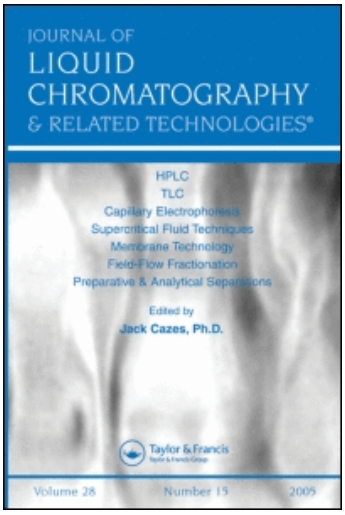

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Reversal of Elution Sequence and Selectivity Resulting from the Use of an Ionic Liquid as a Mobile Phase Modifier

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Reversal of Elution Sequence and Selectivity Resulting from the Use of an Ionic Liquid as a Mobile Phase Modifier

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Abstract: For the binary mixture of N-CBZ-L-amino acid + L-amino acid, the effects of using the ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]), as a mobile phase modifier on the elution sequence and the selectivity were investigated. The aqueous solution of 80% methanol by volume at pH 3.0 was employed as a mobile phase and a reversed-phase C_{18} media as a stationary phase. The use of ionic liquid as a mobile phase modifier caused the reversal of elution sequence for the aforementioned binary mixture when the ionic liquid concentration was increased beyond a certain value (or critical ionic liquid concentration). Such effect of ionic liquid gave rise to the occurrence of a minimum in the selectivity between the mixture components when the ionic liquid concentration reached the critical one. Below the critical ionic liquid concentration, the increase of the ionic liquid concentration reduced the selectivity. Beyond the critical ionic concentration, the selectivity was enhanced as the ionic liquid concentration increased. These results indicate the importance of determining a proper ionic liquid concentration if the ionic liquid is to be chosen as a mobile phase modifier.

Keywords: Ionic liquid, Elution sequence, Selectivity, Reversal, 1-Butyl-3-methylimidazolium tetrafluoroborate

INTRODUCTION

Exploiting a proper mobile phase modifier for higher resolution or selectivity has been an issue of great interest in a liquid chromatographic research area

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during the last few decades. Such an interest has been recently focused on the application of ionic liquids to a reversed-phase C_{18} chromatography, which has been proven effective in several previous studies.[1–5]

Ionic liquids are known to be a broad range of organic salts, whose melting points are close to room temperature.[6,7] These materials are composed of a bulky, asymmetric organic cation and a smaller inorganic anion.[6] The properties of ionic liquids favorable for application's sake include excellent salvation quality, low viscosity, non-volatility, high electrical conductivities, non-flammability, and chemical and thermal stability.[8–13] Ionic liquids have also been regarded as one of the important components in green chemistry.[14,15] To take advantage of such ionic liquid properties in a chromatographic separation area, an extensive effort has been made at elucidating the effect of ionic liquids on the retention behavior and the resultant selectivity as mentioned in the following.

He et al.[1] reported that the addition of ionic liquid to a mobile phase has an advantageous effect on the separation of polar compounds in a high performance liquid chromatography (HPLC) system. Shetty et al.[2] investigated how the presence of ionic liquids in a mobile phase affected the separation of amines. Jin et al.[4] 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.[5] used the same amino acids to investigate the impact of the ionic liquid concentration on the retention factors and the selectivity. Recently, Yoo et al.[16] made an extensive research on the role of ionic liquid in creating specific chromatographic behaviors of various amino acids.

According to all the aforementioned references, the increase of the ionic liquid content in a mobile phase leads to the broadening of the interval between two component peaks, thus improving the selectivity. No exceptions to such cases have been reported in the literature so far. However, the exceptions can be quite within the realms of possibility. This motivated us to explore the exception cases where the relationship between the ionic liquid content and the peak interval (or selectivity) represents a unique behavior that has never been reported in the literature.

For this purpose, we tested four amino acid mixtures while employing a reversed-phase C_{18} media and the ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]), as a stationary phase and a mobile phase modifier, respectively. The amino mixtures used included N-CBZ-L-tyrosine + L-tyrosine, N-CBZ-L-tryptophan + L-tryptophan, N-CBZ-L-phenylalanine + L-phenylalanine, and N-CBZ-L-methionine + L-methionine. Several different mobile phase conditions were tested in order to determine a proper mobile phase condition leading to the aforementioned exception cases. It was ultimately found in this study that, such interesting phenomena occurred when the aqueous solution of 80% methanol by volume at pH 3.0 was employed as a mobile phase.

Under such mobile phase condition, the use of ionic liquid as a mobile phase modifier brings about the reversal of elution sequence for the aforementioned binary mixture when the ionic liquid concentration is increased beyond a certain value (or critical ionic liquid concentration). Such effect of ionic liquid results in the lowest selectivity, i.e., a selectivity of unity when the ionic liquid concentration is equal to the critical one. Beyond the critical ionic concentration, the selectivity becomes higher as the ionic liquid concentration increases. These results indicate the gravity of determining a proper ionic liquid concentration if the ionic liquid is to be chosen as a mobile phase modifier.

Fundamental Principle of Interaction of Ionic Liquid with the Stationary Phase in a Reversed-Phase Column

The stationary phase of a reversed-phase chromatography contains two distinct functional groups, which play a vital role in creating a difference in the retention behaviors of different solute molecules.[15] One of the functional groups is the silanol group that attracts the polar groups of a solute molecule through specific electrostatic interactions (Figure 1). The other functional group is the alkyl group that attracts the alkyl groups of a solute molecule through hydrophobic interactions (Figure 1).

