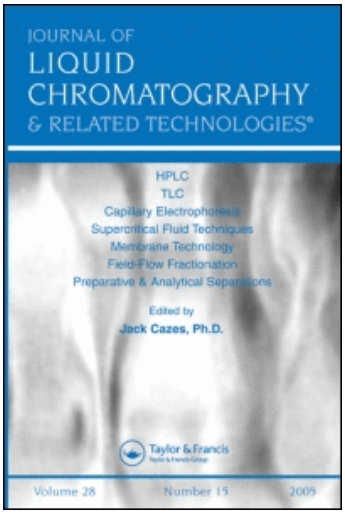

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of Concentration of Ionic Liquid 1-Butyl-3-Methylimidazolium, Tetrafuoroborate, for Retention and Separation of Some Amino and Nucleic Acids

Yulia Polyakova[a]; Yinzhe Jin[a]; Jinzhu Zheng[a]; Kyung Ho Row[a]
[a] Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, Nam-Ku, Incheon, Korea

To cite this Article Polyakova, Yulia , Jin, Yinzhe , Zheng, Jinzhu and Row, Kyung Ho(2006) 'Effect of Concentration of Ionic Liquid 1-Butyl-3-Methylimidazolium, Tetrafuoroborate, for Retention and Separation of Some Amino and Nucleic Acids', Journal of Liquid Chromatography & Related Technologies, 29: 12, 1687 — 1701
To link to this Article: DOI: 10.1080/10826070600716769
URL: http://dx.doi.org/10.1080/10826070600716769

Journal of Liquid Chromatography & Related Technologies®, 29: 1687–1701, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600716769

Effect of Concentration of Ionic Liquid 1-Butyl-3-Methylimidazolium, Tetrafuoroborate, for Retention and Separation of Some Amino and Nucleic Acids

Yulia Polyakova, Yinzhe Jin, Jinzhu Zheng, and Kyung Ho Row
Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, Nam-Ku, Incheon, Korea

Abstract: In this paper, three nucleic acids such as cytosine, cytidine, and thymine and two amino acids, such as D-tryptophan and N-carbobenzyloxy-D-phenylalanine, were chromatographed using ionic liquid as an additive for the mobile phase in high performance liquid chromatography (HPLC). Ionic liquid, 1-butyl-3-methylimidazolium tetrafuloroborate ($[BMIm]^+$ $[BF_4]^-$), was used. The nucleic acid eluent was 5 vol. % of the modifier (methanol:acetonitrile = 95:5 (vol. %)), in 10 mM of sodium phosphate monobasic, with addition of 0.5, 2.0, and 4.0 mmol/L of ionic liquid. Separation of nucleic acids was obtained on a commercially available octadecyl silica column (4.6 × 150 mm i.d., and particle size 5 μm). In the case of D-amino acids, the mobile phase was 65% methanol in water, with additions of 0.5, 1.0, 2.0, 8.0, 12.0, and 15.0 mmol/L of ionic liquid. The experiments were performed on a stainless steel column, 3.9 × 300 mm i.d., and particle size 15 μm, packed with octadecyl-bonded silica at the laboratory. Effects of the concentration of ionic liquid for retention and separation of some nucleic and amino acids were discussed. The results showed the potential application of ionic liquid as a mobile phase additive in liquid chromatography.

Keywords: Ionic liquid, RP-HPLC, Retention factor, Resolution, Nucleic acid, D-Amino acid

Address correspondence to Kyung Ho Row, Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Building 2S 335, Inha University, #253, Yonghyun-Dong, Nam-Ku Incheon 402-751, Korea. E-mail: rowkho@inha.ac.kr

INTRODUCTION

Ionic liquids (ILs) are organic salts which are liquids at ambient temperatures. Unlike traditional solvents, which can be described as molecular liquids, ionic liquids are composed of ions. Their unique properties such as non-volatility, non-flammability, and excellent chemical and thermal stability have made them an environmentally attractive alternative to conventional organic solvents.[1–5] Room temperature ionic liquids are gaining wide recognition as novel solvents in chemistry. Their application in analytical chemistry, especially in separating analytes, is merited because ILs have some unique properties, such as negligible vapor pressure, good thermal stability, tunable viscosity, strong polarity, and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions. Ionic liquids have low melting points ($<100^{\circ}$C) and remain as liquids within a broad temperature window ($<300^{\circ}$C).[2] Some ILs are liquid at over 400°C, and some at as low as -96°C.[1]

