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ORIGINAL ARTICLE

Partitioning of acidic, basic and neutral amino acids into imidazolium-based ionic liquids

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Abstract In this paper, partitioning behaviors of typical neutral (Alanine), acidic (Glutamic acid) and basic (Lysine) amino acids into imidazolium-based ionic liquids [C_4mim][PF_6], [C_6mim][PF_6], [C_8mim][PF_6], [C_6mim][BF_4] and [C_8mim][BF_4] as extracting solvents were examined. [C_6mim][BF_4] showed the best efficiency for partitioning of amino acids. The partition coefficients of amino acids in ionic liquids were found to depend strongly on pH of the aqueous solution, amino acid and ionic liquid chemical structures. Different chemical forms of amino acids in aqueous solutions were pH dependent, so the pH value of the aqueous phase was a determining factor for extraction of amino acids into ionic liquid phase. Both water content of ionic liquids and charge densities of their anionic and cationic parts were important factors for partitioning of cationic and anionic forms of amino acids into ionic liquid phase. Extracted amino acids were back extracted into phosphate buffer solutions adjusted on appropriate pH values. The results showed that ionic liquids could be used as suitable modifiers on the stationary phase of an HPLC column for efficient separation of acidic, basic, and neutral amino acids.

Keywords Neutral amino acid · Acidic amino acid · Basic amino acid · Ionic liquid · Partition coefficient · Back extraction

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Introduction

Amino acids are the building blocks of proteins, as indispensable agents of biological function, which can be obtained by biosynthesis or from protein hydrolysis. Amino acids are amphoteric compounds that can be classified into the following categories: (1) neutral (uncharged) amino acids, (2) acidic amino acids (which have a net negative charge at neutral pH), and (3) basic amino acids (which have a net positive charge at neutral pH). All amino acids have more than one ionizable group that can exist in cationic, anionic, and zwitterionic forms depending on the pH of the aqueous solution (Garrett and Grisham 1999). The study of the pH-dependent partitioning of amino acids is of interest not only for a better understanding of a variety of metabolic processes in biological membranes but also for devising efficient separation and extraction processes (Wang et al. 2005; Ku et al. 2007; Smirnova et al. 2004). During the last century, amino acids partitioning into various phases including reverse micelles and organic solvents have been studied (Nozaki and Tanford 1971; Leodidis and Alan Hatton 1990; Leodidis et al. 1991; Gude et al. 1996; Xun et al. 2001), but little studies on the amino acid partitioning into ionic liquid (IL) phases have been reported (Wang et al. 2005; Smirnova et al. 2004).

Room temperature ionic liquids (RTIL's) or IL's have been composed of an organic cation and either an organic or an inorganic anion. Because of stabilities against air and water, negligible vapor pressures, wide liquid temperature ranges, chemical and thermal stabilities, nonflammability, high-ionic conductivities, wide electrochemical potential window (Welton 1999), and as well as multiple solvation interactions (Anderson et al. 2002); IL's are emerging as alternative green solvents. Therefore, RTIL's are regarded to be used as solvent in

chemical reactions (Sharifi et al. 2008; Shaabani et al. 2007; Mallakpour and Kowsari 2006; Iranpoor et al. 2006), organometallic chemistry (Sievers et al. 2006; Iranpoor et al. 2008), batteries and fuel cells investigations (Sekhon et al. 2006).

In recent years, IL's have also aroused increasing applications in liquid–liquid extractions of inorganic and organic species (Berthod et al. 2005; Absalan et al. 2008). IL's can be hydrophilic and hydrophobic depending on the chemical structures of cations and anions (Suarez et al. 1998; Holbrey and Seddon 1999) but it seems that the anionic part of an ionic liquid is more important for determining its water miscibility (Huddleston et al. 2001). Those IL's based on bis [(trifluoromethyl)sulfonyl] amide ($[Tf_2N]^-$), hexafluorophosphate ($[PF_6]^-$), and tetrafluoroborate ($[BF_4]^-$) are normally water immiscible, therefore, they are selected as solvents for forming biphasic systems in most IL extraction applications(Absalan et al. 2008; Soto et al. 2005). While this property is largely conveyed by the hydrophobicity of the anion, length of the alkyl chain or the temperature of the system are also effective parameters for governing water miscibility of the RTIL's (Suarez et al. 1998; Holbrey and Seddon 1999).