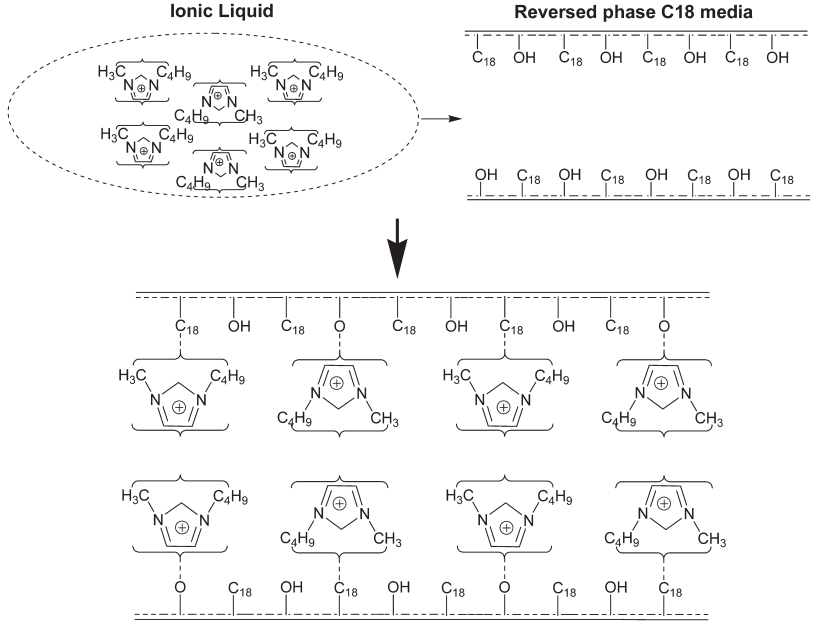

Figure 1. Mechanism of [bmim][BF4] interaction on modified silica surface.[1]

Once ionic liquid is added to a mobile phase, the aforementioned interaction behaviors can enter upon a new phase.[15] This is because 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). Such 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.[16] For this reason, a reduction in the retention times of solute molecules happens if ionic liquid is exploited as a mobile phase modifier in a reversed phase chromatography.

To quantify the effect 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:

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

where t_{void} is the hold-up time, t_R is the retention time, and k is the 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 factors of the two components to be separated ($k_1 \leq k_2$).

EXPERIMENTAL

Apparatus

The experiments were conducted with the HPLC system, which consisted of a HPLC pump (Waters 515), a photodiode array detector (Waters 996), and a Rheodyne injector with 20 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 (USA) was used to obtain distilled deionized water (DDW).

Reagents

The ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4], 99 % purity), was purchased from C-TRI Co. (Gyeonggi-do, Korea), and used as a mobile phase modifier. The amino acids used in this study were N-carbobenzyloxy-L-tyrosine (N-CBZ-L-tyrosine, 97% purity), L-tyrosine (99% purity), N-carbobenzyloxy-L-tryptophan (N-CBZ-L-tryptophan, 99% purity), L-tryptophan (98% purity), N-carbobenzyloxy-L-phenylalanine (N-CBZ-L-phenylalanine, 99% purity), L-phenylalanine (99% purity), N-carbobenzyloxy-L-methionine (N-CBZ-L-methionine, 97% purity), and L-methionine (99% purity) (Figure 2) from Sigma-Aldrich Co. (St. Louis, MO, USA). Mobile phase was prepared by mixing DDW and methanol, which was purchased from J.T. Baker Company (USA). The percentage of methanol in the mobile phase was 80% by volume. Hydrochloric acid was purchased from Samchun Pure Chemical Co. (Gyeonggi-do, Korea) and used to adjust pH of the mobile phase.

Methods

A different content of ionic liquid ([bmim][BF_4]) was added to the mobile phase (methanol: DDW = 80:20 (v/v)), 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 7.0 mmol/L of [bmim][BF_4]. Experiments were carried out at room temperature, and the flow rate of each experiment was 1.0 mL/min.

A series of pulse tests with the amino acid mixtures under investigation were carried out: (mixture of N-CBZ-L-tyrosine (0.1 g/L) + L-tyrosine (0.1 g/L), mixture of N-CBZ-L-tryptophan (0.2 g/L) + L-tryptophan (0.2 g/L), mixture of N-CBZ-L-phenylalanine (1.0 g/L) + L-phenylalanine (1.0 g/L), and mixture of N-CBZ-L-methionine (1.0 g/L) + L-methionine (1.0 g/L)). Effluent from the column was monitored using a photodiode array detector (Waters 996). For the injections of the mixtures of N-CBZ-L-tyrosine + L-tyrosine and N-CBZ-L-methionine + L-methionine, the column effluents were monitored at the wave lengths of 280 nm and 240 nm, respectively. The other amino acid mixtures tested were detected at 254 nm.

RESULTS AND DISCUSSION

Quantitative evaluation of the ionic liquid effect in this study was based on the estimation of a change in the retention factor and selectivity due to the addition of the ionic liquid [bmim][BF4] to the mobile phase. For such an estimation, the retention time of each component was measured from its effluent

