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Complex-precipitation using functionalized chiral ionic liquids with L-proline anion and chromatographic analysis for enantioseparation of racemic amino acids

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Abstract

As one kind of functionalized green medium, chiral ionic liquids (CILs) have been widely applied in fields of asymmetric catalysis, enantioseparation, and so on. In this study, four kinds of amino acid–based CILs were synthesized by using trimethylamine, N-methylpyrrolidine, N-methylimidazole, and tropine as cationic nucleus, respectively. Then their specific optical rotation and solubility in common solvents were determined for further resolution application. The effect of different cations in these CILs was explored on the separation of racemic phenylalanine in complex-precipitation way. Moreover, various factors were systematically investigated for their effects on resolution efficiency, including the type of additive copper salts, the molar ratio of Cu (II) to CIL, pH value, the amount of racemic phenylalanine, and temperature. Under the appropriate conditions, L-phenylalanine mainly existed in solid phase and could be separated from its enantiomers in liquid phase. Furthermore, the mechanism of resolution was studied by thermogravimetric analysis, infrared spectrum, and molecular simulation. The resolution system has characteristics of no organic solvent, fast separation speed, simple resolution process, and easy scale-up.

KEYWORDS

characterization, chiral ligand, complex-precipitation method, ionic liquids, mechanism

1 | INTRODUCTION

Functionalized ionic liquids (FILs) are a kind of ionic liquids with special physicochemical properties, which are widely used in various fields of science and technology in recent years. The design and synthesis of novel functionalized ionic liquids and their special applications are at the forefront of current research. As one kind of functionalized ionic liquids with multifunction, chiral ionic liquids (CILs) have both the properties of chirality and ionic liquids.[1,2] They are widely reported in the application of asymmetric catalysis, enantioseparation, and so on.[3,4] At present, the most widely applied CILs are imidazole CILs,[5-7] and other kinds of CILs include pyridinium CILs,[8-10] thiazole CILs,[11] and quaternary ammonium CILs.[12,13]

The resolution of racemic amino acids (AAs) is an important way to obtain AAs with single configuration. In recent years, the study of their enantioseparation has been attracting more and more researchers, and various methods have been emerging that mainly include chemical resolution,[14] membrane resolution,[15-17]

Huimin Zang and Shun Yao contributed equally to this work.

enzymatic resolution,[18-20] chromatographic resolution,[21-25] extraction,[26,27] and molecular imprinting technique.[28] Among them, the solvent extraction method has the advantages of high separation efficiency, simple equipment, and low energy consumption, but a lot of organic solvents are used. As green medium, CILs are expected to replace these conventional solvents. On the other hand, L-proline (L-Pro)-Cu (II) has been proved as good ligand in chiral catalysis.[29] Inspired by this, Liu et al[30] synthesized a series of immidazolium CILs with the anion of L-proline and used them in enantioseparation on the basis of the principle of chiral ligand exchange in 2009. When CILs are used for chiral extraction, it can be divided into two ways: organic phase extraction and aqueous two-phase extraction. Tang et al[31] used the reaction between $[C_nMim][L\text{-}Pro]$ and Cu^{2+} to obtain the complex with the ability of chiral recognition, which was used to separate D,L-phenylalanine (D,L-Phe) from their ethyl acetate solution and e.e. value of 50% was achieved in extracted phase. Wu et al[32] applied two kinds of chiral immidazolium ILs and sodium sulphate to form aqueous two-phase system (ATPS) in the enantioseparation of D,L-Phe. As the result, L-Phe was enriched in water phase, and e.e. value was 53%. In the preliminary studies of our laboratory, Wu et al[33] used tropine-based CILs and inorganic salt solution to prepare ATPS, and the enantiomeric excess value of L-phenylalanine in solid phase was 65%. The formation of aqueous two-phase system avoids the use of volatile organic solvents, but a large amount of inorganic salts are needed. And there are some difficulties in product recovery and reuse of the whole system. However, complex-precipitation method with CILs in single-phase system is rarely studied which has lower requirement and better reusability, and it is easier to realize fast resolution and recovery. So this method is explored and developed in the following study.