ILs are now widely recognized as important components of green chemistry. The basic concepts behind both green chemistry and ILs is not new; however, due to the increase of interest in ILs and the influence of novel minds, new approaches are generating original solutions and driving changes in industrial and commercial practices, proving that green chemistry alternatives exist. ILs have been used in a variety of different areas in modern chemistry and shown to provide unique properties. ILs have been applied to catalysis,[3,4] biocatalysis,[1] synthesis,[5] analytical,[2] etc. Thus, it suggests that they might also supply exclusive and interesting opportunities for separation science in general, and chromatography, specifically.[6–17] It was already proven that ionic liquids could be used as mobile phase additives in reversed-phase chromatography when mixed with another solvents of a low viscosity. Numerous experiments were performed with water, methanol, acetonitrile, and their mixtures as the mobile phases. These trials show that ionic liquids could be good eluent strength modifiers in reversed-phase high performance liquid chromatography (HPLC). However, one should note, when ILs are diluted or immobilized on a stationary support, they may not possess all the properties of the ordinary ILs. In some cases, they may keep several kinds of intermolecular interactions, which can be useful for chromatographic separations. In liquid chromatography, the mobile phase should be selective for the components and its composition is one of the most necessary variables influencing a separation.[18] The investigations showed that as mobile phase additives, ionic liquids could play a multiplicity of roles, such as blocking of the residual silanols groups, modifying the stationary phase, or to be as active ion-pairing agents. The readily commercial availability of a wide range of ionic liquids is acting as a stimulus to the field, creating the correct climate for innovations in HPLC.

Reversed phase HPLC is widely used as a standard analytical instrument, a numbers of stationary phases are commercially available.[19] In a

chromatographic column, injected materials are separated based on their differences in the solvation and partition in a stationary phase.[20] HPLC is used for the separation, purification, collection of the single components, the qualitative and quantitative analysis of the adsorption, division, ion exchange, and exclusion effects when the mobile phase passes through the solid phase.

We investigated the effect of concentration of the ionic liquid 1-butyl-3-methylimidazolium tetrafuloroborate as a mobile phase modifier on the retention of the nucleic (cytosine, cytidine, and thymine) and amino (N-carbobenzyloxy-D-phenilalanine and D-tryptophan) acids. The name, structure, and molecular weight of the sorbats and ionic liquid are listed in Figure 1. Hydrophilic ionic liquid, 1-butyl-3-methylimidazolium tetrafuloroborate, was miscible in water and methanol, its molecular weight was 226, and its melting point is 75°C.

EXPERIMENTAL

Reagents

Butyl-3-methylimidazolium tetrafluoroborate ionic liquid ($[BMIm]^+[BF_4^-]$) was purchased from C-tri Co. (Namyang, Korea). Cytosine, cytidine, thymine,

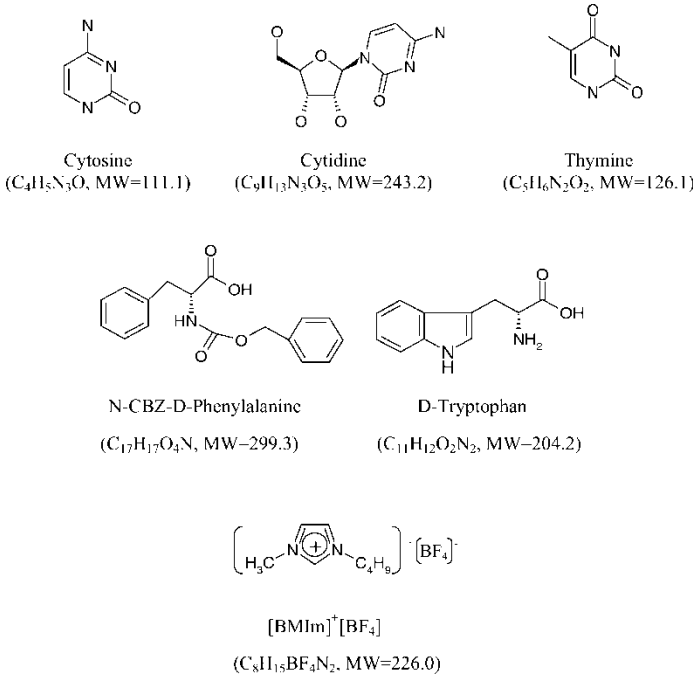

Figure 1. Names, chemical structures, and molecular weights of investigated nucleic and amino acids and ionic liquid.

N-carbobenzyloxy-D-phenylalanine (N-CBZ-D-phenylalanine), and D-tryptophan were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Potassium nitrate (KNO_3) and sodium phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$) were purchased from Kanto Chemical Co. (Japan). Hydrochloric acid (HCl) was purchased from Junsei Chemical Co. (Japan). All these chemicals are of analytical grade purity. HPLC grade methanol (CH_3OH) and acetonitrile (CH_3CN) were purchased from Duksan Pure Chemical Co. (Ansan, Korea). Distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. Each sample was filtered by using a MFS-5, 0.2 μm TF filter fromWhatman, U.S.A., before injection into the HPLC system. The solutions were stored at 4°C, and the working standards were freshly prepared every 2 days to avoid potential errors from decomposition of the targets.