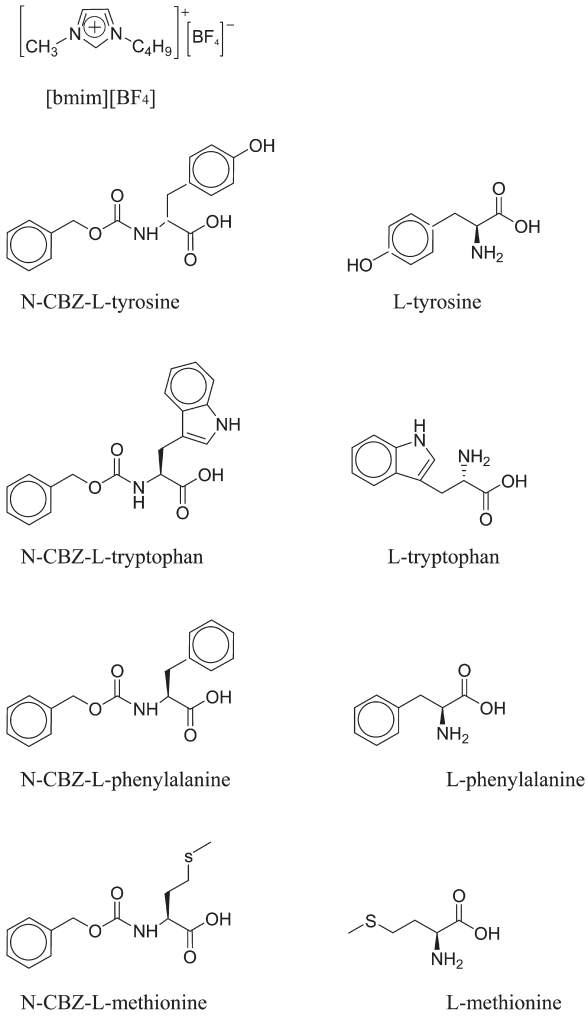

Figure 2. Chemical structures of amino acids and ionic liquid.

chromatogram, followed by plugging the measured value into Eqs. (1) and (2). The retention time is defined here as the time that the peak maximum occurs in the effluent chromatogram.

Effect of Ionic Liquid on the Chromatographic Behaviors for the Mixture of N-CBZ-L-tyrosine + L-tyrosine

For the mixture of N-CBZ-L-tyrosine + L-tyrosine, a series of pulse injection experiments (or pulse tests) were carried out using an aqueous solution

(methanol/DDW = 80/20 (v/v)) at pH 3 as a mobile phase. The experiments began with the pulse test based on the absence of ionic liquid in the mobile phase. Then, the concentration of ionic liquid in the mobile phase was gradually increased to examine the effect of ionic liquid on the retention behaviors of the amino acids under investigation.

First, the retention factors of N-CBZ-L-tyrosine and L-tyrosine were estimated in each pulse test based on different ionic liquid concentration, and the results are presented in Table 1 and Figure 3. We see that the addition of ionic liquid to the mobile phase has a quite different effect on the two components. As the ionic liquid concentration in the mobile phase increases, the retention factor of N-CBZ-L-tyrosine exhibits a slight variation, whereas that of L-tyrosine decreases sharply. These aspects are also well demonstrated in Figure 4, where a series of the experimental chromatograms are arranged in the order of the ionic liquid concentration in the mobile phase. The numbers 1 and 2 in the figures indicate N-CBZ-L-tyrosine and L-tyrosine, respectively. It is easily seen that the exit time of the N-CBZ-L-tyrosine peak is almost unaffected by a change in the ionic liquid concentration, whereas the L-tyrosine peak exits the column earlier with an increase in the ionic liquid concentration.

Figure 4 also shows another interesting phenomenon, which is explained minutely in the following. In a low range of the ionic liquid concentration, the two peaks are closer to each other with an increase in the ionic liquid concentration as shown in Figures 4a to 4c. Eventually, the two peaks are merged into a seemingly single peak when the ionic liquid concentration is increased to 1.0 mmol/L (Figure 4d). Beyond such an ionic liquid concentration, the single peak is split into two peaks again. At this time, it should be note that the elution sequence of the two split peaks is reversed compared to the case

Table 1. Retention factor values of N-CBZ-L-tyrosine and 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)
	N-CBZ-L-tyrosine	L-tyrosine	L-tyrosine/N-CBZ-L-tyrosine
0.0	0.200	0.540	2.702
0.3	0.189	0.352	1.860
0.5	0.191	0.300	1.568
1.0	0.192	0.192	1.000
2.0	0.195	0.105	1.867
3.0	0.196	0.066	2.987
7.0	0.198	0.017	11.974

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

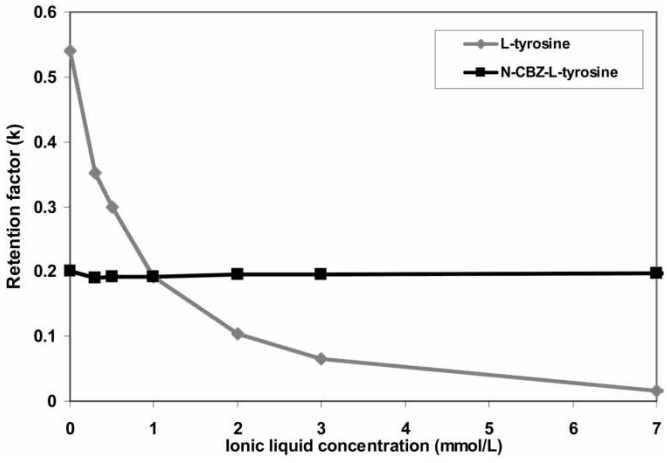

Figure 3. Effect of the ionic liquid concentration on the retention factor of N-CBZ-L-tyrosine and L-tyrosine.

of the ionic concentration below 1.0 mmol/L. The interval between the two reversed peaks becomes broader again as the ionic liquid concentration increases. These results indicate that the selectivity between the two amino acids can reach its minimum value at a certain ionic liquid concentration (Figure 5). Such a trend in the selectivity, together with the reversal of elution sequence, has never been explored in previous studies.