In this study some imidazolium-based IL's: $[C_4mim][PF_6]$, $[C_6mim][PF_6]$, $[C_8mim][PF_6]$, $[C_6mim][BF_4]$ and $[C_8mim][BF_4]$ have been used to study partitioning behaviors of neutral (Alanine), acidic (Glutamic acid) and basic (Lysine) amino acids (Harris 2003) into ionic liquid phase as to the best of our knowledge this investigation has not been studied so far.

Materials and methods

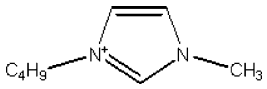
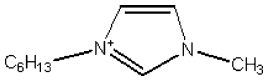
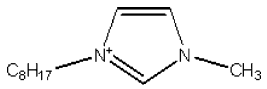
Instruments

UV absorption spectra were recorded against the solvent blank at room temperature, using Ultrospec 4000 spectrophotometer (Pharmacia Biotech) operated in double-beam mode. The pH measurements were made with a Metrohm 780 pH meter using a combined glass electrode. NMR spectra were recorded on a Brucker-Advanced DPX/250 (^{1}H NMR 250 MHZ and ^{13}C NMR 62.9 MHZ) spectrophotometer.

Reagents

1-Bromobutane, 1-bromohexane, 1-bromooctane, 1-methylimidazolium, ammonium hexafluorophosphate, sodium tetrafluoroborate, Alanine, Glutamic acid, Lysine dihydrate, ninhydrin, hydrindantin dihydrate, and other reagents used in this study were of the highest purity available from either Merck or Fluka Chemical Companies and were used without further purification. Ionic liquids (Table 1) $[C_4mim][PF_6]$, $[C_6mim][PF_6]$, $[C_8mim][PF_6]$, $[C_6mim][BF_4]$ and $[C_8mim][BF_4]$ were synthesized as described by Bonhote et al. (1996) and their chemical structures were testified by using NMR spectroscopy. Distilled deionized water was used throughout this work.

Table 1 Chemical structures of cationic constituents of the studied ionic liquids

Cations	Abbreviation
 1-Butyl-3-methylimidazolium	$[C_4mim]^+$
 1-Hexyl-3-methylimidazolium	$[C_6mim]^+$
 1-Octyl-3-methylimidazolium	$[C_8mim]^+$

Software

In this study, molar volumes (cm^3/mol) of cationic and anionic part of IL's were calculated by using Gaussian03 program after optimization of their geometries at density functional level using B3LYP method and 6-311 ++G** basis set and then charge density (e/Å^3), charge ratio to molecular volume, of each cationic or anionic part was calculated.

Procedure

Aqueous solutions containing 1.0×10^{-3} M of amino acids (0.5 ml) were brought into contact with 50 µl of IL at room temperature for extraction purpose. Back extractions were performed into phosphate buffer solutions (0.5 ml) at appropriate pH values. In both extraction and back extraction processes, the ionic strengths of aqueous solutions were kept constant using 0.1 M KCl for all pH values. The phase-contacting experiments were carried out in a carefully stoppered glass test tube at room temperature. The system was vigorously stirred with magnetic stirrer for 30 min and then two phases were carefully separated using a centrifuge device. The

partition coefficients of amino acids between the IL phase and aqueous solution were calculated as: $P_{IL/W} = (C_i - C_f)\ V_{aq}/C_f V_{IL}$ where C_i and C_f refer to the initial and final concentrations of each amino acid in aqueous phase; V_{aq} and V_{IL} refer to the aqueous and IL phase volumes, respectively. Partition coefficients of amino acids in IL's were measured in duplicate by ninhydrine method (Sun et al. 2006).

