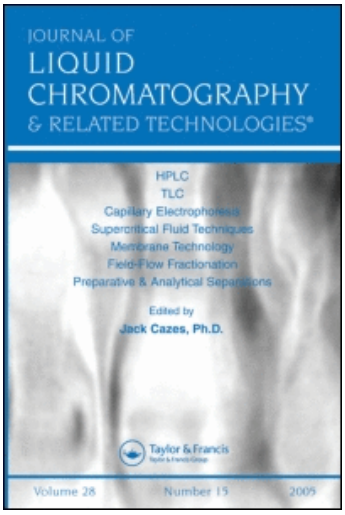

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of Ionic Liquid on the Retention and Separation Behavior of Various Amino Acids in Reversed Phase Chromatography

Chang Geun Yoo[a]; Sung Chul Yi[a]; Sungyong Mun[a]
[a] Department of Chemical Engineering, Hanyang University, Seoul, Korea

To cite this Article Yoo, Chang Geun , Yi, Sung Chul and Mun, Sungyong(2007) 'Effect of Ionic Liquid on the Retention and Separation Behavior of Various Amino Acids in Reversed Phase Chromatography', Journal of Liquid Chromatography & Related Technologies, 30: 20, 2989 — 3006
To link to this Article: DOI: 10.1080/10826070701629838
URL: http://dx.doi.org/10.1080/10826070701629838

Journal of Liquid Chromatography & Related Technologies®, 30: 2989–3006, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701629838

Effect of Ionic Liquid on the Retention and Separation Behavior of Various Amino Acids in Reversed Phase Chromatography

Chang Geun Yoo, Sung Chul Yi, and Sungyong Mun
Department of Chemical Engineering, Hanyang University, Seoul, Korea

Abstract: The chromatographic retention behaviors of various amino acids were investigated using ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4]), as a mobile phase additive and a reversed phase C_{18} media as a stationary phase. The amino acids investigated include L-tyrosine, N-CBZ-L-tyrosine, L-phenylalanine, N-CBZ-L-phenylalanine, L-methionine, and N-CBZ-L-methionine. The effect of ionic liquid on N-CBZ-L-amino acids was found to be different from that on L-amino acids. As the concentration of ionic liquid in mobile phase increased, the retention factors of L-amino acids showed a decreasing trend, whereas those of N-CBZ-L-amino acids exhibited a minute change. The selectivity between L-amino acid and N-CBZ-L-amino acid was, thus, improved by increasing the content of ionic liquid. In addition, the peak shapes of L-amino acids, if asymmetric, were transformed into symmetric ones by increasing the ionic liquid content. The increase of feed concentration causes the overloaded condition, leading to a decrease in the retention factor. If such effect of feed concentration is coupled with the effect of using ionic liquid, one can achieve a better separation between L-amino acid and N-CBZ-L-amino acid.

Keywords: Ionic liquid, RP-HPLC, Retention behavior, 1-butyl-3-methylimidazolium tetrafluoroborate, L-Amino acid, N-CBZ-L-amino acid

INTRODUCTION

Ionic liquids have recently received growing attention as one of the potential mobile phase additives in the chromatographic separation processes. Ionic

Address correspondence to Prof. Sungyong Mun, Department of Chemical Engineering, Hanyang University, Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea. E-mail: munsy@hanyang.ac.kr

liquids are molten salts at ambient temperatures and consist entirely of ionic species. Ionic liquids have both the properties of conventional organic solvents, such as excellent solvation quality and a low viscosity, as well as unique properties, such as non-volatility, high electrical conductivities, non-flammability, and excellent chemical and thermal stability.[1–6] These properties have facilitated the application of ionic liquids to chromatographic separations, which have been reported in the following publications.

He et al.[7] analyzed the chromatographic behaviors of ephedrines by varying the content of ionic liquid in mobile phase. They also tested several ionic liquids with different alkyl substituents on the imidazolium cation or with different counterions as the mobile phase additive. Xiao et al.[8] used ionic liquids as additives for the separation of amines. Shetty et al.[9] reported the applications of alkylammonium nitrate salts as a modifier to adjusting solvent strength in reversed phase liquid chromatography. The development of a fairly robust capillary electrophoresis (CE) method for the separation of polyphenols with ionic liquids was reported by Yanes et al.[10] Polyakova et al.[11] investigated the effects of the content of ionic liquid on the resolution and selectivity of some amino acids. Mwongela et al.[12] accomplished the separations of two achiral mixtures and one chiral mixture using ionic liquids as pseudo stationary phase modifiers.

The latest researches on ionic liquids in chromatographic separation areas have been focused on its application to the separation of some amino acids. Jin et al.[13] analyzed the influence of ionic liquid on the separation of two amino acids, such as D-tryptophan and N-CBZ-D-phenylalanine. Polyakova et al.[14] used the same amino acids to investigate how the ionic liquid content in mobile phase affects the retention factors and the selectivity. According to their publications, an increase in the ionic liquid content in mobile phase improved the selectivity between the two amino acids. The amino acids used in the aforementioned literatures, however, are limited to only D-tryptophan and N-CBZ-D-phenylalanine. No previous studies have hitherto tested other amino acids than D-tryptophan and N-CBZ-D-phenylalanine.

In this study, the target materials of investigation are extended to other amino acids such as L-tyrosine, N-CBZ-L-tyrosine, L-phenylalanine, N-CBZ-L-phenylalanine, L-methionine, and N-CBZ-L-methionine. The chromatographic behaviors of such amino acids are investigated by varying the content of ionic liquid in mobile phase. Furthermore, we investigated how a change in feed concentration affects the chromatographic behaviors and peak shapes by altering the concentration of ionic liquid in mobile phase.