The aforementioned phenomena can be interpreted from the viewpoint of the molecular structure of each component as follows. In the case of N-CBZ-L-tyrosine, it has such a large carbobenzyloxy group as to hinder the interactions between silanol groups of the stationary phase and the cation portion of ionic liquid. By contrast, L-tyrosine is devoid 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. For this reason, 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. Such interpretation is likely to be well founded considering the relevant case in a previous publication by Jin et al.,[1–6] where the effect of ionic liquid on the separation of N-CBZ-D-phenylalanine and D-tryptophan was explained in a similar manner.

Effect of Ionic Liquid on the Chromatographic Behaviors for the Mixture of N-CBZ-L-tryptophan + L-Tryptophan

In the previous section, the reversal of elution sequence was observed for the mixture of N-CBZ-L-tyrosine + L-tyrosine in the course of varying the ionic

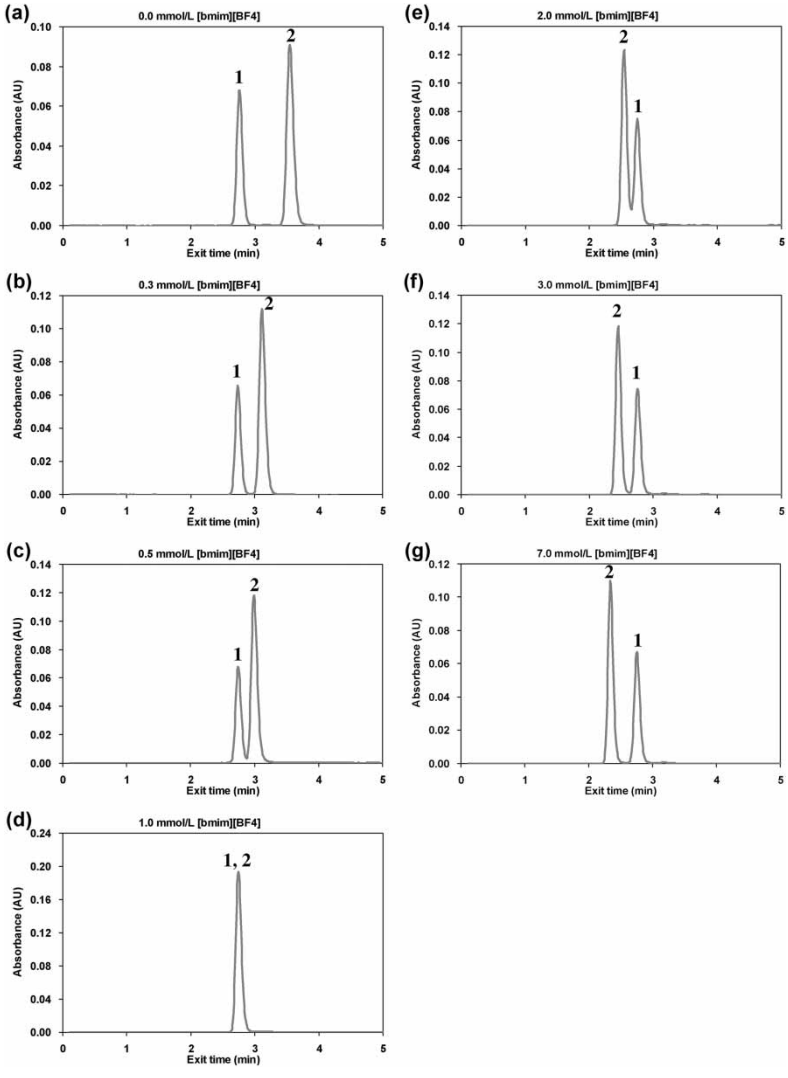

Figure 4. Chromatograms of N-CBZ-L-tyrosine (1) and L-tyrosine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 0.1 g/L, flow rate = 1 mL/min, loading volume = 20 μL, wave length = 280 nm.

liquid content in the mobile phase. To check whether such a trend is also true of other amino acids, the mixture of N-CBZ-L-tryptophan + L-tryptophan was tested first under the same mobile phase condition as before.

Figure 6 presents a series of the experimental chromatograms resulting from the injections of N-CBZ-L-tryptophan and L-tryptophan while increasing the

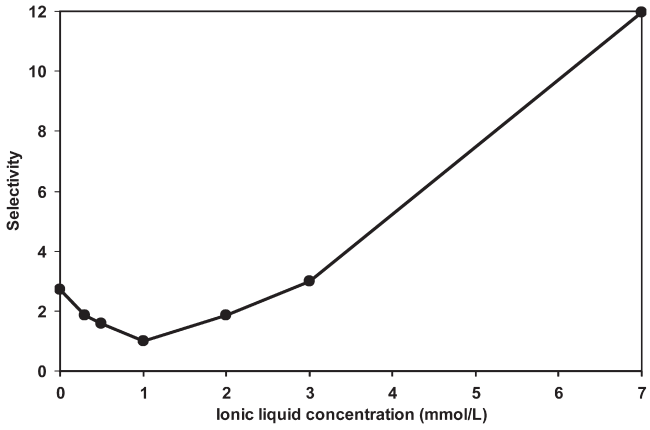

Figure 5. Effect of the ionic liquid concentration on the selectivity between N-CBZ-L-tyrosine and L-tyrosine.

ionic liquid concentration. The overall chromatographic behavior shows a similar pattern to that in the previous section except for the following observation. As shown in Figure 6c, the reversal of elution sequence for the mixture of N-CBZ-L-tryptophan + L-tryptophan occurs at the ionic liquid concentration of 0.5 mmol/L, which is lower than that for the mixture of N-CBZ-L-tyrosine + L-tyrosine (Figure 4d). The major reason for such a phenomenon stems from the occurrence of a narrower interval between the N-CBZ-L-tryptophan and L-tryptophan peaks than between the N-CBZ-L-tyrosine and L-tyrosine peaks in the absence of ionic liquid (compare Figure 4a with 6a). The crossover of the N-CBZ-L-tryptophan and L-tryptophan peaks thus occur earlier than that of the N-CBZ-L-tyrosine and L-tyrosine peaks, when the ionic liquid concentrations in the two cases are increased at the same rate (compare Figure 4d with 6c).

