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Functional amino acid ionic liquids as solvent and selector in chiral extraction

Fei Tang, Qianli Zhang, Dandan Ren, Zhou Nie, Qian Liu, Shouzhuo Yao*

State Key Lab of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, Hunan, China

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ABSTRACT

Amino acid ionic liquids (AAILs) have received great attention due to their potentials in catalysis and separations. In this work, functional AAILs were used as solvent and selector in chiral liquid–liquid extraction for the first time. The AAILs have shown distinct enantioselectivity in amino acid extraction. Using these functional AAILs as acceptor phase and ethylacetate as donor phase, more L-enantiomer of amino acid was extracted into the ionic liquid phase than that of D-enantiomer. The influencing factors, including AAILs structure, copper ion concentration, organic phase and amino acid concentration, were investigated. We found that the enantioselective enrichment of racemic amino acids was achieved through a chiral ligand-exchange mechanism. The enantioselectivity of single-step extraction was up to enantiomeric excess value of 50.6%. Moreover, the functional AAILs were found to be efficient extraction solvents for amino acids. The logarithm of distribution coefficient for L-Phe was in the range of 3.4–3.6 in the ionic liquid–ethylacetate two-phase system. This liquid–liquid extraction approach may extend the application of ionic liquids in chiral separations.

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1. Introduction

In the past decade, ionic liquids (ILs) have received great attention in various fields owing to their potential as green solvent alternatives to conventional organic solvents [1–4]. Currently, the new research interest of ILs is focusing on their tunable physical and chemical properties. The design of ILs with functional cation or anion for specific needs, so called task-specific ILs, is booming [5]. One interesting example is the design and synthesis of task-specific ILs for chiral separations [6–10].

Chiral separation is an important subject in science as well as in technology. Various chiral selectors, such as cyclodextrins, metal complexes, micelles, antibiotics and crown ethers have been widely used in liquid–liquid extractions because of their chiral recognition abilities [11]. However, the application of many current chiral selectors is often limited due to their low solubility, difficult synthesis, thermal instability as well as high cost [12]. In addition, most of selectors need to be dissolved in other solvents or in a solvent system as work solution. Therefore, using chiral ionic liquids simultaneously as solvent and chiral selector is convenient and promising [13]. The chiral ionic liquids have been applied as chiral media in GC [10], CE [14], NIR spectrometry [15], membrane separation [16,17] and enantioselective reaction [9].

In contrast, with other chiral separation methods, liquid–liquid extraction has the potential to be applied in continuous separations and production scale separations [18–23]. This approach involves the extraction of one enantiomer into an acceptor phase by selective coordination with a selector and the uncomplexed enantiomer left in the donor phase. The attraction of this approach is that it can circumvent the crystallization of diastereomeric salts, the time consuming step in production scale separation [24]. As green extractants, ionic liquids have been used to extract metal ions [25], organic molecules [26] and biological macromolecules [27] by the liquid–liquid extraction method.

Some pioneering work has used ionic liquids to extract amino acids. Wang et al. used several hydrophobic ionic liquids to recover amino acids from the aqueous solution [28]. Dibenzo-18-crown-6 ether was added into 1-butyl-3-methylimidazolium hexafluorophosphate [Bmim][PF_6] to improve the extraction of Trp, Phe and His from acidic aqueous solution [29]. Using ionic liquid/supercritical carbon dioxide as media, the chiral separation of amino acid was achieved by a lipase-catalyzed esterification [30]. Moreover, a commercial polyvinylidene fluoride membrane was impregnated with 1-octyl-3-methylimidazolium hexafluorophosphate [Omim][PF_6] for transporting amino acids and amino esters [31]. These work indicated that ionic liquids as extraction solvents have tremendous potential in amino acid chiral separation [32].

AAILs are a class of novel chiral ionic liquids derived from amino acids [33–37]. They are promising platforms for functional ILs due to their low cost, biocompatibility [32], ease of chemical modification [38–41] and excellent chiral stability [42]. AAILs have

* Corresponding author. Tel.: +86 0731 88821968.
E-mail addresses: tf192@sina.com (F. Tang), szyao@hnu.cn (S. Yao).

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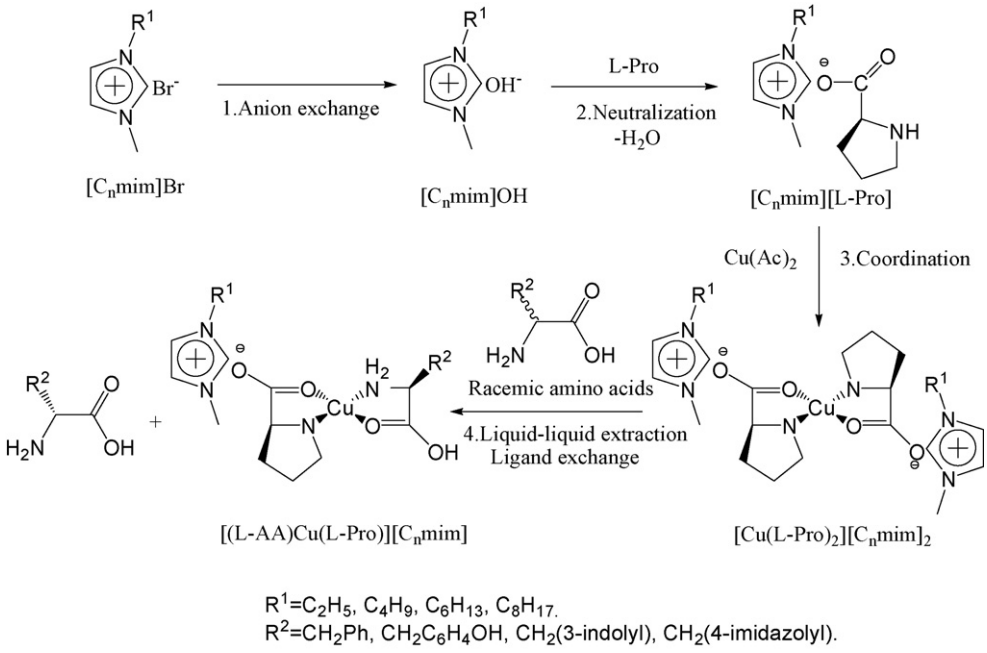