2 | MATERIALS AND METHODS

2.1 | Materials and apparatus

2.1.1 | Materials

All the following chemicals were purchased from Kelong Chemicals Co, Ltd (Chengdu, China): trimethylamine, N-methylimidazole, 1-bromobutane, ethyl acetate, methanol, ethanol, acetonitrile, ammonia, acetic acid, copper acetate, copper sulfate, copper chloride, copper nitrate and 201 × 7 type strongly-alkaline anion-exchange resin (pH range: 0-14, effective particle size: 0.400-0.850 mm, expansivity ≤25%, working exchange capacity ≥450 mmol/l). L-proline (L-Pro), N-methylpyrrolidine, D,L-phenylalanine (D,L-Phe, biological reagent grade, purity ≥99.5%), and cupric bromide were supplied by Aladdin Reagent Co, Ltd (Shanghai, China). Tropine (purity ≥98.9%) was obtained from Huawen Chemicals Co, Ltd (Zhengzhou, China). All of above chemicals are at the level of analysis or above.

2.1.2 | Apparatus

The Shimadzu LC-20AT high performance liquid chromatograph (Kyoto, Japan) consisting of PD-M20A detector and Class VP chromatography workstation was used for the quantitative analysis of the racemic amino acids. A Welchrom-C_{18} column (250 × 4.6 mm, 5 μm) was used as the HPLC analytical column (Welch Materials, Austin). A TGL-16D high speed refrigerated centrifuge was provided by Baita Xinbao Instrument Factory (Changzhou, China). The synthesized ionic liquids were characterized by FT-IR (KBr disc, Perkin Elmer) and 400 MHz ^{1}HNMR (MeOD, Bruker, Switzerland). Deionized water used in the experiment was obtained from the ultra-pure water system produced by Yufei Instrument Co, Ltd (Guangzhou, China).

2.2 | Synthesis of the CILs

Triethylamine, N-methyl pyrrolidine, N-methylimidazole, and tropine reacted with 1-bromobutane to prepare four kinds of ILs with bromonium anion according to previous studies.[29,31] Then they were loaded on strongly basic anion-exchange resin and debromination was achieved. Finally, the target ionic liquids were obtained after neutralized with L-proline. The synthesis procedure was introduced as followings with pyrrolidium-type CIL ([BuPyro][L-Pro]) as an example.

2.2.1 | Synthesis of [BuPyro][Br]

A total of 0.1 mol N-methylpyrrolidine, 0.1 mol N-butyl bromide, and appropriate volume of ethanol were added in a 100 mL flask. After 24 hours refluxing reaction, the solvent was removed by reduced pressure distillation. The residue was washed by ethyl acetate for 2 to 3 times and dried in a vacuum dryer and target product was obtained.

2.2.2 | Synthesis of [BuPyro] [OH]

A total of 0.05 mol [BuPyro][Br] was dissolved in an appropriate amount of methanol in a flask. The solution was transferred to a pretreated strongly basic anion exchange resin packed column (2 × 10 cm). The flow rate of the effluent was controlled at no more than two drops

per second, and methanol was continuously added to the column to ensure that the resin was immersed below the liquid level. Effluent solution with pH greater than 8 was collected, which was [BuPyro][OH] solution.