The aforementioned distinction between the peak intervals for the two mixtures, which happens in the absence of ionic liquid, may be due to a relative difference in the polarity between the two components. Judging from the literature[17] and the molecular structure, it may be inferred that the difference between the polarities of N-CBZ-L-tryptophan and L-tryptophan is smaller compared to the difference between the polarities of N-CBZ-L-tyrosine and L-tyrosine. In the case of a reversed phase chromatographic column, the smaller the difference between the polarities of two components, the narrower the interval between two peaks in the effluent profile. From this point of view, the N-CBZ-L-tryptophan and L-tryptophan peaks are certainly closer to each other under the circumstances devoid of ionic liquid than the N-CBZ-L-tyrosine and L-tyrosine peaks. The former, therefore, undergo crossover at a lower ionic liquid concentration than the latter (compare Table 1 with 2).

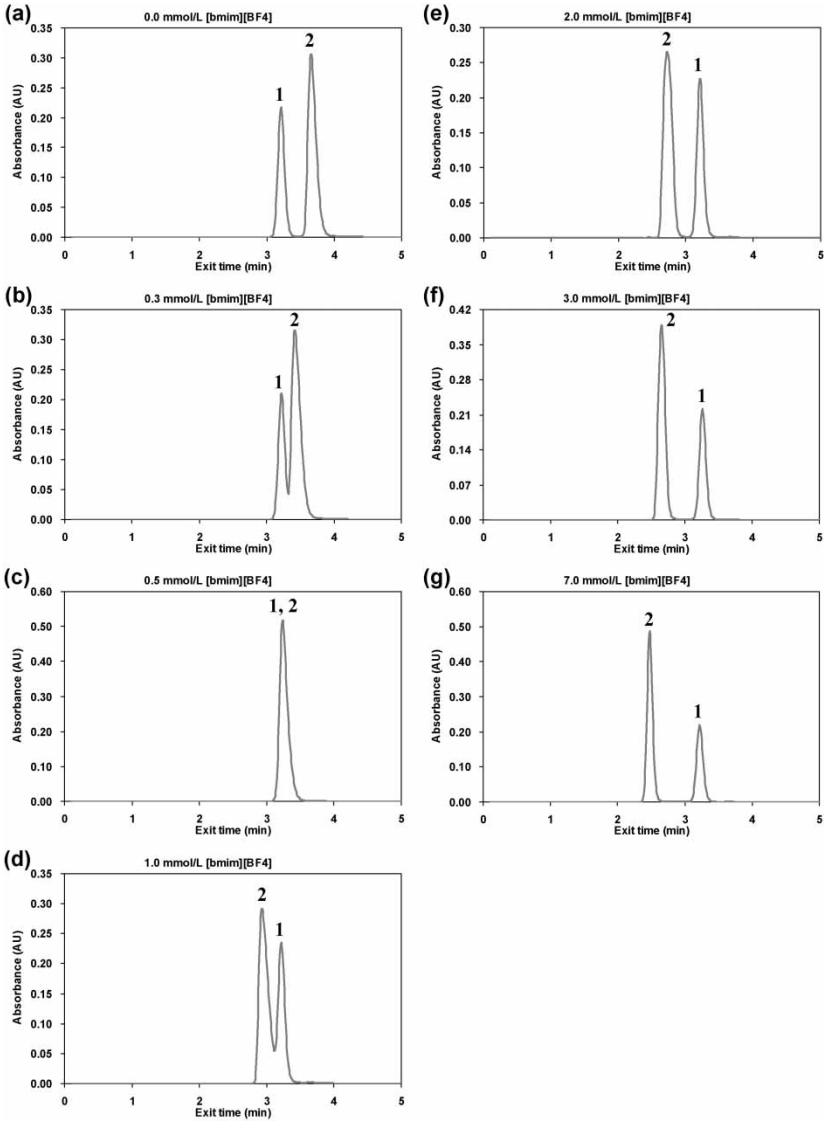

Figure 6. Chromatograms of N-CBZ-L-tryptophan (1) and L-tryptophan (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 0.2 g/L, flow rate = 1 mL/min, loading volume = 20 μL, wave length = 254 nm.

For the reasons above, a minimum in the selectivity between N-CBZ-L-tryptophan and L-tryptophan occurs at a lower ionic liquid concentration than between N-CBZ-L-tyrosine and L-tyrosine (compare Figures 5 and 7). As mentioned in the previous section, the reversal of elution sequence and

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

Ionic liquid concentration (mmol/L)	Retention factor (k)		Selectivity (α) L-tryptophan/ N-CBZ-L-tryptophan
	N-CBZ-L-tryptophan	L-tryptophan	
0.0	0.395	0.587	1.486
0.3	0.400	0.486	1.217
0.5	0.405	0.405	1.000
1.0	0.399	0.272	1.466
2.0	0.393	0.183	2.147
3.0	0.416	0.152	2.740
7.0	0.398	0.076	5.246

[a]The concentrations of N-CBZ-L-tryptophan and L-tryptophan in a feed sample were 0.2 g/L each.

the occurrence of a minimum in the selectivity, as a result of the ionic liquid application, are reported for the first time in this article. No previous researches have dealt with such an issue.