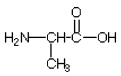
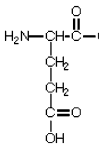
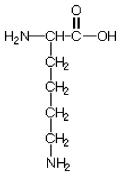
Results and discussion

Effect of amino acid chemical structure and pH

Partitioning of amino acids into IL phase for various forms of amino acids depends on pH of the aqueous solution. Amino acids in the pH's less than pK_a (Table 2) of their carboxylic acid groups are in the cationic or dicationic forms; with the pH increasing, carboxylic acid and ammonium groups start to be dissociated in order, depending on acidic-basic property of amino acids. So various forms of an amino acid exist in aqueous solution and therefore different interactions are expected that must be considered when the partitioning of amino acid between IL's and aqueous solution is studied.

As it is seen in Table 3, partition coefficient of Alanine (Ala) is high in the pH's less than pK_a (=2.348) of its carboxylic acid group. In these pH values, cationic form of Ala is dominant because of its protonated amine group. Electrostatic interaction of cationic form of Ala with the anionic part of IL's is the driving force for higher partitioning of Ala into IL phase at these pH values (Wang et al. 2005). With the pH increasing, carboxylic acid group starts dissociation, zwitterionic form is generated, and Ala partitioning is decreased because of diminishing of electrostatic interactions of Ala and IL's due to the development of a net charge of zero on the amino acid molecule. Moreover, carboxylic acid group dissociation causes to increase hydrophilic property of Ala leading to its lower partition coefficient. This decrease in partition coefficient of Ala is continued up to its isoelectric pH value, i.e. 6.100, after that the partition coefficient is increased again. After the isoelectric point, pH increasing causes dissociation of ammonium group and consequently generation of the anionic form of Ala that finally predominate in the pH's more than pK_a (=9.867) of ammonium group. Since the anionic form of Ala has a higher hydrophobicity than its zwitterionic form and also due to its electrostatic interaction with the cationic part of IL's, a higher partitioning of Ala into IL phase is expected. It is noticeable that electrostatic interaction of cationic form of Ala with anionic part of IL's seems to be stronger than electrostatic interaction of anionic form of Ala with cationic part of IL's. To prove this, charge densities of both cationic and anionic parts of all studied IL's were calculated (Table 6). It is apparent in Table 6 that charge density of the anionic part

Table 2 Chemical structures of the amino acids and their dissociation constants at 25°C

Amino acid	Chemical structure	Carboxylic acid (pK_a)	Ammonium (pK_a)	Substituent (pK_a)	pI
Alanine		2.348	9.867	–	6.100
Glutamic acid		2.230	9.950	4.420	3.320
Lysine		2.040	9.080	10.690	9.880

pI, the pH of isoelectric point

Table 3 Partition coefficients of Alanine between aqueous solutions, with different pH values and diverse ionic liquid phases

Ionic liquid	pH													
	1.39	1.50	2.50	3.50	4.50	5.50	6.10	6.50	7.50	8.50	9.50	10.50	10.82	11.50
$[C_6mim][BF_4]$	2.830	2.740	1.800	0.860	0.450	0.200	0.094	0.100	0.250	0.420	0.530	0.650	0.665	0.700
$[C_8mim][BF_4]$	2.400	2.250	1.430	0.680	0.260	0.090	0.066	0.070	0.130	0.200	0.280	0.360	0.390	0.450
$[C_4mim]$ $[PF_6]$	0.420	0.320	0.200	0.140	0.080	0.074	0.065	0.070	0.200	0.260	0.340	0.530	0.540	0.600
$[C_6mim][PF_6]$	0.058	0.051	0.010	0.008	ND	ND	ND	ND	ND	ND	ND	0.008	0.009	0.010
$[C_8mim][PF_6]$	0.021	0.016	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Experimental conditions: aqueous solution, 0.5 ml of 1.0×10^{-3} M alanine solution with different pH values; IL phase volume, 50 μl; extraction period, 30 min; KCl, 0.1 M