Apparatus and Methods

The instruments used in this study were as follows: a 600 HPLC pump (Waters, U.S.A.), a 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co., Korea), and a Reodyne injection valve with 20 μL sample loop. The experiments were performed with two columns. A commercially available octadecyl silica column (4.6 × 150 mm i.d. and particle size 5 μm) was used with nucleic acids; the stainless steel column, 3.9 × 300 mm i.d., packed with 15 μm octadecyl-bonded silica was tested with amino acids at the laboratory.

In the event that nucleic acid eluents were 5 vol. % of modifier (methanol : acetonitrile = 95:5 (vol. %)) in 10 mM of sodium phosphate monobasic without, and with, additions of 0.5, 2.0, and 4.0 mmol/L of ionic liquid. Mobile phases were 65% methanol in water without, and with, supplements of 0.5, 1.0, 2.0, 8.0, 12.0, and 15.0 mmol/L of the ionic liquid $[BMIm]^+[BF_4]^-$ (used HCl adjust to pH = 3.0, approximately) were tested with amino acids. The isocratic mode at a flow rate of 1.0 mL/min was used in every test. Individual sorbents were dissolved in water with a concentration of 1 mg/mL. A mixture of nucleic acids was prepared as aliquots of individual solutions of cytosine, cytidine, and thymine in the ratio of 1:1:1; the mixture of amino acids was prepared as a combination of individual solution of N-CBZ-D-phenylalanine and D-tryptophan in the ratio 4:1, respectively.

The injection volume was 5 μL and UV wavelengths were set at 254 nm for nucleic acids and 15 μL at 254 nm for amino acids. Chromate software (Ver. 3.0 Interface Eng., Korea) connected to a PC was used as a data acquisition system. All experimental procedures were performed at ambient temperature.

RESULTS AND DISCUSSION

In the development of separation methods by chromatography, there are several factors to be considered, such as inner diameter and length of

column, composition of mobile phase, etc. The type of column and stationary phase depend on a target material, so the optimum separation condition was mainly determined by the composition of mobile phase.[21] Data on the chromatographic behaviors of polar compounds, which is one of the most important fields in modern chemistry, gives useful information for analytical, combinatorial, and medicinal chemistry. In addition, although the liquid chromatography is a modern powerful method, the separation of a very polar compound like an ionizable compound remains difficult for solution. Furthermore, studying the chromatographic behavior of polar compounds has the practical applications and theoretical interest. Nucleic and amino acids are known as biological and medical agents. During the late 20th century, they were introduced as biological activity compounds contained in human and animal bodies. For this reason, recent researches have concentrated on the synthesis and design of the drugs on the basis of nucleic and amino acids. Therefore, the investigation of the new procedures of analysis is very important for pharmacological and biochemical analysis also.

In this work the retention factor was calculated according to eq. (1).

$$k = \frac{t_R - t_m}{t_m} \tag{1}$$

where t_m is the hold-up time, t_R is the retention time, and k is retention factor, respectively. The selectivity (α) can be obtained by eq. (2).

$$\alpha = \frac{k_2}{k_1} \tag{2}$$

where k_1 and k_2 are the retention factors of the first and second peak ($k_1 \leq k_2$), respectively. The resolution (R_s) is calculated using the eq. (3).

$$Rs = \frac{t_{R2} - t_{R1}}{(w_2 + w_1)/2} = \frac{2\Delta t}{w_2 + w_1} \tag{3}$$

where t_{R1} and t_{R2} was the retention times of the first and second peak ($t_{R1} \leq t_{R2}$), respectively, and w_1 and w_2 was the peak widths of the first and second peak, respectively.

The asymmetry factor (a), which is determined from the front (f) and back (b) peak widths at 10% of the peak height is as follows:[22]

$$a = \frac{b_{0.1}}{f_{0.1}} \tag{4}$$

Tailing factor (TF), which uses the front (f) and back (b) widths at 5% of the peak height is as follows:

$$TF = \frac{f_{0.05} + b_{0.05}}{2 \times f_{0.05}} \tag{5}$$

To calculate a retention factor, the holdup time of the HPLC system used should be measured. Potassium nitrate (KNO_3) is used as the holdup time marker (peak taken as the first deviation of the baseline following the injection of 10 μL KNO_3). Three replicate injections were made to determine the retention time of each substance, and the average values were used to calculate the retention factors. In our experiments, we determined the retention dependencies from ionic liquid concentration on the eluent at constant temperature. Evaluation of the results of the chromatographic experiment was carried out by mathematical statistics techniques. The relative error of a single measurement did not exceed 5%.