Fig. 1. Scheme of preparation functional AAILs for chiral extraction.

been proposed for use in phase separation, critical temperature separation, metal scavenging and heterogeneous catalysis [43,44]. However, the study on AAILs is just in the initial stage. To the best of our knowledge, there has been no reported outcome of using AAILs in the chiral liquid–liquid extraction approach.

Our previous work indicated that AAILs could be used successfully as chiral ligands in HPLC and CE separation [45]. The chiral separation of amino acid enantiomers was achieved based on the formation of ternary mixed metal complexes between the AAIL ligand and the target enantiomers. The different complex stabilities of the mixed complexes with D- and L-enantiomers resulted in the enantioseparation of racemic amino acids. Based on the chiral ligand-exchange mechanism, four pairs of amino acid enantiomers were separated by aqueous solutions of AAILs. However, when AAILs were dissolved in water, the properties of AAILs would be changed due to the dissociation of the cations and anions of AAILs. Whether or not pure AAILs can be used in chiral separation is a very interesting issue that we are eager to examine. Therefore, we attempt to use undiluted AAILs for the separation of racemic amino acids.

In this paper, we demonstrate a novel application of functional AAILs in chiral liquid–liquid extraction. The AAILs, with alkylimidazolium cation and Pro anion, were synthesized and modified with Cu^{2+} for the chiral separation of amino acids by chiral ligand exchange. The functional AAILs were used as solvent and selector to separate racemic amino acids in the liquid–liquid extraction approach (Fig. 1). With functional AAILs as acceptor phase and organic solvent as donor phase, the efficiency and the enantioselectivity of the extraction system were investigated. Furthermore, based on a detailed study of influencing factors, the chiral separation mechanism of the extraction is discussed.

2. Experimental

2.1. Chemicals

L-Proline (L-Pro), L-phenylalanine (L-Phe), D-phenylalanine (D-Phe), racemic phenylalanine (D,L-Phe), racemic tyrosine (D,L-Tyr), racemic histidine (D,L-His), racemic tryptophan (D,L-Trp) and racemic β-phenylalanine (D,L-β-Phe) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Copper acetate monohydrate was purchased from Sigma. Anion exchange resin (Amberlite IRC-400) was purchased from Rohm and Haas.

The ionic liquids, including 1-ethyl-3-methylimidazolium bromide ([Emim]Br), 1-butyl-3-methylimidazolium bromide ([Bmim]Br), 1-hexyl-3-methylimidazolium bromide ([Hmim]Br) and 1-octyl-3-methylimidazolium bromide ([Omim]Br) were purchased from Chemer Chemical (Hangzhou, China). Their marked purities were over 97%.

2.2. Preparation and characterization of functional AAILs

High quality and colorless [$C_n$mim]Br (50 mmol) was used as raw material to synthesize AAILs. We used an ion exchange reaction of [$C_n$mim]Br with anion exchange resin to obtain [$C_n$mim]OH. The neutralization reaction of imidazolium hydroxide and amino acids was used to prepare AAILs (Fig. 1, Steps 1 and 2) [46,47]. A slight excess of equimolar proline aqueous solution was added to neutralize [$C_n$mim]OH to produce AAILs. 90 ml acetonitrile and 10 ml methanol were added to the solution of AAIL to separate out excess amino acid (L-Pro, 55 mmol) and then the mixture was filtered out. The filtrate was concentrated and dried under vacuum for 24 h at 80 °C to obtain the final product of AAILs. Through a similar process, we prepared 1-ethyl-3-methylimidazolium prolinate ([Emim][L-Pro]), 1-butyl-3-methylimidazolium prolinate ([Bmim][L-Pro]), 1-hexyl-3-methylimidazolium prolinate ([Hmim][L-Pro]) and 1-octyl-3-methylimidazolium prolinate ([Omim][L-Pro]). All the AAILs are hydrophilic and supercooled liquids at room temperature. The chemical structures of AAILs were characterized by ^{1}H NMR and ESI mass spectrometry (Fig. S1). Their purities were determined by our proposed LC–MS method [48] and found to be over 87%.