ND not detected

Table 4 Partition coefficients of Glutamic acid between aqueous solutions, with different pH values and diverse ionic liquid phases

Ionic liquid	pH													
	1.27	1.50	2.50	3.32	3.50	4.50	5.36	6.50	7.50	8.50	9.50	10.50	10.90	11.50
$[C_6mim][BF_4]$	2.11	1.93	1.500	0.780	0.700	0.340	0.090	0.100	0.170	0.370	0.400	0.460	0.507	0.530
$[C_8mim][BF_4]$	1.93	1.820	1.300	0.210	0.120	0.080	0.010	0.040	0.120	0.210	0.250	0.310	0.324	0.360
$[C_4mim]$ $[PF_6]$	0.350	0.020	0.150	0.125	0.100	0.060	0.017	0.060	0.150	0.270	0.330	0.400	0.430	0.440
$[C_6mim][PF_6]$	0.041	0.010	0.008	0.007	0.006	ND	ND	ND	ND	ND	ND	ND	0.005	0.008
$[C_8mim][PF_6]$	0.015	0.007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Experimental conditions: aqueous solution, 0.5 ml of 1.0×10^{-3} M Glutamic acid solution with different pH values; IL phase volume, 50 μl; extraction period, 30 min; KCl, 0.1 M

ND not detected

of an IL is higher than the charge density of its cationic part. Therefore, it can effectively provide a stronger electrostatic interaction with cationic form of amino acid. The results are more partitioning of amino acid in acidic pH values where the cationic form of amino acid is dominated. For example, partition coefficient of Ala in $[C_6mim][BF_4]$ at pH 1.39 is 2.830 which is higher than its partition coefficient at pH 11.5 that is 0.700.

As it is found in Table 4, Glutamic acid (Glu), an amino acid with a substituted acidic group, has the highest partition coefficient in pH's less than pK_a (=2.230) of its carboxylic acid group. In these pH values, dominant form of Glu is its cationic form, with two undissociated carboxylic acid groups, so that its electrostatic interaction with anionic constituent of IL is driving force for extraction of Glu into ionic liquid phase. With pH increasing, carboxylic acid group starts dissociation, zwitterionic form is generated and partitioning of Glu is decreased. The result is due to formation of less hydrophobic species of Glu with a net charge of zero that consequently have low tendency toward extraction into IL phase. With more pH increasing, substituted carboxylic acid group of Glu (with $pK_a = 4.420$) begins to be dissociated, another anionic group is generated, hydrophobicity is decreased and partitioning of Glu is more diminished. At pH values higher than 5.36, ammonium group dissociation causes generation of a dianionic form of Glu with a more lypophilic tendency. This consequently is a driving force for more partitioning of the amino acid at pH values more than 5.36. It is remarkable that chemical interaction of cationic form of Glu (which are dominated at pH values less than pK_a of its carboxylic acid group) with the IL's is the strongest and this results in a highest partitioning of Glu. For example, the partition coefficient of cationic form of Glu at pH 1.27 in $[C_6mim][BF_4]$ is 2.11 which is higher than the partition coefficient of its anionic form at pH 11.5, i.e. 0.530. Again, the charge density of the anionic part of the ionic liquid, as discussed for Ala, could be considered as a logical criterion for interpretation of the observed data.