2.2.3 | Synthesis of [BuPyro] [L-Pro]

The [BuPyro][OH] solution was directly dropped in methanol solution containing 0.06 mol L-proline and then stirred for 8 hours at room temperature (20°C). Then the solvent was removed under vacuum, and frozen acetonitrile was added into the remaining liquid. As a result, redundant L-proline was precipitated and removed by filtering, and then viscous yellowish liquid was obtained after concentration, which was the target product of [BuPyro][L-Pro] and preserved in a cold drying environment. Its structure was proved by spectral data, and FT-IR (KBr disc) showed absorbance at 3351, 3296 cm^{-1} ($\nu_{O\text{-}H}$); 3045, 3012 cm^{-1} ($\nu_{as\ C\text{-}H}$ and $\nu_{s\ C\text{-}H}$ of N^+CH_3); 2952, 2909 cm^{-1} ($\nu_{as\ C\text{-}H}$, $\nu_{s\ C\text{-}H}$ of N^+CH_2, N^+CH); 2881 cm^{-1} ($\nu_{\ C\text{-}H}$); 1491, 1473, 1442, 1405 cm^{-1} ($\delta_{as\ C\text{-}H}$, $\delta_{s\ C\text{-}H}$); 1577, 1431 cm^{-1} ($\nu_{as\ C\text{-}O}$, $\nu_{s\ C\text{-}O}$); 1533 cm^{-1} ($\delta_{\ N\text{-}H}$) and 869 cm^{-1} ($\omega_{C\text{-}H}$); meanwhile 1H NMR spectrum (400 MHz, MeOD) exhibited proton signals at 3.83 ppm (1H, dd, J = 8.6, 6.2 Hz, -OH), 3.78~3.27 (8H, m, $-CH_2 \times 4$ on pyrrolidine ring), 3.13~3.04 (4H, m, $-CH_2 \times 2$ on L-proline ring), 2.24 (5H, m, $-CH_2$ and $-CH_3$ on alkyl chain), 1.90 (2H, dd, J = 10.4, 4.3 Hz, $-CH_2$ on alkyl chain), 1.81 (1H, s, -NH), 1.45 (2H, dd, J = 15.0, 7.5 Hz, $-CH_2$ on C-4 of L-proline), and 1.04 (3H, t, J = 7.4 Hz, $N\text{-}CH_3$ on L-proline ring). Other CILs [$BuEt_3N$][L-Pro], [BuIm][L-Pro] and [BuTr][L-Pro] were also synthesized in similar way. The synthetic route of these CILs and their structures are shown in Figure 1.

2.3 | Determination of specific optical rotation and solubility of CILs

For chiral compounds, the specific optical rotation of a given substance is a constant which is equal to the optical rotation when the concentration is 1 g/mL. In this study, 0.2 g CILs was dissolved in 20 mL distilled water, and then the CILs solutions with the concentration of 0.01 g/mL were prepared. The specific optical rotation degree was measured in a 100 mm polarimeter tube at 25°C and 589 nm by using Autopol I polarimeter (Rudolph Research Analytical). Moreover, in the investigation for dissolution behavior of CILs, a certain amount of CILs was placed in a dry glass jacketed reaction bottle; the temperature of jacket was controlled at 25 ± 0.05°C by thermostatic water bath, and the solvent was slowly added into the jacketed reaction bottle until all the CILs was thoroughly dissolved. Then the solubility of the CILs in the solvent can be observed by weighing the mass of the solvent added accurately. In this study, the solubility properties of four kinds of CILs in 12 common organic solvents (methanol, ethanol, acetone, dichloromethane, acetonitrile, ethyl acetate, toluene, chloroform,