To achieve chiral extraction, copper acetate monohydrate (10 mmol) was dissolved in [$C_n$mim][L-Pro] (20 mmol). One copper ion can coordinate with two L-Pro anions to form copper–proline complex as the chiral recognition center (Fig. 1, Step 3). These ionic liquid mixtures were centrifuged for 5 min at 10,000 r/min and filtrated through 0.22 μm nylon membrane to remove the

undissolved copper acetate. All of them were deep blue and remained in liquid state at room temperature. The existence of the proline–copper complex was confirmed by infrared spectra (Fig. S2) and UV spectra (Fig. S3). In the infrared spectra, we found that the metal–ligand band is at 527–623 cm^{-1}, which is consistent with the results reported by Herlinger et al. [49].

2.3. Liquid–liquid extraction process

An IL-organic solvent system was chosen for liquid–liquid extraction of amino acids because functional AAILs are hydrophilic. Ethyl acetate was selected as donor phase based on solvent immiscibility. 1.0 ml ethyl acetate and 20 mg solid racemic amino acid were added to 100 μl functional AAILs in a 1.5 ml centrifuge tube. The above-mentioned mixture was stirred by a vortex mixer to achieve complete extraction. After stirring for 30 min, the mixture was separated to two phases by a centrifuge at 20,000 r/min for 5 min.

2.4. Chiral HPLC analysis

The enantioselectivity of the liquid–liquid extraction was estimated by determining the enantiomeric excess value (e.e.) of amino acid enantiomers in the extracted ionic liquid phase. The e.e. value was calculated according to e.e. = $|C_L - C_D|/|C_L + C_D|$, where C_L is the concentration of L-amino acids and C_D is the concentration of D-amino acids. The C_L and C_D were determined by chiral HPLC analysis. A Shimadzu LC-20AT HPLC series with a RF-10AXL fluorescence detector at Ex 215 nm and Em 295 nm was used. The HPLC column was an Ultimate XB-C18 column (5 μm, 250 mm × 4.6 mm ID). The mobile phase was an aqueous solution of acetonitrile 7.5% at pH 5 and flow rate was 1 ml/min. The optimization of separation conditions has been discussed in detail in our previous work [45]. 1 mM copper acetate and 2 mM L-Pro were added into the mobile phase for chiral separation. The extracted ionic liquids were diluted 500-fold and 10 μl of the dilution was injected into HPLC directly.

2.5. Recycle of AAILs

To reduce consumption of functional AAILs, we attempted to recycle AAILs. Using 1 M HCl to adjust pH to 7.0, L-Pro anion and target amino acid were changed to free amino acids. Then, L-Pro and target amino acid precipitated out by centrifugation at 10,000 r/min for 5 min. Based on the discrepancy of solubility, target amino acid and L-Pro were separated by washing with water three times. The recovery of L-Pro is 63–77% and e.e. of recovered L-Pro is over 98%. L-Pro and the supernatant (containing $[C_nMIM]^+$ and Cl^-) were used as raw material to synthesize AAILs by the method described in Section 2.2.

3. Results and discussion

In chiral liquid–liquid extraction, functional AAILs would play two distinct roles. One is the solvent of acceptor phase, which is immiscible with donor phase and can extract amino acids from donor phase. The other function is the chiral selector, which can recognize and coordinate with the target enantiomer. Therefore, in this work, the two roles of AAILs in chiral extraction were discussed, respectively.

3.1. AAILs as solvent in liquid–liquid extraction

3.1.1. Selection of solvents for donor phase

In preliminary studies, various solvents were mixed with [Bmim][Pro] to test the potential as suitable donor phase. The miscibilities of AAILs with different solvents are listed in Table 1. AAILs are always immiscible with low polar solvents and miscible with high polar solvents. It has been shown that AAILs are highly polar ionic liquid. However, most of amino acids are indissolvable in low polar organic solvents. Considering the miscibility and the solubility of amino acids, ethyl acetate and dioxane are satisfactory candidates. The density of AAILs is in the range of 1.14–1.22 g/ml. Ethyl acetate has the advantage of phase separation because of its lower density. Thus, in this work, ethyl acetate was selected as the solvent of donor phase.

Table 1
Miscibility of [Bmim][Pro] with several solvents.

Solvent	Polarity index[a]	Density (g/ml[a])	Miscibility[b]
n-Hexane	0.0	0.659	Immiscible
Tetrachloromethane	1.6	1.589	Immiscible
Toluene	2.3	0.866	Immiscible
Dichloromethane	3.1	1.327	Immiscible
Chloroform	4.1	1.483	Immiscible
Ethyl acetate	4.3	0.900	Immiscible
Dioxane	4.8	1.032	Immiscible
Ethanol	5.2	0.789	Miscible
Acetonitrile	5.8	0.788	Miscible
Methanol	6.6	0.791	Miscible
Water	9.0	1.000	Miscible

[a] The data are cited from Ref. [56].
[b] 1 ml [BMIM][Pro] and 1 ml organic solvents were mixed. This result was determined at equivalent volume composition at 298 K.