As it is shown in Table 5, Lysine (Lys) partitioning into ionic liquid phase is the most in the pH's less than pK_a (=2.040) of its carboxylic acid group. In these pH values, dicationic form of Lys with undissociated carboxylic acid group is dominant form; therefore, the electrostatic interaction of Lys with IL's is expected to be higher. By pH increasing up to the value of 4.5, decrease in partition coefficients because of the generation of less hydrophobic cationic form of Lys, due to its carboxylic acid group dissociation, is observed. With the more pH increasing, ammonium group starts to be dissociated so that the hydrophobicity and consequently partition coefficients are increased. It should be mentioned that at

Table 5 Partition coefficients of Lysine between aqueous solutions, with different pH values and diverse ionic liquid phases

Ionic liquid	pH													
	1.08	1.50	2.50	3.00	3.50	4.50	5.50	6.50	7.50	8.50	9.88	10.50	11.50	11.65
$[C_6mim][BF_4]$	2.704	2.600	1.300	0.067	0.070	0.200	0.320	0.370	0.400	0.400	0.460	0.500	0.610	0.622
$[C_8mim][BF_4]$	2.215	2.190	0.970	0.005	0.007	0.025	0.040	0.050	0.054	0.065	0.073	0.250	0.350	0.361
$[C_4mim]$ $[PF_6]$	0.370	0.250	0.100	ND	ND	ND	0.010	0.010	0.030	0.047	0.067	0.340	0.570	0.580
$[C_6mim][PF_6]$	0.048	0.020	0.008	ND	ND	ND	ND	ND	ND	ND	ND	0.007	0.010	0.015
$[C_8mim][PF_6]$	0.017	0.010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Experimental conditions: aqueous solution, 0.5 ml of 1.0×10^{-3} M Lysine solution with different pH values; IL phase volume, 50 µl; extraction period, 30 min; KCl, 0.1 M

ND not detected

pH = *p*I = 9.88, zwitterionic form of Lys with a more hydrophobic character is dominant, the result of which is observing higher partition coefficients (Table 5). By increasing pH to values more than *p*I, hydrophobicity property still increases due to the dissociation of the substituted ammonium group; this leads to stronger interactions between Lys and IL's and consequently higher partition coefficients are obtained (Table 5). It could be concluded that any difference in partitioning behavior of amino acids into IL phase can definitely be related to their different substituted chains rather than definitive skeleton groups common to all amino acids (Leodidis and Alan Hatton 1990). Both carboxylate and ammonium groups provide more hydrophilic character for amino acids in contrast to their conjugated forms, i.e. carboxylic acid and amine groups, respectively (Gulyaeva et al. 2003), that must be considered as additional factors for discussing the obtained results. The presence of these chemical forms of amino acids in aqueous solutions are pH dependent so the pH value of the aqueous phase determines the quantity of the extraction of amino acids into IL phase.

Effect of chemical structure of the ionic liquids

The behaviors of IL's for extraction of amino acids are dependent on both the chemical forms of amino acids and the chemical structures of IL's (Tables 3, 4, 5). It is also anticipated that solubility of water in IL's and charge densities of cationic and anionic parts of IL's in interaction with different forms of amino acids could show significant effects on the amino acids partitioning into IL phase. In the pH values that amino acids are in their cationic or dicationic forms, partition coefficients directly depend on the miscibility of water in IL's (i.e. the water content of IL). Literature survey (Freire et al. 2007) shows that water contents of IL's decrease in the order of $[C_6mim][BF_4] > [C_8mim][BF_4] > [C_4mim][PF_6] > [C_6mim][PF_6] > [C_8mim][PF_6]$. It has been reported (Cammarata et al. 2001) that in imidazolium-based IL's, water molecules (in the concentration range of 0.2–1.0 mole of water per liter of IL) are mostly in the free (not self-associated) state, bounded via H-bond to PF_6^-, BF_4^-. The water content and the strength of hydrogen bonding between water molecules and BF_4^- in BF_4^--based IL's has been shown to be more than PF_6^--based IL's. Therefore, presence of water partially disrupts the ion-pair association between cationic and anionic constituents of the IL's. This increases the number of free ions of constitution in the IL phase. This situation would consequently increase the electrostatic interactions between the cationic forms of the amino acids and the anion of the IL's.