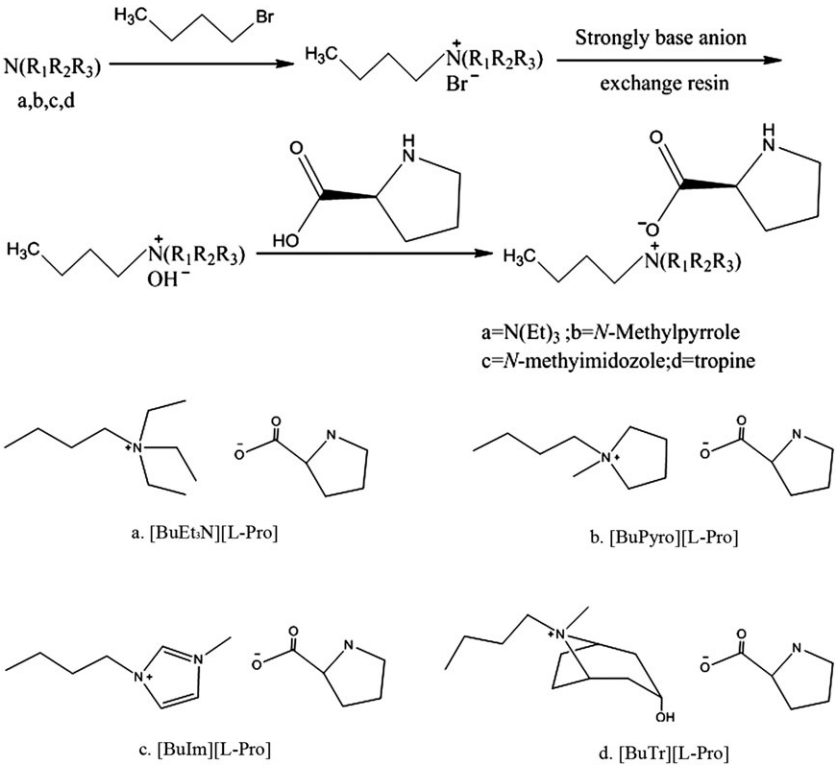

FIGURE 1 Synthesis route of chiral ionic liquids and their structures

petroleum ether, hexane, ether, and water) were studied, and related grading standard was according to Chinese pharmacopoeia (2015 edition).

2.4 | Quantitative analysis of amino acid enantiomers by HPLC

Chiral HPLC method was used for qualitative and quantitative analysis of D,L-Phe. A Welchrom-C_{18} column (5 μm, 250 × 4.6 mm) was used as the HPLC analytical column (Welch Materials, Austin). The chiral mobile phase was composed of L-Pro (12×10^{-4} mol/L), copper acetate (6.0×10^{-4} mol/L) and the volume ratio 1:4 of methanol to water which was adjusted to pH 5 with acetic acid. Other HPLC conditions were as follows: flow rate of 1 mL/min, injection volume of 20 μL, column temperature of 25°C, and detection wavelength of 212 nm.[34] The HPLC determination of D,L-Phe was mainly based on the comparison with retention time of standard compounds. In detail, the retention time of D-Phe was 8.17 minutes and the retention time of L-Phe was 11.25 minutes; D-Phe had shorter retention time than L-Phe. The mechanism is that D-type amino acid is more difficult to form complex with L-Pro and Cu^{2+} in the chiral mobile phase compared to L-type amino acid and would be eluted from the column with priority. On the contrary, L-type amino acid is eluted later and has longer retention time.[35] The chromatogram is shown in Figure S1.

2.5 | Resolution of amino acid enantiomers with CIL by complex-precipitation method

The whole resolution method consisted of several processes is shown in Figure S2. A total of 6.0×10^{-4} mol CIL and 7.2×10^{-4} mol copper salt was mixed in a 5 mL centrifugal tube, and then 1 mL deionized water was added into the mixture. After that, hydrochloric acid or sodium hydroxide was used to adjust the water solution to the specific pH value. The CIL and copper salt could be thoroughly dissolved to form a blue clear transparent solution through the assistance of ultrasound wave for 2 minutes. Finally, 10 mg racemic mixture of D,L-Phe was added in the blue solution and dissolved completely by oscillating. After kept in constant temperature water bath for 30 minutes, the biphase formation completed. The solid phase was obtained through high speed centrifuge for 5 minutes (6000 r/min) and then diluted with deionized water to 25 mL. A total of 20 μL of this solution was injected into the HPLC system for quantitative analysis of related enantiomers in order to evaluate resolution result. Finally, the enantiomeric excess values (e.e., %) of D- and L-Phe in the solid or liquid phase were analyzed.