3.1.2. Distribution coefficient of Phe in liquid–liquid extraction

To investigate the extraction efficiency of AAILs, the partition coefficient of Phe in ionic liquid–ethyl acetate system was determined by the shaking flask method. Using functional [Bmim][Pro] as acceptor phase and ethyl acetate as donor phase, the equilibrium concentrations of L-Phe in ionic liquid phase and ethyl acetate phase were determined by HPLC analysis, respectively. The initial concentration of L-Phe in ethyl acetate phase was 2 mg/ml. The distribution coefficient is calculated according to $D = C_{IL}/C_{EA}$, where C_{IL} is the concentration of L-Phe in ionic liquid phase and C_{EA} is the concentration of L-Phe in ethyl acetate phase. We showed that the logarithm of distribution coefficient ($\log D$) for L-Phe is in the range of 3.4–3.6 in the ionic liquid–ethyl acetate two-phase system. It demonstrates that AAILs can dissolve amino acids excellently and extract more than 99% of amino acids from donor phase. In the same condition, the logarithm of distribution coefficient for D-Phe is in the range of 3.4–3.5, which is similar to that of L-Phe. This data also shows that the extraction system has very low or no enantioselectivity at the relative low initial concentration of Phe (2 mg/ml).

In contrast to previous reports, the extraction efficiency of AAILs is much better than that of hydrophobic ionic liquid ($\log D = -0.12$) [28] and alkyl-L-proline ($\log D = 0.23$) [50]. This result may attribute to high polarity and strong dipolarity/polarizability (π^*) interaction of AAILs. The high polarity of AAILs can improve the dissolution of amino acid via ion-pairing. Like most of imidazolium ionic liquids, the imidazolium rings of AAILs may interact with the benzene rings of Phe to improve the extraction of amino acids via π–π interaction.

3.1.3. Copper ion stability in ionic liquid phase

To achieve enantioselective extraction, copper acetate was dissolved into AAILs to form copper–proline complexes. In other words, functional AAILs are ionic liquid solutions of copper acetate. In the extraction process, Cu^{2+} may release into donor phase, which would make the functional AAILs unstable and pollute the donor phase. Thus, the releasing of Cu^{2+} should be controlled. Using sodium diethyldithiocarbamate as chelant, the released Cu^{2+} were determined at 475 nm by the photometric method. We revealed that only millimolar levels of Cu^{2+} (0.34–2.3 mM) were released

into ethyl acetate phase. Compared with the concentrations of Cu^{2+} in AAILs (1.40–1.83 M), the released Cu^{2+} is negligible as the concentration of Cu^{2+} in EA phase is only 0.07–0.12% of that in IL phase. These results indicate that AAILs have high metal affinity and Cu^{2+} in AAILs is relatively stable. The high metal affinity may rely on strong synergistic effect of coordination with the imino group and the carboxyl group of L-Pro moiety. A similar result was reported by You and co-workers [44] and the metal affinity of AAILs has been used in metal scavenging. Moreover, we find that the released Cu^{2+} into donor phase increases in the order as following:

[Emim][L-Pro] (0.34 mM) < [Bmim][L-Pro] (1.3 mM)

< [Hmim][L-Pro] (1.4 mM) < [Omim][L-Pro] (2.3 mM)

For the imidazolium based AAILs, it is clear that the polarity corresponds to the alkyl chain length. The AAILs with higher polarity, such as [Emim][L-Pro], may have stronger electrostatic interaction with Cu^{2+} and exhibit higher metal ion affinity than that of AAILs with lower polarity.

In summary, functional AAILs are found to be efficient and stable solvents for liquid–liquid extraction of amino acids. However, the binding between amino acid and Cu^{2+} is too strong. In back-extraction experiment, we have not found suitable solvent to back-extract the amino acids from the AAILs, which would be our effort in the near future.

3.2. AAILs as selectors in chiral extraction

In chiral separation, the enantioselectivity is the most important parameter for chiral selector. Although functional AAILs have been successfully used as chiral selectors in HPLC and CE separation, we are also interested in the enantioselectivity of functional AAILs in liquid–liquid extraction. Therefore, the effect factors of chiral extraction are investigated to optimize the enantioselectivity and to understand the separation mechanism of the chiral extraction.

3.2.1. Effect of copper ion concentration

Copper ion, as a central ion, is an indispensable part of the chiral ligand complex. Thus, the effect of Cu^{2+} was studied at first. As shown in Fig. 2, the enantioselectivity of extraction is influenced strongly by the concentration of Cu^{2+}. In the absence of the copper ion, the enantioselectivity of the two-phase system was not observed. However, with the increasing amount of Cu^{2+}, the enantioselectivity increased accordingly. When the molar ratio of Cu^{2+} to L-Pro changed from 0.5 to 1.0, the enantioselectivity increased dramatically at first and then changes slowly to reach a plateau. These interesting results suggested the function of Cu^{2+} in the chiral ligand exchange.

When cupric ion is added into AAILs, it should be chelated with L-Pro to form copper–proline complex. In the presence of the target amino acid, the copper–proline complex can be transformed to a mixed ligand complex with 1 mol of L-Pro and 1 mol target amino acid via ligand exchange [50]. The ligand-exchange reaction of the chiral extraction can be considered as:

$$[AA]_{IL} + [Cu(L\text{-}Pro)_2]_{IL} \rightarrow [Cu(L\text{-}Pro)(AA)]_{IL} + [L\text{-}Pro]_{IL} \quad (1)$$

