatic amines is shown in figure 10.

CONCLUSION

All obtained results in this study have demonstrated that the ionic liquids have a potential to solve the problem of aromatic amines separation such as band tailing and band broadening. It is due to that ionic liquids can play a multiplicity of roles, such as blocking the residual silanol groups, modifying the stationary phase, acting as ion-pairing agents and cation exchanger. The retention behavior of aromatic amines is greatly affected by the ionic liquid concentration, alkyl chain length on the imidazolium cation and anion type of ionic liquid, and mobile phase pH. The increase of the ionic liquid concentration resulted in the drastic decrease of the retention factors of all eight aromatic amines studied. This attributes the competition between imidazolium cations and protonated aromatic amines in the adsorbed imidazolium cations layer (pseudo-positive stationary phase) with the octadecyl and residual silanol groups on octadecylsilica surface. The increase of the alkyl chain length on the imidazolium cation of the ionic liquids caused the drastic decrease of the retention factors of all studied eight aromatic amines, because this factor leads to the increase of electrostatic repulsion. On the contrary, the increase of the mobile phase pH caused the increase of the retention factors of most aromatic amines, this is due to most aromatic amines have a decreased dissociation ability, which causes a decrease of electrostatic repulsion force between the adsorbed imidazolium cations layer (pseudo-positive stationary phase) and the protonated aromatic amines. Moreover, the anion types ([EMIm] [BF_4] and [EMIm] [CF_3SO_3]) of the ionic liquids exhibited the different retention factors, and larger retention factors were obtained with [EMIm] [CF_3SO_3] than with [EMIm] [BF_4]. The increased ratio of retention factor is clear for the BA, which is the strongest base in all studied aromatic amines. The separation mechanism involves electrostatic repulsion, hydrophobic, cation exchange and ion-pairing interactions. These results are extremely useful to apply this system for analysis of other compounds in the future.

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