Effect of Ionic Liquid on the Chromatographic Behaviors for the Mixtures of N-CBZ-L-phenylalanine + L-Phenylalanine and N-CBZ-L-methionine + L-Methionine

Subsequently to the mixture of N-CBZ-L-tryptophan + L-tryptophan tested above, other two mixtures such as N-CBZ-L-phenylalanine + L-phenylalanine

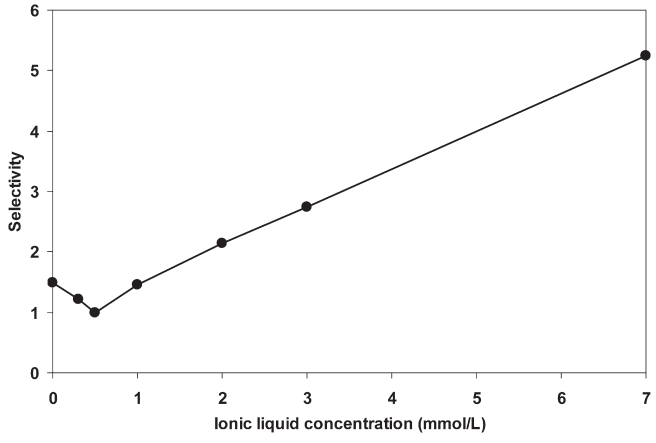

Figure 7. Effect of the ionic liquid concentration on the selectivity between N-CBZ-L-tryptophan and L-tryptophan.

and N-CBZ-L-methionine + L-methionine are investigated in this section as to the reversal of elution sequence and the occurrence of a minimum in the selectivity, due to the addition of ionic liquid to the mobile phase.

The experimental results for the pulse tests with the aforementioned two mixtures are presented in Figures 8 and 9, respectively. Like the mixtures tested in the previous sections, the mixtures of N-CBZ-L-phenylalanine + L-phenylalanine and N-CBZ-L-methionine + L-methionine represent the phenomenon of an overlap and crossover between the two corresponding peaks in its effluent profile when the ionic liquid concentration is gradually increased (Figure 8 and 9). As a result, the elution sequences of the two peaks in each mixture are reversed at the ionic liquid concentrations of 0.3 mmol/L and 1.0 mmol/L, respectively, as shown in Figures 8b and 9d.

A change in the peak shape, together with the reversal of elution sequence, is also worthy of attention as one of the phenomena connected with the role of ionic liquid in this study. As shown in Figure 8, the L-phenylalanine peak is asymmetric in the absence of ionic liquid but gradually closer to a symmetric peak as the ionic liquid concentration increases. Eventually, the peak becomes almost symmetric when the ionic concentration is beyond 3.0 mmol/L. A similar trend is also observed in the shape of the L-methionine peak, whose transformation process with respect to the ionic liquid concentration is well demonstrated in Figure 9. The asymmetry observed in the L-methionine peak gradually vanishes accordingly as the ionic liquid concentration increases. The symmetric peak is attained when the ionic liquid concentration is increased, such that the elution sequence between L-methionine and N-CBZ-L-methionine is completely reversed.

Comparison of the Ionic Liquid Effects on the Selectivities of all the Amino Acid Mixtures Tested

For all the amino acid mixtures tested, the changes in the selectivities resulting from the use of ionic liquid are compared in Figure 10. It is obvious that for each mixture, a selectivity of unity, i.e., the lowest selectivity occurs at a certain ionic liquid concentration, which is defined here as a critical ionic concentration. According to the comparison results in Figure 10, the critical ionic concentration for the mixture of N-CBZ-L-tyrosine + L-tyrosine is the highest among the four mixtures tested. This implies that the addition of ionic liquid to the mobile phase may be ineffective in improving the selectivity between N-CBZ-L-tyrosine and L-tyrosine, if the amount of the added ionic liquid is relatively small.

Beyond the critical ionic concentration, the selectivity of each mixture again shows an increasing trend with respect to the ionic liquid concentration (Figure 10, Tables 1–4), which is similar to the trends reported in previous studies.[5,15,16] Among the mixtures of comparison in Figure 10, the highest