The results of this work show that the ionic liquid content in mobile phase has a significant effect on the retention factors of a series of L-amino acids, but almost no effect on those of a series of N-CBZ-L-amino acids. In addition, the use of ionic liquid as a mobile phase additive promotes the transformation of some amino acid peaks into symmetric ones, which is accompanied by peak compression. The increase of feed concentration causes the overloaded condition, leading to a decrease in the retention factor due to a nonlinear

adsorption behavior. If such effect of feed concentration is coupled with the effect of using ionic liquid, one can achieve a better separation between L-amino acid and N-CBZ-L-amino acid.

Mechanism of Interaction of Ionic Liquid with the Stationary Phase in a Reversed Phase Column

In a liquid chromatography, different solute molecules are separated based on the difference in their residence times, which are mostly controlled by the specific interactions of the solute molecules with a certain functional group of a stationary phase.[11]

In case of a reversed phase chromatography, the stationary phase usually has two distinct functional groups such as silanol groups and alkyl groups (Figure 1). The former attracts the polar groups of a solute molecule through specific electrostatic interactions, while the latter attracts the alkyl groups of a solute molecule through hydrophobic interactions.[11] Such interaction behaviors between the solute molecules and the stationary phase can be disturbed when ionic liquid is added to a mobile phase. The reason is that the cation portion and the alkyl group of ionic liquid can also have interactions with the silanol groups and the alkyl groups of the stationary phase, respectively (Figure 1). This phenomenon causes the solute molecules to compete with ionic liquid for the functional groups of the stationary phase, leading to a decrease in the strength of attraction between the solute molecules and the stationary phase.[11] Consequently, the addition of ionic liquid to the mobile phase of a reversed phase chromatography decreases the chromatographic retention times of solute molecules.[11]

To investigate the effects of ionic liquid on the solute retention behaviors, the retention factor of each component and the selectivity between two different components in a feed mixture are estimated in this study. First, the retention factor is calculated from the retention time using the following equation:

$$k = \frac{t_R - t_{void}}{t_{void}} \tag{1}$$

where t_{void} is the hold-up time, t_R is the retention time, and k is retention factor of a component, respectively.

The selectivity (α) between the two components to be separated is calculated from the following equation:

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

where k_1 and k_2 are the retention factor of the two components to be separated ($k_1 \leq k_2$).[14]

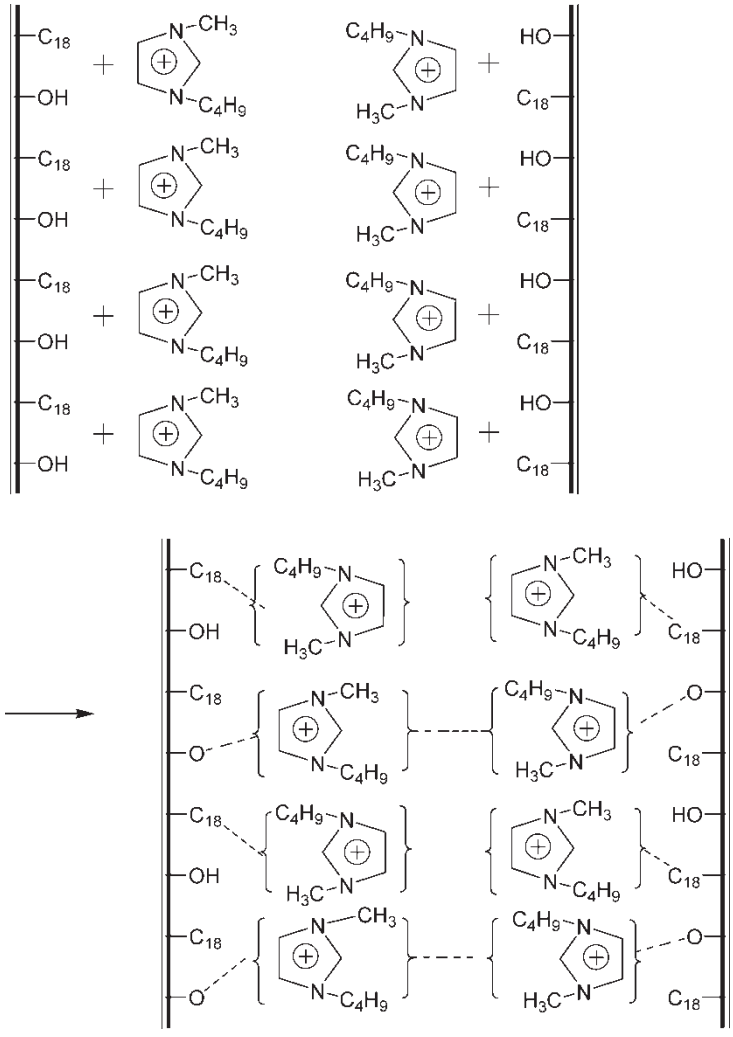

Figure 1. Mechanism of [bmim][BF4] interaction on modified silica surface.

EXPERIMENTAL

Apparatus

All the experiments in this study were conducted with the HPLC system, which consisted of a HPLC pump (Waters 515), a photodiode array detector (Waters 996), and Rheodyne injector with 50 μL sample loop. The experimental data from the HPLC system were collected and analyzed with the

help of Waters Millennium software operating in the Windows environment. The reversed phase chromatographic column installed in the HPLC system was a Waters Spherisorb® C-18 column (250 × 4.6 mm I.D. and particle size 10 μm). A Milli-Q system by Millipore (U.S.A.) was used to obtain distilled deionized water (DDW).