where $[AA]_{IL}$, $[L\text{-}Pro]_{IL}$, $[Cu[L\text{-}Pro]_2]_{IL}$ and $[Cu[L\text{-}Pro][AA]]_{IL}$ are target amino acid, free L-Pro, copper complex with 2 molecular of Pro and the mixed ligand complex with 1 molecular of L-Pro and 1 molecular target amino acid in the ionic liquid phase, respectively. The chiral extraction can be explained by the ligand-exchange reaction as follows: at the low concentration, target amino acid is difficult to chelate with Cu^{2+} due to the excess of L-Pro. Thus, no enantioselectivity is observed. When the molar ratio of Cu^{2+} to L-Pro reached to 0.5, the concentration of $Cu[L\text{-}Pro]_2$ as well as the reaction rate of ligand exchange attain the maximum, which illustrates the substantial increase of enantioselectivity. Then, with the increase of Cu^{2+} concentration, the target amino acid and $Cu[L\text{-}Pro]_2$ forms Cu[L-Pro][AA]. When their molar ratio is close to 1, the concentration of Cu[L-Pro][AA] reaches the maximum, and the best of enantioselectivity is achieved. Finally, the e.e. value tends to be stable at 39%. However, in the absence of target amino acids, the AAILs dissolved so much copper acetate may be unstable or supersaturated. Therefore, the employed ratio of Cu/Pro is 0.5 despite the optimal e.e. value should be obtained at 1.

3.2.2. Effect of racemic amino acid concentration

In the ligand-exchange reaction, the enantioselectivity of extraction is also influenced by the added amount of solid racemic amino acid. As shown in Fig. 3, at low concentration, no enantioselectivity is observed. Interestingly, when the amino acid enantiomer exceeds 6.3 g/l, the e.e. value increases dramatically, then it changes slowly to reach a plateau and remains stable at 38%. For better understanding of this result, the concentrations of amino acid enantiomers in AAILs phase varies with the added amount of racemic Phe are also shown in Fig. 3. We found that the D-Phe and L-Phe were extracted equally into ionic liquid phase

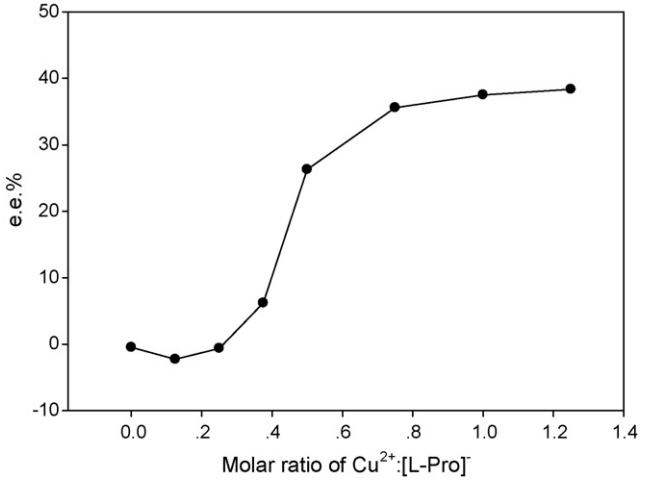

Fig. 2. Effect of Cu^{2+} on enantioselectivity of the extraction. The e.e. value was calculated according to e.e. = $|C_L - C_D|/|C_L - C_D|$, where C_L, C_D are the concentration of L-amino acids and D-amino acids in ionic liquid phase, respectively. The C_L and C_D were determined by chiral HPLC analysis (see Section 2.4).

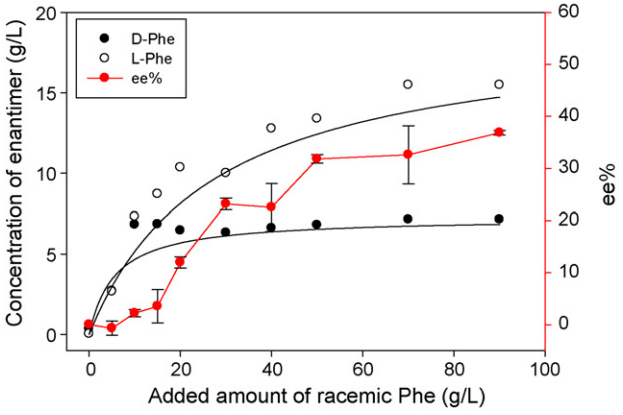

Fig. 3. Extraction of racemic Phe using the Cu^{2+} modified [Emim][L-Pro]. The influence of the added amount of racemic Phe on the extraction enantioselectivity (left axis) and the enantiomer concentration in ionic liquid phase (right axis). The molar ratio of Cu^{2+} to $[L\text{-}Pro]^-$ is 1:2.