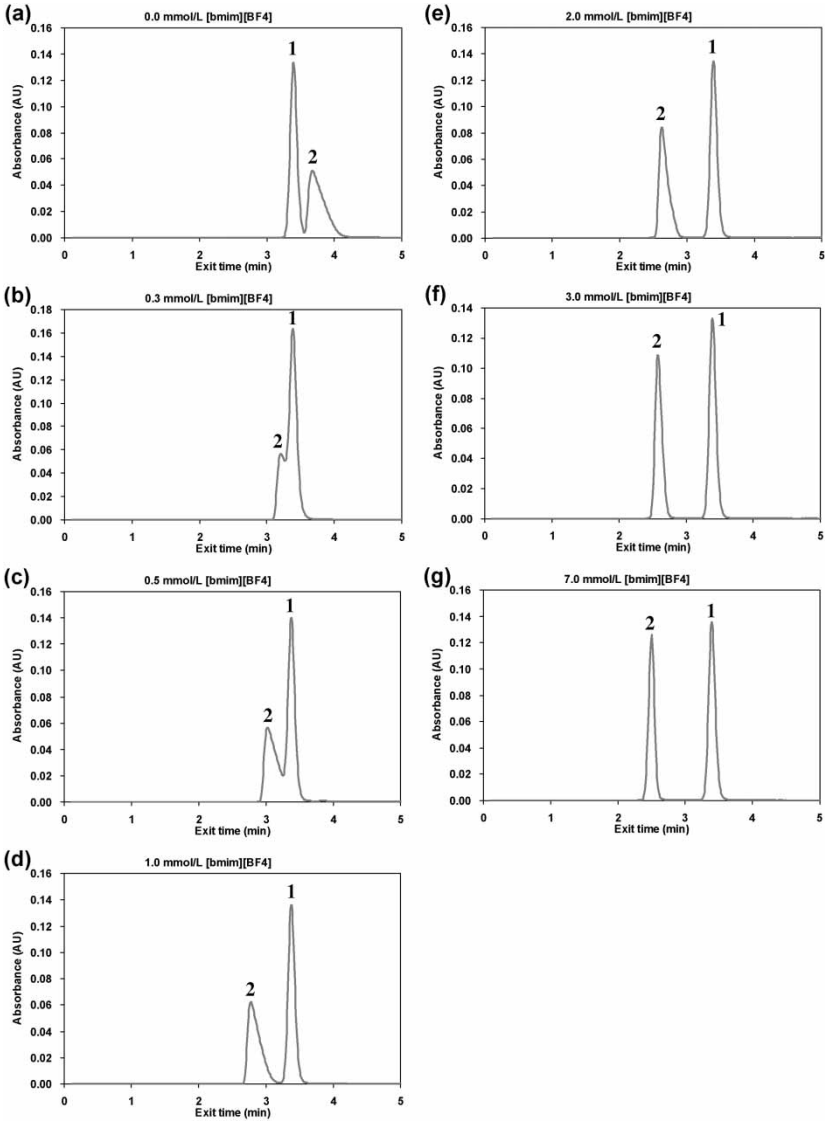

Figure 8. Chromatograms of N-CBZ-L-phenylalanine (1) and L-phenylalanine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 1.0 g/L, flow rate = 1 mL/min, loading volume = 20 μL, wave length = 254 nm.

rate of selectivity increase with respect to the ionic concentration beyond the critical one is observed in the mixture of N-CBZ-L-tyrosine + L-tyrosine. This is mostly attributed to the following phenomenon that the retention factor of L-tyrosine undergoes a sharp reduction with increasing the ionic liquid

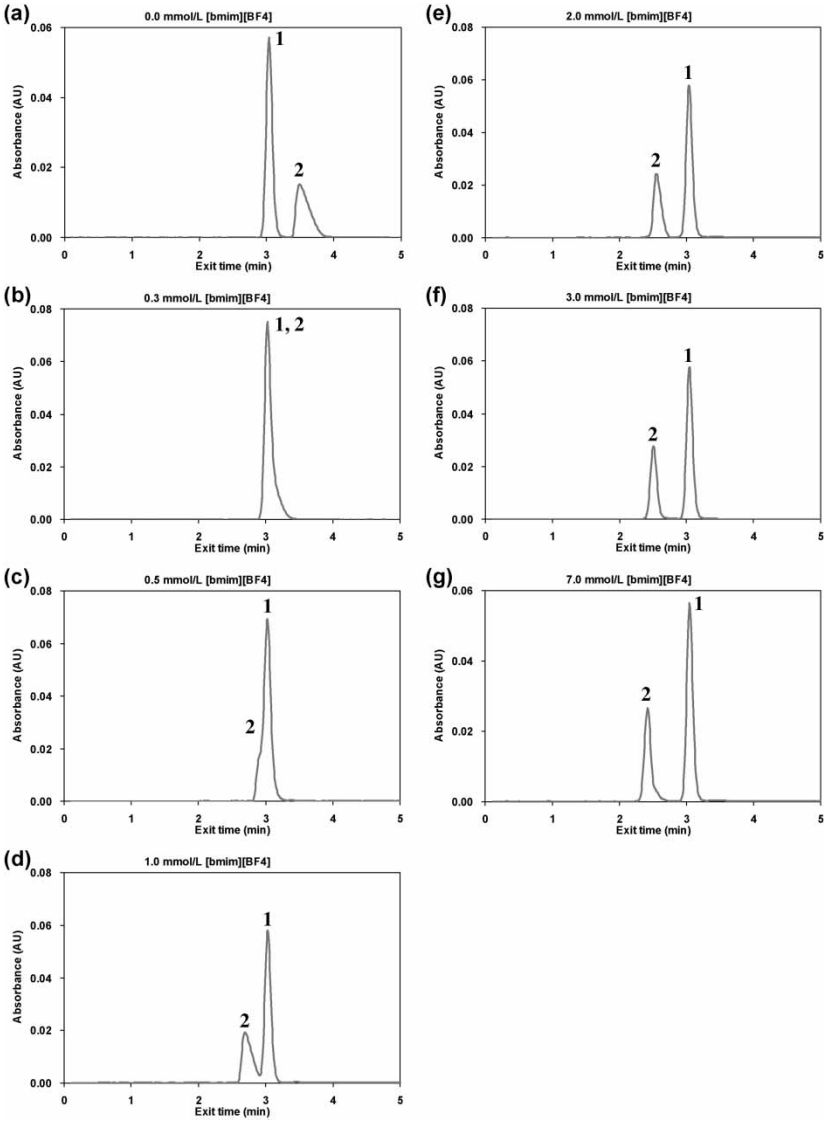

Figure 9. Chromatograms of N-CBZ-L-methionine (1) and L-methionine (2) under different concentrations of [bmim][BF4] in the mobile phase. The experimental conditions are as follows: feed concentration = 1.0 g/L, flow rate = 1 mL/min, loading volume = 20 μL, wave length = 240 nm.

concentration (Table 1), which in turn leads to a rapid increase in the selectivity as expected from Eq. (2).