Experimental values of the retention factors and selectivity of nucleic acids are shown in Table 1, and obtained chromatograms of the mixture demonstrated in Figure 2. It is easy to see that the efficiency and shapes of nucleic acid peaks were similar, each containing ionic liquid and non-ionic liquid in the eluent; the elution order was same also. Retention times of cytosine were stable and retention times of cytidine were close also. Unfortunately, the addition of ionic liquid did not practically influence their retention. At the same time, the addition of the ionic liquid to the eluent renders minor influence on the chromatographic behavior of thymine. Retention factors of thymine were negligibly changed as the concentration of ionic liquid increased from 0.0 mmol/L to 2.0 mmol/L (Table 1). The following treatment of mobile phase by 4.0 mmol/L of ionic liquid did not change thymine retention.

No special effects between were indicated in relation to the selectivity and resolution (Figure 3) between closed peaks. In this case, this implies the chromatographic retention should be determined, generally, by nonspecific dispersion interactions of sorbents with the stationary phase. This proves the high contribution of dispersion interactions to the nucleic acids retention. This phenomenon has been shown to influence the contribution of specific interaction sorbate–sorbent, leading to the conclusion that the dipole-dipole and Van der Waals intermolecular interactions with the stationary phase are more important than specific interactions with the eluent in retention of the investigated nucleic acids. We also checked several parameters such as peak asymmetry and peak tailing (Table 2). These two parameters are related: a low peak height may be indicative of a tailing or a wide peak and tailing peaks provide high asymmetry factor values. So, as can be deduced from Table 2, the mobile phase with 2 mmol/L of ionic liquid gave the best asymmetry factors for every investigated nucleic acid.

Thus, we obtained that the tested ionic liquid does not influence positively on the chromatographic separation of the studied nucleic acids. As a result, the concentrations of ionic liquid more than 4.0 mmol/L were not subsequently tested.

Another part of our studies was devoted to the chromatographing of two amino acids. The obtained values of retention factors and selectivity are present in Table 1. Data indicate that N-CBZ-D-phenylalanine's retention were quite stable with the different mobile phase compositions.

Table 1. Retention factor and selectivity of investigated substances with and without ionic liquid in mobile phase

	Retention factor (k)					Selectivity (α)		
Ionic liquid concentration (mmol/L)	Cytosine	Cytidine	Thymine	D-tryptophan	N-CBZ-D-phenylalanine	Cytosine/ cytidine	Cytidine/ thymine	D-tryptophan/ N-CBZ-D-phenylalanine
0.0	0.01	0.15	0.87	4.07	4.07	14.00	5.82	1.00
0.5	0.04	0.21	1.01	2.06	4.15	6.00	4.79	2.01
2.0	0.04	0.21	0.98	1.51	4.42	6.00	4.67	2.93
4.0	0.04	0.22	0.98	—	—	5.00	4.48	—
15.0	—	—	—	1.22	4.26	—	—	3.48

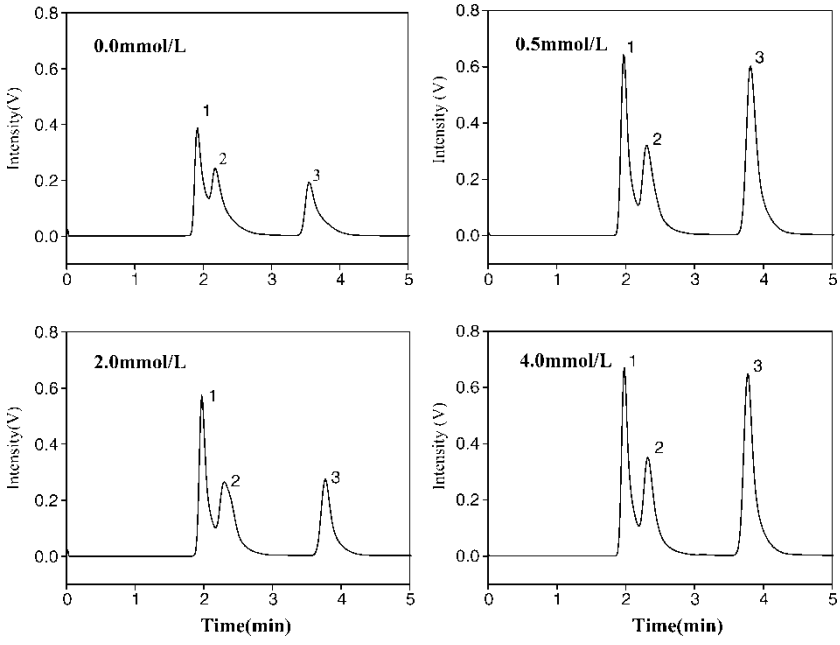

Figure 2. Chromatograms of nucleic acids with different concentrations of $[BMIm]^+[BF_4]^-$ in mobile phase. 1 = cytosine; 2 = cytidine; 3 = thymine (1 mL/min flow rate, 5 μL injection volume).