Table 6 Calculated charge density of ionic parts of studied ionic liquids

Ionic part of ionic liquids	Charge density (e/Å^3)
1-Butyl-3-methylimidazolium	0.00541
1-Hexyl-3-methylimidazolium	0.00372
1-Octyl-3-methylimidazolium	0.00306
PF_6^-	0.00941
BF_4^-	0.01471

In addition to water content, charge densities and effective negative charges of anionic part of IL's for interpreting their electrostatic interactions with cationic form of an amino acid, are important factors to be considered. Calculated charge density of BF_4^- is higher than PF_6^- anion (Table 6). Quantum chemical calculations also indicated that the effective negative charge (Tsunekawa et al. 2003) in BF_4^- is much more than that in PF_6^-. Therefore, stronger electrostatic interaction between the cationic forms of an amino acid and BF_4^-, and consequently a higher extraction into BF_4^--based IL's, is expected. It is concluded that water contents of IL's and charge densities of their anionic parts make cationic and dicationic forms of amino acids to partition into IL phase in the same manner.

In the pH ranges that amino acids are in their anionic or dianionic forms, water content and charge density of cationic form of IL's are driving forces for partitioning of amino acids into IL phase. As it is shown in Tables 3, 4, 5, the order of IL's for partitioning of anionic or dianionic forms of amino acids is $[C_6mim][BF_4] > [C_4mim][PF_6] > [C_8mim][BF_4] > [C_6mim][PF_6] > [C_8mim][PF_6]$; which is not consistent with the order of charge densities of cationic parts of IL's and the order of their water contents. Order of IL's, based on charge density of their cationic part is $[C_4mim][PF_6] > [C_6mim][PF_6] = [C_6mim][BF_4] > [C_8mim][PF_6] = [C_8mim][BF_4]$ and the order of IL's based on their water contents was already mentioned in the previous paragraph. Thus, in some cases, parameter of charge density of cationic part of IL's governs the order of partition coefficients of anionic forms of amino acids, and in the other cases, the inverse is true. This observation can be verified where $[C_6mim][BF_4]$ with a lower charge density of its cationic part but a higher water content than $[C_4mim][PF_6]$ has shown a higher partitioning for anionic forms of amino acids; and $[C_4mim][PF_6]$ with a higher charge density of its cationic part but a lower water content than $[C_8mim][BF_4]$ has shown a higher partitioning for anionic forms of amino acids. In addition, despite of lower cationic charge density of $[C_8mim][BF_4]$ than $[C_6mim][PF_6]$, partition coefficients of anionic forms of amino acids are higher into the former which is in agreement with the more water content of $[C_8mim][BF_4]$. Finally, Partitioning of anionic forms of amino acids into $[C_8mim][PF_6]$ phase is the least because of its least water content and its lower cationic charge density which shows that the effects of its water content and its cationic charge density on the partitioning of anionic forms of amino acids is almost the same.

Partitioning of zwitterionic forms of amino acids into ionic liquid phase is almost in order of $[C_6mim][BF_4] > [C_8mim][BF_4] \geq [C_4mim][PF_6] > [C_6mim][PF_6] > [C_8mim][PF_6]$. Water content of an IL is a parameter that could be considered for explanation of the above observation.

Extraction mechanism of amino acids into IL phase

An ion-exchange mechanism was proposed for extraction of amino acids into the IL phase. The following equilibria show the schemes of this type of exchanges:

$$\text{Scheme 1}: [RNH_3]^+_{(aq)} + [C_6mim]^+[BF_4]^- \rightleftarrows [C_6mim]^+_{(aq)} + [RNH_3]^+[BF_4]^-$$

$$\text{Scheme 2}: [RCO_2]^-_{(aq)} + [C_6mim]^+[BF_4]^- \rightleftarrows [BF_4]^-_{(aq)} + [C_6mim]^+[RCO_2]^-$$

where $[RNH_3]^+_{(aq)}$ and $[RCO_2]^-_{(aq)}$ refer to the cationic and anionic forms of an amino acid in aqueous solution, respectively. $[RNH_3]^+[BF_4]^-$ and $[C_6mim]^+[RCO_2]^-$ refer to the extracted amino acids into the ionic liquid phase in cationic and anionic forms, in order. To prove the validity of the proposed mechanism, some experimental works were performed for extraction of amino acids into $[C_6mim][BF_4]$ at pH values that cationic or anionic forms of the tested amino acids were the dominant species while the ionic strength was kept constant.