Among the results presented above, what deserves the most emphasis is indeed the existence of a critical ionic liquid concentration leading to the

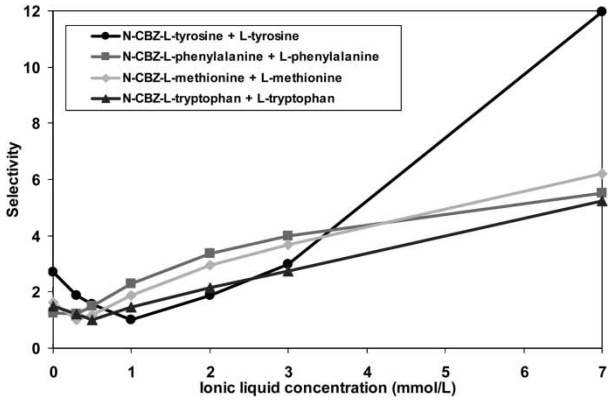

Figure 10. Comparison of the ionic liquid effects on the selectivities of all the amino acid mixtures tested.

lowest selectivity. It is, therefore, worth discussing which factors bring about such a phenomenon. The principal factor may be the different effects of ionic liquid on L-amino acid and N-CBZ-L-amino acid, which arise from the difference between the molecular structures of the two components. As stated previously, the elution time of L-amino acid is reduced by increasing the ionic liquid concentration, whereas that of N-CBZ-L-amino acid is little affected by the ionic liquid. However, this factor alone cannot elucidate the occurrence of a critical ionic concentration. One more factor needs to be mentioned, which may be closely connected with the elution sequence of each component in the mobile phase devoid of ionic liquid. If the employed mobile phase condition creates an earlier elution of L-amino acid than

Table 3. Retention factor values of N-CBZ-L-phenylalanine and 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)
	N-CBZ-L-phenylalanine	L-phenylalanine	L-phenylalanine/ N-CBZ-L-phenylalanine
0.0	0.475	0.594	1.252
0.3	0.471	0.391	1.204
0.5	0.467	0.312	1.499
1.0	0.465	0.204	2.277
2.0	0.475	0.142	3.353
3.0	0.474	0.119	3.993
7.0	0.474	0.086	5.505

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

Table 4. Retention factor values of N-CBZ-L-methionine and 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)
	N-CBZ-L-methionine	L-methionine	L-methionine/ N-CBZ-L-methionine
0.0	0.318	0.517	1.628
0.3	0.315	0.315	1.000
0.5	0.315	0.268	1.177
1.0	0.317	0.169	1.881
2.0	0.318	0.109	2.932
3.0	0.324	0.088	3.670
7.0	0.324	0.052	6.208

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

N-CBZ-L-amino acid in the absence of ionic liquid, the interval between the two component peaks will increase continuously with increasing the ionic liquid concentration. In this case, a minimum in the selectivity with respect to the ionic liquid concentration does not happen, resulting in the non-existence of a critical ionic liquid concentration. By contrast, if the mobile phase condition used facilitates a later elution of L-amino acid than N-CBZ-L-amino acid in the absence of ionic liquid, the interval between the two component peaks will decrease first and then increase with increasing the ionic liquid concentration. Such trend gives rise to the occurrence of a minimum in the selectivity and thus the existence of a critical ionic liquid concentration. These results indicate the importance of determining a proper ionic liquid concentration if the ionic liquid is to be chosen as a mobile phase modifier.

CONCLUSION

For a binary amino acid mixture migrating through the reversed phase chromatographic column, the effects of using the ionic liquid as a mobile phase modifier on the elution sequence and the selectivity were investigated. The mobile phase employed for this study was the aqueous solution of 80% methanol by volume with a pH of 3.0. The ionic liquid used was 1-butyl-3-methylimidazolium tetrafluoroborate, and the four feed samples tested were comprised of N-CBZ-L-amino acid and L-amino acid as follows: (1) N-CBZ-L-tyrosine + L-tyrosine, (2) N-CBZ-L-tryptophan + L-tryptophan, (3) N-CBZ-L-phenylalanine + L-phenylalanine, and (4) N-CBZ-L-methionine + L-methionine.

In the absence of ionic liquid, L-amino acid was eluted later than N-CBZ-L-amino under the employed mobile phase condition. Such an elution sequence is

mostly due to the difference in the polarity between the two components. As the content of the ionic liquid added to the mobile phase was increased, the retention behaviors of N-CBZ-L-amino acid and L-amino acid showed quite different behaviors, which may be ascribed to the difference between the molecular structures of the two components. According to the results, increasing the ionic liquid concentration promoted a reduction in the retention time of L-amino acid but had little effect on that of N-CBZ-L-amino acid. The two component peaks were therefore closer to each other as the ionic liquid concentration increased. Eventually, the two component peaks underwent overlap and crossover when the ionic liquid concentration reached its critical value. Beyond the critical ionic concentration, the elution sequences of the two peaks were reversed and the interval between such reversed peaks was increased with an increase in the ionic liquid concentration.

Due to the aforementioned effect of ionic liquid, the selectivity between N-CBZ-L-amino acid and L-amino acid showed its minimum value (unity) when the ionic liquid concentration reached the critical one. While the ionic liquid concentration is below the critical one, the increase of the ionic liquid concentration had an undesirable effect on the selectivity. Beyond the critical ionic concentration, the selectivity became higher as the ionic liquid concentration increased. These results indicate the gravity of determining a proper ionic liquid concentration if the ionic liquid is to be chosen as a mobile phase modifier.

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