Reagents

The ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4]), was purchased from Sigma-Aldrich Co. (U.S.A), and used as a mobile phase modifier. The amino acids tested were N-carbobenzyloxy-L-phenylalanine (N-CBZ-L-phenylalanine), L-phenylalanine, N-carbobenzyloxy-L-methionine (N-CBZ-L-methionine), L-methionine, N-carbobenzyloxy-L-tyrosine (N-CBZ-L-tyrosine), and L-tyrosine (Figure 2) from Sigma-Aldrich Co. (U.S.A.). Mobile phase was prepared by mixing DDW and methanol, which was purchased from J. T. Baker Company (U.S.A.). The

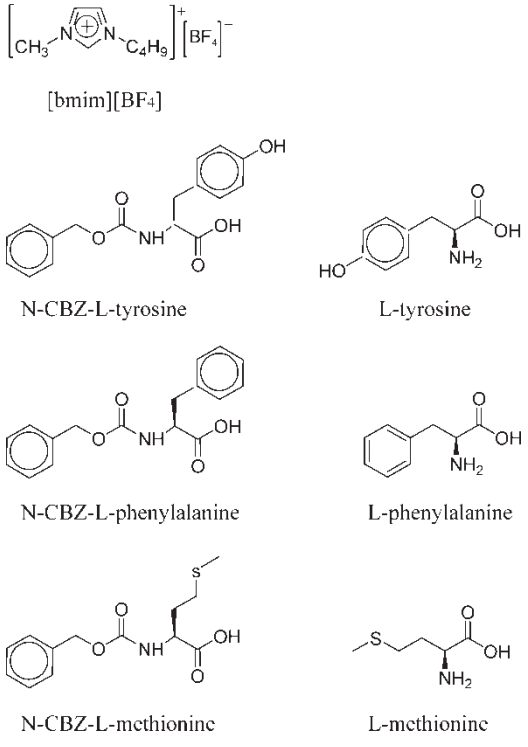

Figure 2. Chemical structures of amino acids and ionic liquid.

percentage of methanol in the mobile phase was 65% by volume. Hydrochloric acid was purchased from Samchun Pure Chemical Co. (Korea) and used to adjust the pH of the mobile phase.

Methods

A different content of ionic liquid ([bmim][BF4]) was added to the mobile phase (methanol:DDW = 65:35 (vol.%)), whose pH was adjusted to 3.0 by hydrochloric acid. The range of ionic liquid concentration in the mobile phase used was extended from 0.0 mmol/L through 5.0 mmol/L of [bmim][BF4]. All the experiments were carried out at ambient temperature, and the flow rate in each experiment was fixed at 1.0 mL/min.

A series of pulse tests with the amino acid mixtures under investigation were carried out: (mixture of L-tyrosine (0.1 g/L) + N-CBZ-L-tyrosine (0.1 g/L), mixture of L-phenylalanine (1.0 g/L) + N-CBZ-L-phenylalanine (1.0 g/L), and mixture of L-methionine (1.0 g/L) + N-CBZ-L-methionine (1.0 g/L)). To check the effect of a change in feed concentration, additional pulse tests were carried out with the mixture of N-CBZ-L- phenylalanine and L-phenylalanine by varying the feed concentration. Effluent from the column was monitored using a photodiode array detector (Waters 996). For the injection of the mixture of L-tyrosine and N-CBZ-L-tyrosine, the column effluent was monitored at the wave length of 280 nm. The other amino acid mixtures tested were detected at 254 nm.

RESULTS AND DISCUSSION

To quantify the effects of the ionic liquid [bmim][BF4] on the chromatographic behaviors of the amino acids under investigation, the retention factor and the selectivity need to be estimated. This requires the determination of retention time from the respective effluent chromatogram. If a symmetric peak shape was obtained in the effluent chromatogram, the time that the peak maximum occurs was taken as the retention time. In case the effluent chromatogram resulted in an asymmetric peak shape, the mass center time that corresponds to the first moment of concentration profile was determined as the retention time.

Effect of Ionic Liquid on the Separation of L-Tyrosine and N-CBZ-L-Tyrosine

To measure the influence of ionic liquid on the separation of L-tyrosine and N-CBZ-L-tyrosine, a series of pulse tests were conducted by varying the concentration of ionic liquid in the mobile phase. The experimental results are

Table 1. Retention factor values of L-tyrosine and N-CBZ-L-tyrosine[a] and the corresponding selectivity values with respect to the ionic liquid concentration

Ionic liquid concentration (mmol/L)	Retention factor (k)		Selectivity (α)
	L-Tyrosine	N-CBZ-L-Tyrosine	L-Tyrosine/ N-CBZ-L-tyrosine
0.0	0.405	0.735	1.812
0.3	0.289	0.719	2.490
0.5	0.258	0.706	2.742
1.0	0.191	0.709	3.707
2.0	0.134	0.709	5.302
3.0	0.103	0.712	6.882
5.0	0.067	0.715	10.619

[a]The concentrations of L-tyrosine and N-CBZ-L-tyrosine in a feed sample were 0.1 g/L each.

presented in Table 1 and Figure 3. As the concentration of the ionic liquid ([bmim][BF4]) increases, the retention factor of N-CBZ-L-tyrosine shows a minute change, whereas that of L-tyrosine decreases significantly. Such trends can also be observed in Figure 4, where a series of the experimental chromatograms are arranged in the order of the ionic liquid concentration in the mobile phase. No appreciable change in the retention time of N-CBZ-L-tyrosine is observed, while L-tyrosine is eluted earlier with the increase of ionic liquid concentration. Such aspects will lead to broadening of the

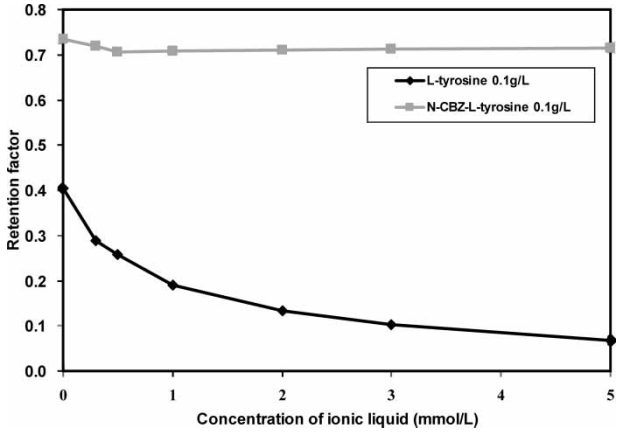

Figure 3. Effect of the ionic liquid concentration on the retention factor of L-tyrosine and N-CBZ-L-tyrosine.