At the same time, the addition of ionic liquid to the eluent influences the chromatographic behavior of D-tryptophan. Retention evidently changed as the concentration of ionic liquid increased from 0.0 mmol/L to 0.5 mmol/L. The concentration 2.0 mmol/L of ionic liquid in the mobile phase already leads to the expressed reduction of retention. An increase of content of ionic liquid in the eluent from 2.0 mmol/L to 15.0 mmol/L leads to the gradual decrease of D-tryptophan's retention.

In the mobile phase composed of 65% methanol without addition of the ionic liquid, the value of the retention factor of D-tryptophan was 4.07, but the smallest value of the retention factor of N-CBZ-D-phenylalanine also was 4.07. These experiments indicated that separation of these two substances was not accomplished using the eluent (65% methanol in water, pH = 3.0) without ionic liquid, and the selectivity value was 1.0. But with the mobile phase adjusted by ionic liquid, the optimum value of selectivity of these two amino acids can reach 3.48 (Table 1).

Figure 4 shows the chromatograms of mixtures of D-tryptophan and N-CBZ-D-phenylalanine, with a mobile phase of 65% methanol, containing the different concentrations of $[BMIm]^+[BF_4]^-$ at pH 3.0, and without ionic liquid. The elution order of two amino acids was D-tryptophan and N-CBZ-D-phenylalanine, respectively, and it was not changed with mobile phase

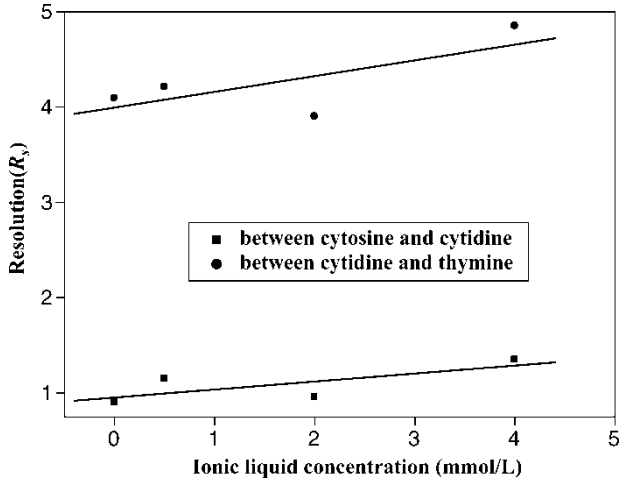

Figure 3. The resolution of nucleic acids with different concentrations of $[BMIm]^+[BF_4]^-$ in mobile phase.

compositions. It is obvious that the addition of ionic liquid to 65% methanol's eluent results in a better separation of these two amino acids. The resolution of the investigated analytes were increased with the concentration of $[BMIm]^+[BF_4]^-$ as an additive of the used mobile phase (Figure 5). The addition of ionic liquid positively affected the resolution between the D-tryptophan and N-CBZ-D-phenylalanine in the used mobile phase. The resolution was improved with the larger concentration of ionic liquid. The peak shapes of N-CBZ-D-phenylalanine were also improved with the concentration of $[BMIm]^+[BF_4]^-$ was increased in mobile phase. At the same time, the peak shape of D-tryptophan without ionic liquid in the mobile phase was better than those obtained with ionic liquid in the mobile phase.

It is common knowledge that the chromatographic behavior is affected by intermolecular forces, which include Van der Waals, ion–dipole, dipole–dipole, dipole–induced dipole interactions, etc. The chromatographic partition, in turn, results from adsorption–desorption of the compounds on the stationary phase, during which the degree of freedom of the molecule changes.

Accordingly, chromatographic retention depends mainly on the factors such as hydrophobicity, charge of the solute molecule, and nature of eluent. As a rule, modifying additives are a substantial influence on equilibrium establishment in the sorbent- sorbate and eluent-sorbate systems. Furthermore, interaction of the modifier with the sorbent and sorbate often occurs by very complex natures, and it leads to the realization of completely new mechanisms of separation. Several authors show that the various ionic liquids form the layer on the surface of the modified silica gel.[14,23,24]

Table 2. Asymmetry factor and peak tailing of nucleic and amino acids with different mobile phases