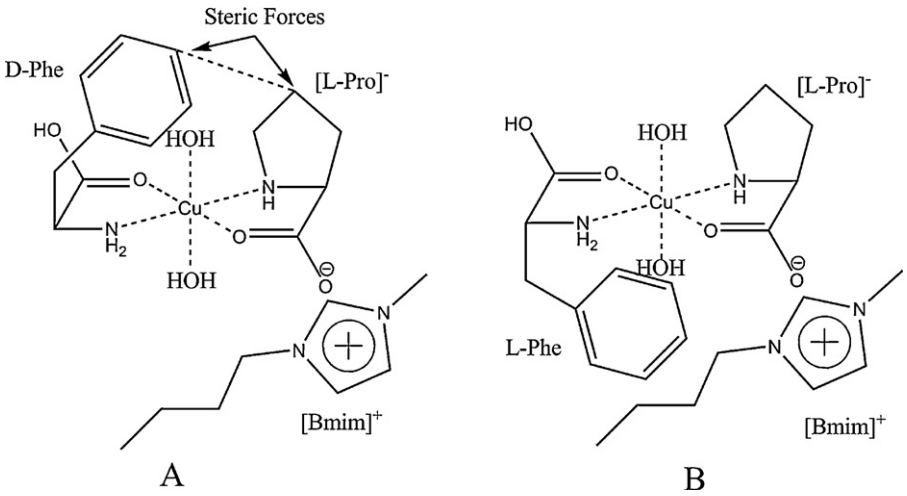

Fig. 4. Chiral recognition mechanism of functional AAILs in liquid–liquid extraction. The structures of the ternary complex of Cu[L-Pro][D-Phe] (A) and Cu[L-Pro][L-Phe] (B) are shown in the scheme. The doted line between D-Phe and L-Pro represents the steric force. The complex stability constant of A is less than B because of the steric effect. Based on the electrostatic interaction, the ion-pairs of alkylimidazolium cations and L-Pro anion are formed.

at low concentration. When the concentration of Phe exceeded the threshold value (6.3 g/l), the concentration of D-Phe did not increase. It seems that the functional AAIL is saturated with D-Phe. However, more L-Phe can be extracted into ionic liquid phase until reaching the saturation solubility for L-Phe (19.3 g/l). In this case, the functional AAILs demonstrate distinct enantioselectivity for amino acid enantiomers. The enantiomeric resolution of the racemic amino acids may be explained through the ligand-exchange mechanism (as illustrated in Fig. 4). The amino and carboxyl groups of amino acids are coordinated with Cu^{2+} to form the ternary complexes of Cu[L-Pro][L-Phe]. Due to the presence of steric forces, the diastereoisomeric complex with D-enantiomer is less stable [51,52]. Therefore, the chiral extraction of amino acid enantiomers was observed. Moreover, the alkylimidazolium cations and L-Pro anion can form ion-pairs by electrostatic interaction. The ion-pairs can enhance the solubility of diastereoisomeric complexes.

We also determined the saturation limit and solubility of D- and L-Phe in ionic liquid phase, respectively to confirm the enantioselectivity. At 25 °C, the saturation solubility of [Emim][Pro] was 9.3 g/l for D-Phe and 22.5 g/l for L-Phe, respectively. This data confirmed that the undiluted functional AAILs have distinct enantioselectivity for amino acid enantiomers.

In the extraction process, the concentrations of Phe in donor phase are stable in the range of 6.3–6.9 μg/ml, thus undissolved amino acid may exist in donor phase. The excess of D-enantiomer might be present in the undissolved amino acid fraction.

Notably, we found that the functional AAILs can achieve enantioselective extraction at very high amino acids concentration (112–155 mM), which is much higher than the reported work for the chiral extraction of amino acids (1–20 mM) [24,50,53]. In most chiral extraction, the chiral separation capacity is always limited by the low solubility of selectors, such as salenes, crown ethers and deoxyguanosine derivatives. However, the functional AAILs are chiral selectors themselves and they show much higher separation capacity in the IL phase. In preparation of scale chiral extraction, functional AAILs as separation media with high chiral separation capacity have obvious advantages to improve efficiency and reduce solvent consumption.

3.2.3. Effect of AAILs cation

The effect of alkyl chain length of imidazolium cations on the enantioselectivity was studied by using the chiral HPLC method and the result is shown in Fig. 5. With lengthening of the alkyl chain, the enantioselectivity was increased from 37.2% to 50.6% in e.e. value and the extracted amino acids in ionic liquid phase decreased. It showed that the cations of functional AAILs can influence the stability of the chiral complexes. According to Koska's report [54], the stability difference of copper–proline complexes increased with the reduction of solvent polarity. Thus, the functional AAILs with longer alkyl chain showed better enantioselectivity than those with shorter one.

3.2.4. Effect of chemical structure of amino acids

To investigate the effect of chemical structure of amino acids on the enantioselectivity, five racemic amino acids were extracted by the functional AAILs. The extraction results are listed in Table 2. For all tested racemic α-amino acids, more L-enantiomers were extracted into ionic liquid phase than D-enantiomers (Fig. S4). However, the enantioselectivity of liquid–liquid extraction system was different, which implied that amino acid residue can influence the stability of the mixed ligand complex to change the enantioselectivity. We also found that the e.e. values of four racemic amino acids

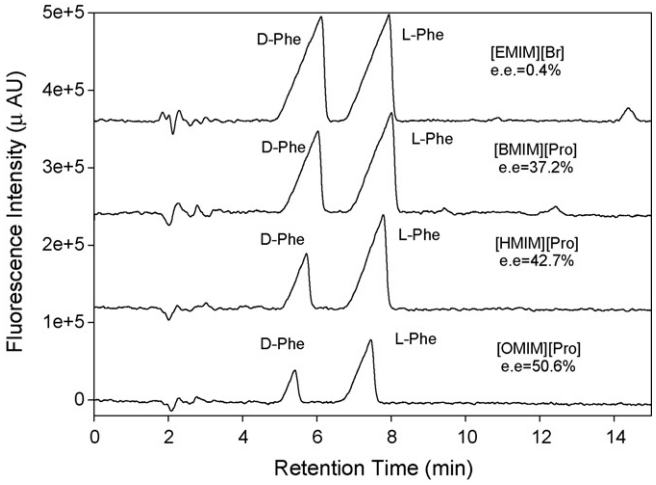

Fig. 5. Chromatograms of the chiral HPLC analysis for Phe enantiomers. *Chromatographic condition*: Column, Ultimate XB-C18 150 mm × 4.6 mm; *Mobile phase*: Aqueous solution of copper acetate (1 mM), Pro (2 mM) and acetonitrile 7.5%, pH 5; flow rate: 1 ml/min. 100 μl ionic liquid was used to extract 20 mg racemic Phe. The ionic liquids were 500-fold diluted and injected directly into HPLC.