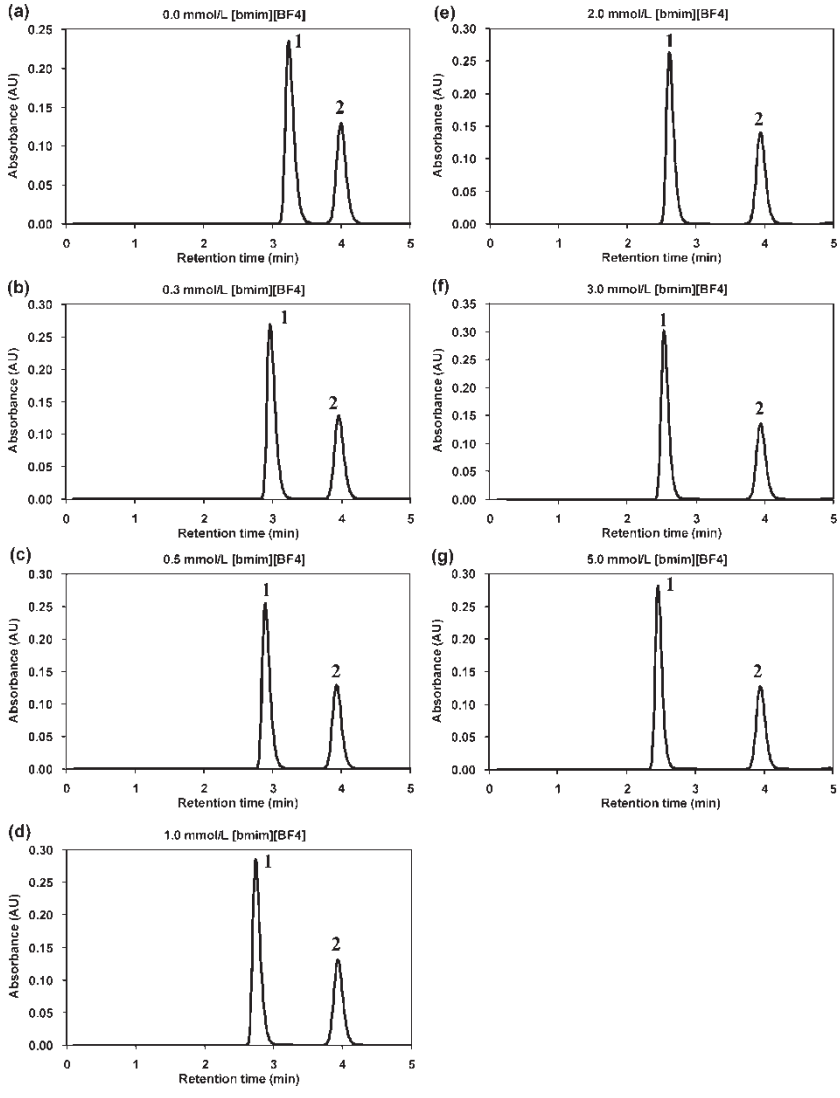

Figure 4. Chromatograms of L-tyrosine (1) and N-CBZ-L-tyrosine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 0.1 g/L, flow rate = 1 mL/min, loading volume = 50 μL, wave length = 280 nm.

interval between the two peaks, which in turn improves the selectivity between the two components as shown in Figure 5.

The selectivity is enhanced with the increase of ionic liquid concentration (Figure 5), which is similar to the trends reported in the previous studies.[14]

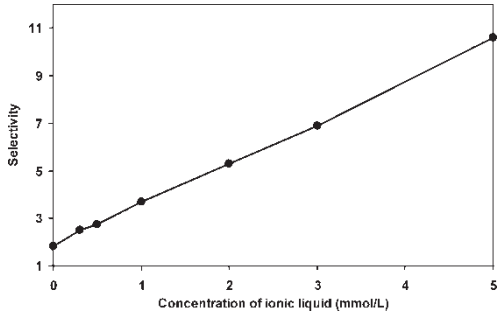

Figure 5. Effect of the ionic liquid concentration on the selectivity between L-tyrosine (0.1 g/L) and N-CBZ-L-tyrosine (0.1 g/L).

The occurrence of the above phenomena may be ascribed to the difference between the molecular structures of L-tyrosine and N-CBZ-L-tyrosine. Such inference stemmed from some explanations in the previous publication,[13] in which the effect of ionic liquid on the separation of N-CBZ-D-phenylalanine and D-tryptophan was studied. Based on the context of the previous report,[13] the following interpretation can be made on the phenomena occurring in our system. N-CBZ-L-tyrosine has a sufficiently large carbobenzyloxy group to hinder the interactions between silanol groups of the stationary phase and the cation portion of ionic liquid. On the contrary, L-tyrosine is free of such a large carbobenzyloxy group, which leads to a stronger interaction of the stationary phase with ionic liquid during the elution of L-tyrosine than during the elution of N-CBZ-L-tyrosine. This is why the presence of ionic liquid in the mobile phase has a significant impact on the retention behavior of L-tyrosine, but has little effect on that of N-CBZ-L-tyrosine.

Effect of Ionic Liquid on the Separation of L-Amino Acids and N-CBZ-L-Amino Acids

In the previous section, the effect of using ionic liquid as a mobile phase additive in the separation between L-tyrosine and N-CBZ-L-tyrosine was well understood. In this section, we investigated whether such ionic liquid effect is also true of the separation between L-amino acid and N-CBZ-L-amino acid of other kinds. For this purpose, we carried out a series of pulse tests with the following two mixtures: (1) L-phenylalanine + N-CBZ-L-phenylalanine and (2) L-methionine + N-CBZ-L-methionine. The retention factors of two components in each mixture was then measured by increasing the concentration of the ionic liquid [bmim][BF4] in the mobile phase, and the results are presented in Tables 2 and 3.