Ionic liquid concentration (mmol/L)	Asymmetry factor (*a*)					Peak tailing (*TF*)				
	Cytosine	Cytidine	Thymine	D-tryptophan	N-CBZ-D-phenylalanine	Cytosine	Cytidine	Thymine	D-tryptophan	N-CBZ-D-phenylalanine
0.0	5.72	2.95	3.57	1.43	1.43	3.78	2.45	2.38	1.24	1.24
0.5	4.01	2.51	2.15	1.90	1.48	2.85	2.15	1.78	1.53	1.30
2.0	2.84	2.18	2.20	1.76	1.19	2.70	1.89	1.82	1.45	1.12
4.0	3.53	2.22	2.42	—	—	2.56	1.87	1.80	—	—
15.0	—	—	—	1.74	1.22	—	—	—	1.43	1.19

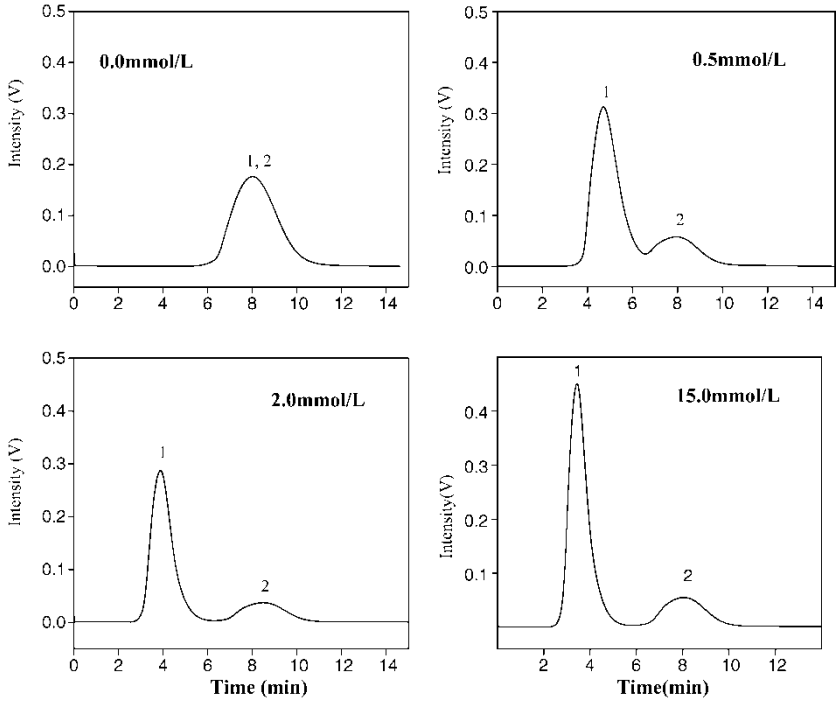

Figure 4. Chromatograms of D-tryptophan and N-CBZ-D-phenylalanine with different concentrations of $[BMIm]^+[BF_4]^-$ in mobile phase. 1 = D-tryptophan; 2 = N-CBZ-D-phenylalanine (1 mL/min flow rate, 10 μL injection volume).

With an increase in the concentration of an ionic liquid modifier in the mobile phase, cations interact with the silanols groups through electrostatic interactions, producing a weak layer electronic structure, and interact with the alkyl groups through hydrophobic and non-specific interactions. Thus, it is possible to tell about the realization of the version of dynamic modification of sorbent by ionic liquid and formation of the pseudo-stationary phase. Therefore, this phenomenon can efficiently shield the residual silanols and improve the peak shapes and also influence the chromatographic retention times of the sorbates.

Xiao and coworkers showed that the retention of some amines is greatly affected by the pH of the mobile phase and nature of modifiers.[14] When amines are retained in the stationary phase, residual silanols on the surface of the stationary phase would interact with the analytes, and result in a poor separation of these basic compounds if no additive is added to in the mobile phase. The retention of based amines is shortened because of the repulsion between the imidazolium cation and the ionized amines. On the other hand, ionic liquids have different effects on the separation of phthalic acids owing

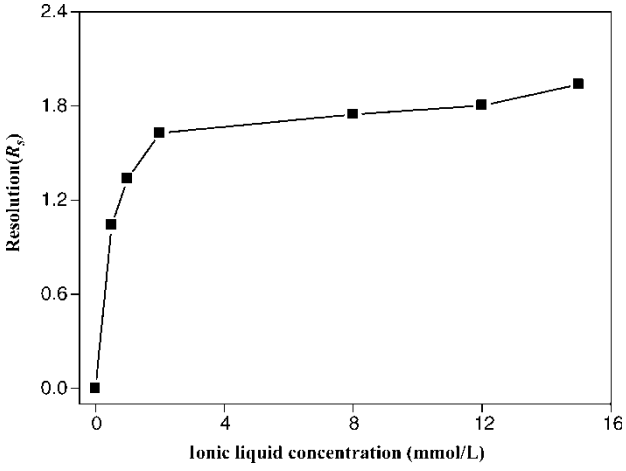

Figure 5. The resolution of D-tryptophan and N-carbobenzyloxy-D-phenylalanine with different concentrations of $[BMIm]^+[BF_4]^-$ in mobile phase.