Table 2
Chiral extraction results of racemic amino acids using the functional ionic liquids.

Entry	Amino acid[a]	Ionic liquid	e.e.%[b]
1	α-Phe	[Emim][Pro]	35.8 ± 0.1
2	α-Tyr	[Emim][Pro]	21.9 ± 0.3
3	α-His	[Emim][Pro]	4.0 ± 0.6
4	α-Trp	[Emim][Pro]	5.9 ± 0.3
5	β-Phe	[Emim][Pro]	0.3 ± 0.1
6	α-Phe	[Emim][Br]	0.6 ± 0.1

[a] Excess racemic amino acid (20 mg) was added into 100 μl ionic liquid.
[b] Cu^{2+}: Pro^- is the molar ratio of $Cu(Ac)_2$ to [$C_n$mim][L-Pro].

were in the order of:

[Phe] > [Tyr] > [Trp] > [His]

This phenomenon may attribute to the coordination ability of amino acid residue. Phe and Tyr were separated with high enantioselectivity because their residues have weaker coordination ability and greater steric effect. However, for His and Trp, their residues (imidazolyl and indolyl) have imine groups, in which the nitrogen atom can form a strong ligand bond with Cu^{2+}. This will change the relative disposition of the chiral reorganization center and reduce the enantioselectivity. A similar explanation has been reported on the poor separation factor of His by chiral HPLC [55]. The same order has been observed in the separation factor of these amino acids by chiral chromatographic separation [45]. This result confirms that the liquid–liquid extraction and the chromatographic separation have the same separation mechanism: chiral ligand exchange.

Racemic β-amino acid was also extracted by functional AAILs (Table 2, entry 5). We found that the functional AAILs had no enantioselectivity for β-Phe, which is probably due to the reason that the β-amino group and carboxyl are difficult to chelate with Cu^{2+} simultaneously.

4. Conclusion

In this work, we designed and synthesized novel functional AAILs for chiral separation. These AAILs performed two roles in the liquid–liquid extraction approach. As solvent, they act as an efficient and stable acceptor phase for amino acids. As selector, AAILs coupled with Cu^{2+} exhibit distinct enantioselectivity in the separation of racemic amino acids. According to the study of the influencing factors, we revealed that the amino acid enantiomers were separated based on the chiral ligand-exchange mechanism.

The present work not only extended the application of amino acid ionic liquids but also provided some useful information to design task-specific ionic liquids for chiral separation. We believe functional AAILs are promising materials for chiral resolution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.05.013.