Table 2. Retention factor values of L-phenylalanine and N-CBZ-L-phenylalanine[a] and the corresponding selectivity values with respect to the ionic liquid concentration

Ionic liquid concentration (mmol/L)	Retention factor (k)		Selectivity (α) L-phenylalanine/ N-CBZ-L-phenylalanine
	L-Phenylalanine	N-CBZ-L-phenylalanine	
0.0	0.528	2.207	4.182
0.3	0.387	2.201	5.686
0.5	0.351	2.175	6.198
1.0	0.286	2.165	7.573
2.0	0.250	2.147	8.582
3.0	0.223	2.134	9.577
5.0	0.187	2.182	11.65

[a]The concentrations of L-phenylalanine and N-CBZ-L-phenylalanine in a feed sample were 1.0 g/L each.

It can be easily seen that the retention factors of N-CBZ-L-phenylalanine and N-CBZ-L-methionine is less affected by the addition of ionic liquid than those of L-phenylalanine and L-methionine. Such trends are similar to those observed in the previous section, where the materials investigated were L-tyrosine and N-CBZ-L-tyrosine. All of these results support the following hypothesis[13] that the presence of a large functional group in N-CBZ-L-amino acids hinders the interaction between ionic liquid and the stationary phase, thus minimizing the interfering effect of ionic liquid on the interaction between N-CBZ-L-amino acids and the stationary phase.

According to the results in Tables 2 and 3, L-amino acid is the less retained component (or low-affinity component), while N-CBZ-L-amino

Table 3. Retention factor values of L-methionine and N-CBZ-L-methionine[a] and the corresponding selectivity values with respect to the ionic liquid concentration

Ionic liquid concentration (mmol/L)	Retention factor (k)		Selectivity (α) L-methionine/ N-CBZ-L-methionine
	L-Methionine	N-CBZ-L-methionine	
0.0	0.373	1.176	3.156
0.5	0.235	1.173	4.991
2.0	0.130	1.166	8.943
3.0	0.111	1.161	10.441
5.0	0.094	1.184	12.616

[a]The concentrations of L-methionine and N-CBZ-L-methionine in a feed sample were 1.0 g/L each.

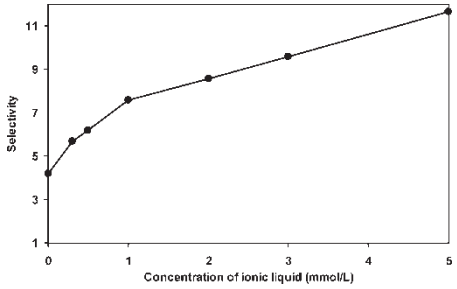

Figure 6. Effect of the ionic liquid concentration on the selectivity between L-phenylalanine (1.0 g/L) and N-CBZ-L-phenylalanine (1.0 g/L).

acid the more retained component (or high-affinity component). As the ionic liquid concentration increases, the retention factor of the less retained component shows a decreasing trend, whereas that of the more retained component remains almost the same as in the absence of ionic liquid. Hence, the use of ionic liquid as a mobile phase additive leads to the improvement of the selectivity between L-amino acid and N-CBZ-L-amino acid (Figures 6 and 7), which was also observed in the mixture of L-tyrosine and N-CBZ-L-tyrosine.

Effect of Ionic Liquid on the Peak Shapes of L-Amino Acids and N-CBZ-L-Amino Acids

To investigate the effect of ionic liquid on the peak shapes of L-amino acids and N-CBZ-L-amino acids, a series of experimental chromatograms were

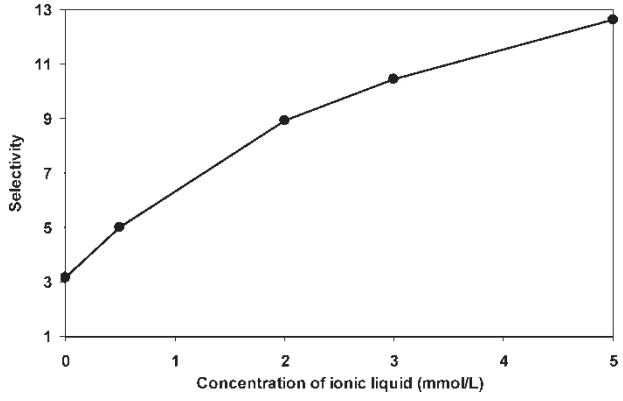

Figure 7. Effect of the ionic liquid concentration on the selectivity between L-methionine (1.0 g/L) and N-CBZ-L-methionine (1.0 g/L).

obtained by increasing the ionic liquid concentration. First, the chromatograms for the mixture of L-methionine and N-CBZ-L-methionine are arranged in the order of ionic liquid concentration and shown in Figure 8.

Note that the peak shape of N-CBZ-L-methionine remains almost unchanged regardless of ionic liquid concentration. On the contrary, a significant change is observed in the peak shape of L-methionine. In the absence of ionic liquid, the peak of L-methionine exhibits a quite asymmetric shape. Such asymmetry in peak shape, however, seems to vanish with the increase of ionic liquid concentration. The peak of L-methionine ultimately becomes symmetric when the ionic liquid concentration is 5 mmol/L (Figure 8). Such a series of peak shape transformations also affect the maximum peak concentration (C_{peak}^{max}) of L-methionine. It is easily seen that the C_{peak}^{max} becomes higher as the ionic liquid concentration increases and the peak