to the delocalization of the charge.[14] He et al.[10] noted the differences between triethylamine (TEA) and ionic liquids on the separation of ephedrines and confirmed that ionic liquids are better additives. Imidazolium cations can interact with silanol groups and compete for the silanol groups on the alkylsilica surface with the polar group of the analytes. Therefore, it can effectively shield the residual silanols and improve the peak shapes, while also decreasing the retention time of the analytes. Furthermore, interaction of the modifier with the sorbent and sorbate often occurs by their very complex nature, and leads to the realization of completely new mechanisms of separation. The role of ionic liquids is multiplex, and further investigation is needed in order to quantitatively explain some of the phenomena. However, ionic liquids showed a promising performance as an additive in HPLC.

In practice, short times and low costs are the very key factors for obtaining the final product. As a rule, in chromatographic experiments, the optimum separation condition is determined by the factors such as resolution value above 1.5, reduction of concentration of additive, and time of analysis.

We disclosed these parameters for analytical separation of D-tryptophan and N-CBZ-D-phenylalanine. The resolution value of D-tryptophan and N-CBZ-D-phenylalanine was 1.04 at 65% methanol containing 0.5 mmol/L of $[BMIm]^+[BF_4]^-$ at pH 3.0. This resolution value was evidently insufficient. At the same time, the resolution values above 1.5 can already be obtained at 2.0 mmol/L of ionic liquid in the eluent. Subsequent increase of the concentration of ionic liquid in the mobile phase does not lead to the distinction on the effect of separation. The mobile phase with 65% methanol containing 2.0 mmol/L of $[BMIm]^+[BF_4]^-$ at pH 3.0 was the optimum result for analytical separation. These conditions were proposed, and already successfully

used, for separation of D-tryptophan and N-CBZ-D-phenylalanine[25] by the SMB (simulation moving bad chromatography) method.[26]

According to these results, we deduced that ionic liquids have certain potential as a possible modifier in HPLC. Although the use of ILs as modifiers is in its infancy, they have already demonstrated certain potential in HPLC. One should especially note that at present, unfortunately, the influence of the ionic liquids on the separation mechanism is not clearly understood. The latter fact, together with the already existing successful examples of the application of ionic liquids for chromatographic separations, is a powerful stimulus for further studies in this direction in the future.

CONCLUSION

In this paper, we investigated the effect of the concentration of the ionic liquid $[BMIm]^+[BF_4]^-$ as a mobile phase modifier on the retention of the nucleic (cytosine, cytidine, and thymine) and amino (N-CBZ-D-phenilalanine and D-tryptophan) acids. The addition of the ionic liquid to the eluent influences the chromatographic behavior of thymine; at the same time the retention times of cytosine and cytidine were close, with and without ionic liquid in the eluent. No special effects with various concentrations of ionic liquid were indicated, relating to the selectivity and resolution of nucleic acids. This implies that, in this case, retention should be determined generally by nonspecific (dispersion) interactions of sorbants with the stationary phase. The contents of the ionic liquid affected the resolution between the D-tryptophan and N-CBZ-D-phenylalanine in the mobile phase. The resolution was improved with the larger concentration of ionic liquid. The mobile phase, with 65% methanol containing 2.0 mmol/L of $[BMIm]^+[BF_4]^-$ at pH = 3.0, had the optimum effect for analytical separation of the investigated amino acids. This experiment also showed that the shape of peaks (asymmetry and peak tailing parameters) could be improved at the mobile phase using the ionic liquid $[BMIm]^+[BF_4]^-$ as an additive.

ACKNOWLEDGMENT

This work was supported by Inha University Research Grant (INHA–2006).

REFERENCES

1. Yang, Z.; Pan, W. Ionic liquids: Green solvents for nonaqueous biocatalysis. Enzyme Microb. Technol. **2005**, *37*, 19–28.
2. Liu, J.; Jonsson, J.; Jiang, G. Application of ionic liquids in analytical chemistry. TrAC Trend Anal. Chem. **2005**, *24*, 20–27.