References

[1] T. Welton, Chem. Rev. 99 (1999) 2071.
[2] X. Han, D.W. Armstrong, Acc. Chem. Res. 40 (2007) 1079.
[3] A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, J. Chromatogr. A 1184 (2008) 6.
[4] J.L. Anderson, D.W. Armstrong, G.T. Wei, Anal. Chem. 78 (2006) 2892.
[5] M. Smiglak, A. Metlen, R.D. Rogers, Acc. Chem. Res. 40 (2007) 1182.
[6] E. Miyako, T. Maruyama, N. Kamiya, M. Goto, Chem. Commun. (2003) 2926.
[7] S. Qi, Y. Li, Y. Deng, Y. Cheng, X. Chen, Z. Hu, J. Chromatogr. A 1109 (2006) 300.
[8] S.A. Rizvi, S.A. Shamsi, Anal. Chem. 78 (2006) 7061.
[9] M.L. Patil, H. Sasai, Chem. Rec. 8 (2008) 98.
[10] J. Ding, T. Welton, D.W. Armstrong, Anal. Chem. 76 (2004) 6819.
[11] T.J. Ward, Anal. Chem. 78 (2006) 3947.
[12] D.K. Bwambok, H.M. Marwani, V.E. Fernand, S.O. Fakayode, M. Lowry, I. Negulescu, R.M. Strongin, I.M. Warner, Chirality 20 (2008) 151.
[13] T.J. Ward, B.A. Baker, Anal. Chem. 80 (2008) 4363.
[14] C.D. Tran, I. Mejac, J. Chromatogr. A 1204 (2008) 204.
[15] C.D. Tran, D. Oliveira, S. Yu, Anal. Chem. 78 (2006) 1349.
[16] R. Xie, L.Y. Chu, J.G. Deng, Chem. Soc. Rev. 37 (2008) 1243.
[17] C.A.M. Afonso, J.G. Crespo, Angew. Chem. Int. Ed. 43 (2004) 5293.
[18] N. Demirel, Y. Bulut, H. Hosgoren, Chirality 16 (2004) 347.
[19] P. Dzygiel, T.B. Reeve, U. Piarulli, M. Krupicka, I. Tvaroska, C. Gennari, Eur. J. Org. Chem. (2008) 1253.
[20] B. Schuur, J. Floure, A.J. Hallett, J.G.M. Winkelman, J.G. de Vries, H.J. Heeres, Org. Process. Res. Dev. 12 (2008) 950.
[21] B. Schuur, J.G.M. Winkelman, H.J. Heeres, Ind. Eng. Chem. Res. 47 (2008) 10027.
[22] L.J. Tang, H. Ga, J. Kim, S. Choi, R. Nandhakumar, K.M. Kim, Tetrahedron Lett. 49 (2008) 6914.
[23] B.J.V. Verkuijl, A.J. Minnaard, J.G. de Vries, B.L. Feringa, J. Org. Chem. 74 (2009) 6526.
[24] T.B. Reeve, J.P. Cros, C. Gennari, U. Piarulli, J.G. de Vries, Angew. Chem. Int. Ed. 45 (2006) 2449.
[25] T. Nakashima, T. Kawai, Chem. Commun. (2005) 1643.
[26] H.D. Willauer, J.G. Huddleston, R.P. Swatloski, A.E. Visser, R.D. Rogers, Chem. Commun (1998) 1765.
[27] K. Shimojo, K. Nakashima, N. Kamiya, M. Goto, Biomacromolecules 7 (2006) 2.
[28] J.J. Wang, Y.C. Pei, Y. Zhao, Z.G. Hu, Green Chem. 7 (2005) 196.
[29] S. Carda-Broch, A. Berthod, D.W. Armstrong, Anal. Bioanal. Chem. 375 (2003) 191.
[30] M.T. Reetz, W. Wiesenhofer, G. Francio, W. Leitner, Adv. Synth. Catal. 345 (2003) 1221.
[31] R. Fortunato, M.J. Gonzalez-Munoz, M. Kubasiewicz, S. Luque, J.R. Alvarez, C.A.M. Afonso, I.M. Coelhosoa, J.G. Crespoa, J. Membr. Sci. 249 (2005) 153.
[32] J.C. Plaquevent, J. Levillain, F. Guillen, C. Malhiac, A.C. Gaumont, Chem. Rev. 108 (2008) 5035.
[33] D. Bregeon, J. Levillain, F. Guillen, J.C. Plaquevent, A.C. Gaumont, Amino Acids 35 (2008) 175.
[34] B. Ni, A.D. Headley, G. Li, J. Org. Chem. 70 (2005) 10600.
[35] W. Bao, Z. Wang, Y. Li, J. Org. Chem. 68 (2003) 591.
[36] G.-H. Tao, L. He, W.-S. Liu, L. Xu, W. Xiong, T. Wang, Y. Kou, Green Chem. 8 (2006) 639.
[37] G.H. Tao, L. He, N. Sun, Y. Kou, Chem. Commun. (2005) 3562.
[38] J. Kagimoto, K. Fukumoto, H. Ohno, Chem. Commun. (2006) 2254.
[39] J.Z. Yang, Q.G. Zhang, B. Wang, J. Tong, J. Phys. Chem. B 110 (2006) 22521.
[40] W. Guan, W.-F. Xue, N. Li, J. Tong, J. Chem. Eng. Data 53 (2008) 1401.
[41] Y.-Y. Jiang, G.-N. Wang, Z. Zhou, Y.-T. Wu, J. Geng, Z.-B. Zhang, Chem. Commun. (2008) 505.
[42] K. Fukumoto, Y. Kohno, H. Ohno, Chem. Lett. 35 (2006) 1252.
[43] K. Fukumoto, H. Ohno, Angew. Chem. Int. Ed. 46 (2007) 1852.
[44] W. Chen, Y. Zhang, L. Zhu, J. Lan, R. Xie, J. You, J. Am. Chem. Soc. 129 (2007) 13879.
[45] Q. Liu, K. Wu, F. Tang, L. Yao, F. Yang, Z. Nie, S. Yao, Chem. Eur. J. 38 (2009) 9889.
[46] K. Fukumoto, M. Yoshizawa, H. Ohno, J. Am. Chem. Soc. 127 (2005) 2398.
[47] H. Ohno, K. Fukumoto, Acc. Chem. Res. 40 (2007) 1122.
[48] F. Tang, K. Wu, Z. Nie, L. Ding, Q. Liu, J. Yuan, M. Guo, S. Yao, J. Chromatogr. A 1208 (2008) 175.
[49] A.W. Herlinger, S.L. Wenhold, T.V. Long 2nd., J. Am. Chem. Soc. 92 (1970) 6674.
[50] T. Takeuchi, R. Horikawa, T. Tanimura, Anal. Chem. 56 (1984) 1152.
[51] G. Gubitz, W. Jellenz, W. Santi, J. Chromatogr. 203 (1981) 377.
[52] H.Y. Aboul-Enein, I. Ali, Chromatographia 54 (2001) 200.
[53] J. Lacour, C. Goujon-Ginglinger, S. Torche-Haldimann, J.J. Jodry, Angew. Chem. Int. Ed. 39 (2000) 3695.
[54] J. Koska, C. Mui, C.A. Haynes, Chem. Eng. Sci. 56 (2001) 29.
[55] A.T.E. Gil-Av, P.E. Hare, J. Am. Chem. Soc. 102 (1980) 5115.
[56] J.G. Speight, Lange's Handbook of Chemistry, McGraw-Hill, New York, 2005.