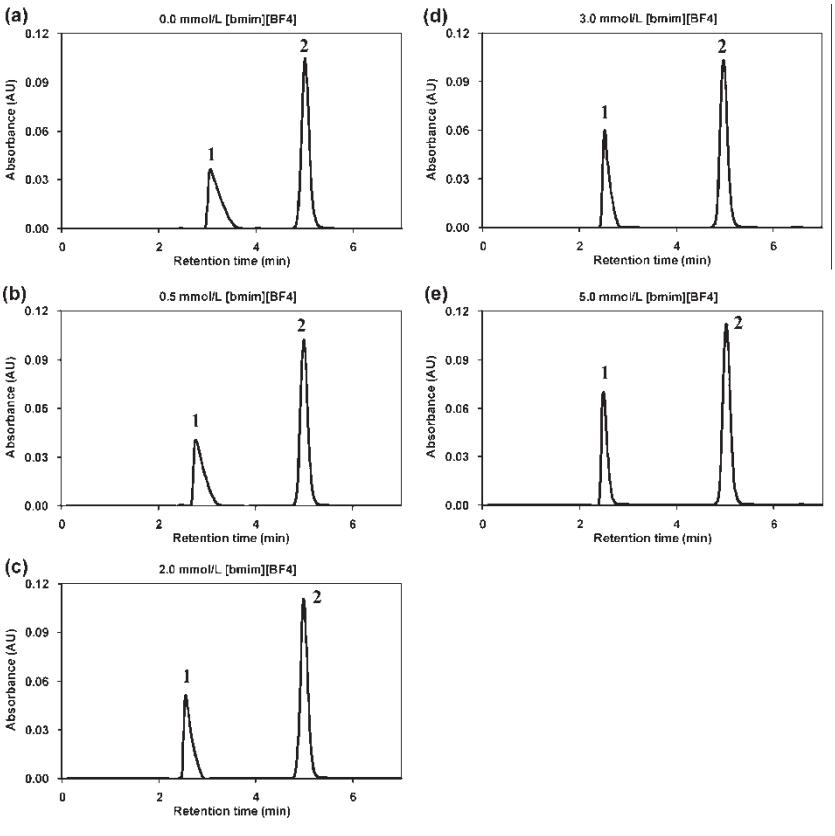

Figure 8. Chromatograms of L-methionine (1) and N-CBZ-L-methionine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 1.0 g/L, flow rate = 1 mL/min, loading volume = 50 μL, wave length = 254 nm.

shape is thus closer to a symmetric one. Compared to the C_{peak}^{max} under the absence of ionic liquid (Figure 8a), the C_{peak}^{max} under the presence of 5 mmol/L of ionic liquid (Figure 8e) is increased by about two times. This is mainly due to the peak compression, which is facilitated during the transformation processes of an asymmetric peak into a symmetric one. It is obvious that such peak compression is favorable for the separation between L-methionine and N-CBZ-L-methionine (Figure 8).

The experimental chromatograms for the mixture of L-phenylalanine and N-CBZ-L-phenylalanine are also arranged in the order of ionic liquid concentration and they are shown in Figure 9. One can see that the overall trend in peak shape transformations is similar to that for the mixture of L-methionine and N-CBZ-L-methionine (Figures 8 and 9). Only a little difference is observed when the ionic liquid concentration is beyond 2.0 mmol/L

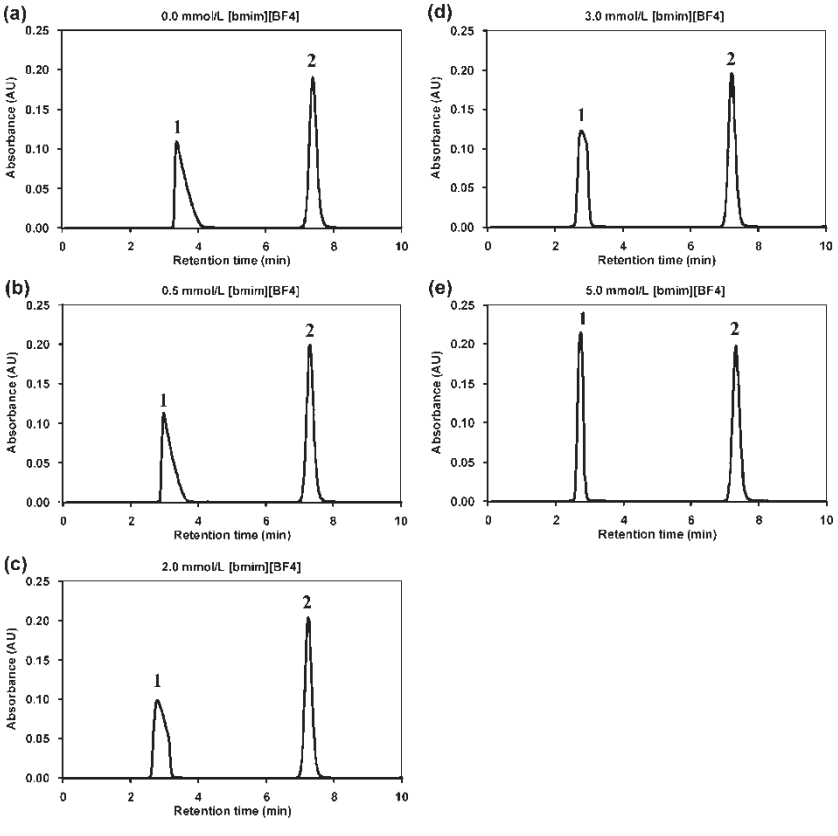

Figure 9. Chromatograms of L-phenylalanine (1) and N-CBZ-L-phenylalanine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 1.0 g/L, flow rate = 1 mL/min, loading volume = 50 μL, wave length = 254 nm.

(Figures 8c to 8e and 9c to 9e). The peak of L-methionine undergoes a gradual compression with the increase of ionic liquid concentration, leading to a gradual increase in the C_{peak}^{max} of L-methionine as shown in Figures 8c to 8e. By contrast, the peak of L-phenylalanine does not show a gradual compression but a sharp compression in a certain range of ionic liquid concentration, i.e., when the ionic liquid concentration is changed from 3.0 mmol/L to 5.0 mmol/L. In such a range of ionic liquid concentration, a significant increase in the C_{peak}^{max} of L-phenylalanine occurs as a result of peak compression, as shown in Figures 9d and 9e.