3. Zhao, D.; Wu, M.; Kou, Y.; Min, E. Ionic liquids: Applications in catalysis. Catal. Today **2002**, *74*, 157–189.
4. Gordon, C.M. New developments in catalysis using ionic liquids. Appl. Catal. **2001**, *A222*, 101–117.
5. Sun, J.; Fujita, S.; Arai, M. Development in the green synthesis of cyclic carbonate from carbon dioxide using ionic liquids. J. Organomet. Chem. **2005** (in press).
6. Liu, J.; Li, N.; Jiang, G.; Liu, J.; Jonsson, J.A.; Wen, M. Disposable ionic liquid coating for headspace solid-phase microextraction of benzene, toluene, ethylbenzene, and xylenes in paints followed by gas chromatography-flame ionization detection. J. Chromatogr. A **2005**, *1066*, 27–32.
7. Peng, J.; Liu, J.; Jiang, G.; Tai, C.; Huang, M. Ionic liquid for high temperature headspace liquid-phase microextraction of chlorinated anilines in environmental water samples. J. Chromatogr. A **2005**, *1072*, 3–6.
8. Tian, K.; Qi, S.; Cheng, Y.; Chen, X.; Hu, Z. Separation and determination of lignans from seeds of Schisandra species by micellar electrokinetic capillary chromatography using ionic liquid as modifier. J. Chromatogr. A **2005**, *1072*, 181–187.
9. He, C.; Li, S.; Liu, H.; Li, K.; Liu, F. Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt. J. Chromatogr. A **2005**, *1072*, 143–149.
10. He, L.; Zhang, W.; Zhao, L.; Liu, X.; Jiang, S.X. Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as the eluent on the separation of ephedrines by liquid chromatography. J. Chromatogr. A **2003**, *1007*, 39–45.
11. He, L.J.; Zhang, W.Z.; Wen, B.; Liu, X.; Lang, S.X. Separation of ephedrines using 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids as eluent in high-performance liquid chromatography (HPLC). Chin. Chem. Lett. **2003**, *14*, 603–604.
12. Marszałł, M.P.; Baczek, T.; Kaliszan, R. Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids. Anal. Chim. Acta **2005**, *547*, 172–178.
13. Waichigo, M.M.; Riechel, T.L.; Danielson, N.D. Ethylammonium acetate as mobile phase modifier for reversed phase liquid chromatography. Chromatogr. **2004**, *61*, 17–23.
14. Xiao, X.; Liang, Z.; Xia, L.; Jiang, S. Ionic liquids as additives in high performance liquid chromatography analysis of amines and the interaction mechanism of ionic liquids. Anal. Chim. Acta **2004**, *519*, 207–211.
15. Zhang, W.; He, L.J.; Xia, L.; Jiang, S.X. Ionic liquids as mobile phase additives for separation of nucleotides in HPLC. Chin. J. Chem. **2004**, *22*, 549–552.
16. Kaliszan, R.; Marszall, M.P.; Markuszewski, M.J.; Baczek, T.; Pernak, J. Suppression of deleterious effects of free silanols in liquid chromatography by imidazolium tetrafluoroborate ionic liquids. J. Chromatogr. A **2004**, *1030*, 263–271.
17. Berthod, A.; Ruiz-Angel, M.J.; Huguet, S. Nonmolecular solvents in separation methods: Dual nature of room temperature ionic liquids. Anal. Chem. **2005**, *77* (13), 4071–4080.
18. Lee, C.H.; Lee, J.W.; Row, K.H. Optimum solvent selectivity and gradient mode for deoxyribonucleosides in reversed-phase high-performance liquid chromatography. J. Chromatogr. A **1998**, *1072*, 337–344.
19. Lee, Y.W.; So, M.S.; Lee, J.W.; Chung, S.T.; Row, K.H. Retention models of capacity factor with different compositions of organic modifier in RP-HPLC. Korean J. Chem. Eng. **1996**, *13*, 578–584.

20. Choi, Y.J.; Han, S.K.; Chung, S.T.; Row, K.H. Chromatographic separation of bupivacaine racemate by mathematical model with competitive Langmuir isotherm. Korean J. Chem. Eng. **2004**, *21*, 829–835.
21. Han, S.K.; Jin, Y.; Row, K.H. Correlation equation for retention and resolution of ibuprofen in SFC. Bull. Korean Chem. Soc. **2004**, *25*, 1–5.
22. *United States Pharmacopoeia*; United States Pharmacopoeial Convention: Maryland, USA, 1984.
23. Yanes, E.G.; Gratz, S.R.; Baldwin, M.J.; Robison, S.E.; Stalcup, A.M. Capillary electrophoretic application of 1-alkyl-3-methylimidazolium-based ionic liquids. Anal. Chem. **2001**, *73*, 3838–3844.
24. Imaz, C.; Navajas, R.; Carreras, D.; Rodrıguez, C.; Rodrıguez, A.F. Comparison of various reversed-phase columns for the simultaneous determination of ephedrines in urine by high-performance liquid chromatography. J. Chromatogr. A **2000**, *870*, 23–28.
25. Koo, Y.M. Application of ionic liquid in biotechnology: Biotoxicity, bioconversion and separation, In *The 6th Inha ERC International Symposium*; Inha University: Incheon, Korea, 2005; 50.
26. Broughton, D.B.; Gerhold, C.G. (U.S. Patent No 2,985,589).

Received February 12, 2006
Accepted March 20, 2006
Manuscript 6829