In the case of cationic forms of the amino acids, the partition coefficients were decreasing when increasing amounts of $[C_6mim]^+Br^-$ were added to the aqueous sample solutions which proves that the equilibrium shown in Scheme 1 was shifted to left as the result of a cation-exchange process. Similar changes in partition coefficients of anionic forms of the amino acids were observed when different amounts of $NaBF_4$ were added to the aqueous sample solutions which certified the occurrence of an anion-exchange process. A similar mechanism has been reported in the synthesis of some amino acid ionic liquids (Ohno and Fukumoto 2007; Plaquevent et al. 2008; Chen et al. 2008).

Back extraction of amino acids

In order to know how amino acids can be back extracted from IL phase, three amino acids were individually extracted into $[C_6mim][BF_4]$ phase in the pH values less than pK_a of the corresponding carboxylic acid group. Then every amino acid was back extracted into an aqueous solution adjusted with an appropriate pH value. The pH of aqueous solution was the value at which a special ionic form of the amino acid was in its dominant form, i.e. with a fraction of 0.9. It seems (Table 7) that the back extraction efficiency of each amino acid is lower at pH values given the higher partition coefficient. After back extraction, the IL phase has been used for three more extraction protocol that shows IL is recyclable.

Conclusions

It was found that electrostatic interactions of cationic and anionic sites of IL's with anionic and cationic forms of amino acids, respectively, resulted in partitioning of amino acids into IL phase. It is noticeable that electrostatic interactions of anionic part of IL's with cationic forms of amino acids are stronger than electrostatic interactions of cationic part of IL's with anionic forms of amino acids which causes higher partitioning of cationic forms of amino acids into IL phase. In particular, it was shown that

Table 7 Back extraction of amino acids from $[C_6mim][BF_4]$ phase into aqueous solutions with different pH values

(a) Alanine (was extracted into IL at pH 1.39)				
pH	1.39	6.10	10.82	
Back extraction (%)	ND	41.30	12.40	
Partition coefficient	ND	13.00	21.60	
(b) Glutamic acid (was extracted into IL at pH 1.27)				
pH	1.27	3.32	5.36	10.90
Back extraction (%)	ND	24.00	53.50	17.00
Partition coefficient	ND	18.04	8.90	21.02
(c) Lysine (was extracted into IL at pH 1.08)				
pH	1.08	3.00	9.88	11.65
Back extraction (%)	ND	60.00	32.70	15.20
Partition coefficient	ND	6.91	15.35	20.76

Experimental conditions: aqueous solution, 0.5 ml of 1.0×10^{-3} M amino acid solution with different pH values (a: 1.39, b: 1.27, c: 1.08); KCl 0.1 M IL phase, 50 μl; extraction period, 30 min; phosphate buffer solution, 1.0×10^{-3} M; KCl 0.1 M; back extraction period, 30 min

ND not detected

the chemical structures and chemical properties of side chains of amino acids explained the specific behavior of any amino acid for partitioning into IL phase. It was shown that extraction of amino acids into IL phase followed an ion-exchange mechanism and back-extraction efficiency of each amino acid, at a specific pH value, was inversely related to the value of its partition coefficient at that pH. With regard to the different partitioning behaviors of amino acids into IL's we are going to use IL's as suitable modifiers on stationary phase of HPLC column to separate acidic, basic and neutral amino acids from each other.

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