Coupled Effect of Ionic Liquid and Feed Concentration on the Separation of L-Phenylalanine and N-CBZ-L-Phenylalanine

Besides the addition of ionic liquid to the mobile phase, a change in feed concentration can also affect the selectivity and the peak shape. To examine such effect, a series of pulse tests with the mixture of L-phenylalanine and N-CBZ-L-phenylalanine were carried out by increasing the feed concentration while keeping the ionic liquid concentration constant. Such pulse tests were then repeated by varying the ionic liquid concentration.

From the pulse test results, the selectivity was estimated for each experimental condition. Figure 10 shows the resulting selectivity values as a function of feed concentration and ionic liquid concentration. As expected, the selectivity shows an increasing trend with the increase of ionic liquid concentration and such trend is maintained over the entire feed concentration range (Figure 10).

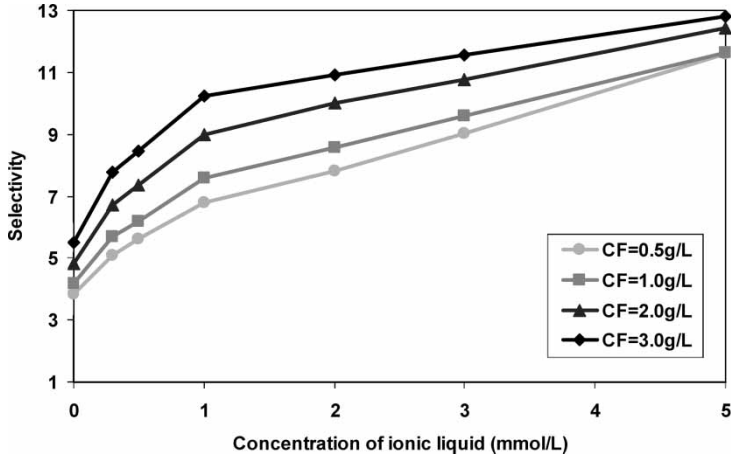

Figure 10. Coupled effect of the ionic liquid concentration and the feed concentration (C_F) on the selectivity between L-phenylalanine and N-CBZ-L-phenylalanine.

At a fixed ionic liquid concentration, the selectivity becomes higher as the feed concentration increases (Figure 10). Such effect of feed concentration on the selectivity is the most pronounced when the ionic liquid concentration is maintained at 1.0 mmol/L (Figure 10). Under such a condition, the selectivity is increased by about 60% if the feed concentration is changed from 0.5 g/L to 3.0 g/L.

To understand the above phenomenon, the two corresponding experimental chromatograms based on the feed concentrations of 0.5 g/L and 3.0 g/L under the same ionic liquid concentration are compared in Figure 11. Notice that the L-phenylalanine peak from a higher feed concentration is much more asymmetric than that from a lower feed concentration. This phenomenon implies that the peak asymmetry may result from a nonlinear adsorption behavior of L-phenylalanine on the stationary phase, which usually occurs under the overloaded condition such as high feed concentration or large feed volume.

In other words, all the L-phenylalanine molecules are not accessible to the adsorption sites on the stationary phase if the concentration of L-phenylalanine in a feed sample is too high. In that case, the adsorbed L-phenylalanine molecules, like ionic liquid, can hinder the interaction of the stationary phase with the remaining L-phenylalanine molecules in the mobile phase. Obviously, the hindered L-phenylalanine molecules will have shorter retention times than the adsorbed L-phenylalanine molecules. As a result, the L-phenylalanine peak becomes asymmetric, its average migration velocity increases, and its retention factor decreases (Table 4 and Figure 11). Since the retention factor of N-CBZ-L-phenylalanine is little

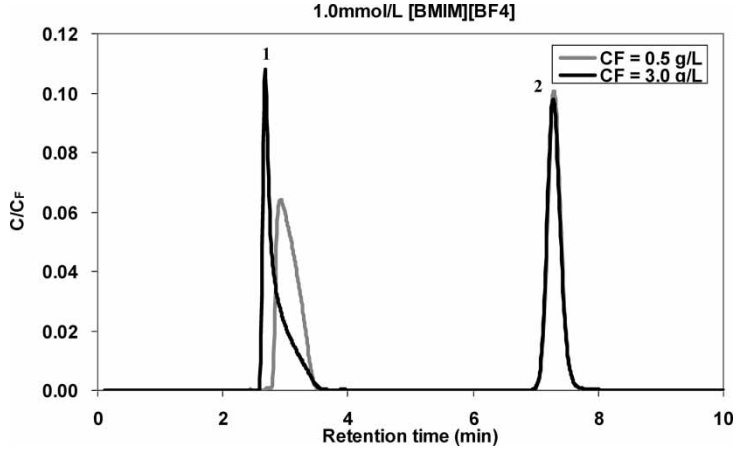

Figure 11. Comparison of the two experimental chromatograms based on the feed concentrations (C_F) of 0.5 g/L and 3.0 g/L. The feed sample is the mixture of L-phenylalanine (1) and N-CBZ-L-phenylalanine (2), and the ionic liquid concentration is maintained at 1.0 mmol/L.

Table 4. Retention factor values of L-phenylalanine and N-CBZ-L-phenylalanine and the corresponding selectivity values with respect to the ionic liquid concentration and the feed concentration

Ionic liquid concentration (mmol/L)	Retention factor (k)								Selectivity (α)			
	L-phenylalanine				N-CBZ-L-phenylalanine				L-phenylalanine/N-CBZ-L-phenylalanine			
	0.5 g/L	1.0 g/L	2.0 g/L	3.0 g/L	0.5 g/L	1.0 g/L	2.0 g/L	3.0 g/L	0.5 g/L	1.0 g/L	2.0 g/L	3.0 g/L
0.0	0.569	0.528	0.462	0.395	2.180	2.207	2.225	2.183	3.831	4.182	4.818	5.523
0.3	0.432	0.387	0.328	0.281	2.199	2.201	2.200	2.189	5.086	5.686	6.700	7.778
0.5	0.387	0.351	0.293	0.255	2.172	2.175	2.157	2.159	5.612	6.198	7.357	8.454
1.0	0.320	0.286	0.241	0.211	2.166	2.165	2.167	2.162	6.775	7.573	8.993	10.24
2.0	0.275	0.250	0.214	0.195	2.154	2.147	2.147	2.136	7.833	8.582	10.03	10.92
3.0	0.237	0.223	0.198	0.184	2.142	2.134	2.131	2.128	9.029	9.577	10.78	11.58
5.0	0.187	0.187	0.175	0.169	2.195	2.182	2.173	2.171	11.59	11.65	12.41	12.82

affected by feed concentration (Table 4 and Figure 11), the selectivity between L-phenylalanine and N-CBZ-L-phenylalanine becomes higher with the increase of feed concentration (Figure 10).

CONCLUSIONS

The effects of adding ionic liquid to the mobile phase on the retention behaviors of various amino acids were investigated with a reversed phase chromatographic column. The ionic liquid used was 1-butyl-3-methylimidazolium tetrafluoroborate, and the tested amino acid mixtures included L-tyrosine + N-CBZ-L-tyrosine, L-phenylalanine + N-CBZ-L-phenylalanine, and L-methionine + N-CBZ-L-methionine. The addition of ionic liquid to the mobile phase promoted a decrease in the retention behaviors of L-amino acids but had little effect on those of N-CBZ-L-amino acids. Higher selectivity can thus be attained between L-amino acid and N-CBZ-L-amino acid by increasing the content of ionic liquid in the mobile phase.

The effect of using ionic liquid on the peak shape was also investigated for the two mixtures of L-methionine + N-CBZ-L-methionine and L-phenylalanine + N-CBZ-L-phenylalanine. The peak shapes of N-CBZ-L-methionine and N-CBZ-L-phenylalanine remained almost the same as in the absence of ionic liquid. However, the peak shapes of L-methionine and L-phenylalanine were transformed from asymmetric ones to symmetric ones as the ionic liquid concentration increases. Such peak shape transformations were accompanied by peak compression, which in turn increased its maximum peak concentration and also improved the separation between L-amino acid and N-CBZ-L-amino acid.

In addition to the use of ionic liquid, the increase of feed concentration was also found to improve the selectivity between L-phenylalanine and N-CBZ-L-phenylalanine. This phenomenon occurred because high feed concentration led to the overloaded condition for L-phenylalanine. Since this condition facilitated the earlier elution of a portion of the L-phenylalanine molecules, the selectivity between L-phenylalanine and N-CBZ-L-phenylalanine was enhanced. Such effect of feed concentration on the selectivity was maximized under a certain ionic liquid concentration (1.0 mmol/L) instead of under the highest ionic concentration tested (5.0 mmol/L).

ACKNOWLEDGMENT

This work was supported by the ERC for Advanced Bioseparation Technology, KOSEF.

REFERENCES

1. Qi, S.; Cui, S.; Chen, X.; Hu, Z. Rapid and sensitive determination of anthraquinones in Chinese herb using 1-butyl-3-methylimidazolium-based ionic liquid with β-cyclodextrin as modifier in capillary zone electrophoresis. J. Chromatogr. A **2004**, *1059*, 191–198.
2. Fannin, A.A.; Floreani, D.A.; King, L.A.; Landers, J.S.; Piersma, B.J.; Stech, D.J.; Vaughn, R.L.; Wilkes, J.S.; Williarm, J.L. Properties of 1,3-dialkylimidazolium chloride aluminum-chloride ionic liquids. 2. phase-transitions, densities, electrical conductivities, and viscosities. J. Phys. Chem. **1984**, *88*, 2614–2621.
3. Hussey, C.L. Room-temperature haloaluminate ionic liquids–Novel solvents for transition–metal solution chemistry. Pure Appl. Chem. **1988**, *60*, 1763–1772.
4. Adams, C.J.; Earle, M.J.; Seddon, K.R. Catalytic cracking reactions of polyethylene to light alkanes in ionic liquids. Green Chem. **2000**, 21–23.
5. Anderson, J.L.; Ding, J.; Welton, T.; Armstrong, D.W.J. Characterizing ionic liquids on the basis of multiple salvation interactions. Am. Chem. Soc. **2002**, *124*, 14247–14254.
6. Wilkes, J.S.; Zaworotko, M.J. Air and water stable 1-ethyl-3-methylimidazolium based ionic liquids. J. Chem. Soc. Chem. Commun. **1992**, *13*, 965–967.
7. He, L.; Zhang, W.; Zhao, L.; Liu, X.; Jiang, S. 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.
8. Xiao, X.; Zhao, L.; Liu, X.; 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.
9. Shetty, P.H.; Youngberg, P.J.; Kersten, B.R.; Poole, C.F. Solvent properties of liquid organic salts used as mobile phases in microcolumn reversed phase liquid chromatography. J. Chromatogr. **1987**, *411*, 61–79.
10. 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.
11. Polyakova, Y.; Koo, Y.M.; Row, K.H. Application of ionic liquids as mobile phase modifer in HPLC. Biotechnol. Bioproc. Eng. **2006**, *11*, 1–6.
12. Mwongela, S.M.; Numan, A.; Gill, N.L.; Agbaria, R.A.; Warner, I.M. Separation of achiral and chiral analytes using polymeric surfactants with ionic liquids as modifiers in micellar electrokinetic chromatography. Anal. Chem. **2003**, *75*, 6089–6096.
13. Jin, Y.; Zheng, J.; Polyakova, Y.; Koo, Y.M.; Row, K.H. Influence of ionic liquid for separation of D-tryptophan and N-CBZ-D-phenylalanine. Korean Chem. Eng. Res. **2006**, *44* (95), 453–459.
14. Polyakova, Y.; Jin, Y.; Zheng, J.; Row, K.H. Effect of concentration of ionic liquid 1-butyl-3methylimidazolium, tetrafuoroborate, for retention and separation of some amino and nucleic acids. J. Liq. Chromatogr. & Rel. Technol. **2006**, *29*, 1687–1701.

Received April 30, 2007
Accepted May 18, 2007
Manuscript 6125