The percentages of D,L-Phe in different phases were obtained under above conditions. The e.e. value and yield of enantiomers were calculated according to Equations (1) to (3) as following:

$$e.e._{L-Phe(D-Phe)} = |\omega_{L-Phe\ in\ solid\ (liquid)} - \omega_{D-Phe\ in\ solid\ (liquid)}| / |\omega_{L-Phe\ in\ solid\ (liquid)} + \omega_{D-Phe\ in\ solid\ (liquid)}| \times 100\%, \quad (1)$$

$$Y_{L-Phe} = m_{L-Phe\ in\ solid} / \left(m_{L-Phe\ in\ solid} + m_{L-Phe\ in\ liquid}\right) \times 100\%, \quad (2)$$

$$Y_{D-Phe} = m_{D-Phe\ in\ liquid} / \left(m_{D-Phe\ in\ solid} + m_{D-Phe\ in\ liquid}\right) \times 100, \quad (3)$$

where ω and m are the mass fraction and mass of L-Phe and D-Phe in solid or liquid phase, respectively.

On the other hand, the evaluation of racemic compounds resolution is mainly based on the enantiomer excess value, that is, the e.e. value, but it can only be used as the optical purity parameter of the target product and cannot reflect the actual production capacity of the resolution system and the yield of the target enantiomers. Therefore, the concept of enantioseparation effectiveness (α) is proposed according to Schuur et al,[36] which is calculated according to Equation (4):

$$\alpha = e.e. \times Y, \quad (4)$$

where Y represents the yield of L-Phe in the solid phase, $0 \le Y \le 1$; e.e. is the excess value of enantiomer in the solid phase, $0 \le$ e.e. ≤ 1. D-Phe is the target product in the liquid phase and also has α value corresponding to the solid phase. The resolution effect can be evaluated through the combination of above indexes, which can reflect the application value of the resolution system comprehensively.

2.6 | Resolution mechanism research

The mechanism of complex-precipitation of racemic amino acids was analyzed by the following methods. The solid phase were washed with deionized water, then dried and the dark blue powders were obtained. The product was firstly analyzed by FT-IR analysis (KBr disc) in the wavenumber range of 400 to 4000 cm^{-1}; then thermogravimetric analysis (TG) was performed on STA 449 F3 simultaneous thermal analyzer (NETZSCH, Selb, Germany), and heating rate was set at 10°C/min in the temperature range of 30°C to 800°C and under nitrogen

atmosphere. Finally, and a manual docking was carried out among three ligands in the obtained complexes, and the binding energy of the two kinds of complexes with different configuration was calculated.

3 | RESULTS AND DISCUSSION

3.1 | Specific optical rotation and solubility of CILs

The measurable specific optical rotation ($[\alpha]_D^{20}{}_{ILexp}$) of four ionic liquids is −27.75 ([$BuEt_3N$][L-Pro]), −25.27 ([BuPyro][L-Pro]), −27.06 ([BuIm][L-Pro]), and − 30.18 ([BuTr][L-Pro]), respectively. The results indicate the specific optical rotation values of amino acid based ionic liquids with different cations are relatively close. So it can be concluded that the optical activity mainly originates from the anion of L-proline. Moreover, the solubility properties of four kinds of CILs in 12 common organic solvents are listed in Table 1. Four kinds of amino acid ionic liquids are all soluble in polar and intermediate polar solvents and insoluble in weak polar solvents. It has been proved that these ionic liquids possess high polarity, and all of them can be dissolved in the listed solvents with the polarity higher than or equal to that of acetone. They have the same side chain length and L-proline anion, so their difference in solubility is mainly determined by the cationic parent structure. Triethylamine, N-methylpyrrolidine, N-methylimidazole, and tropine as cationic nucleuses have different polarity and interaction with solvent molecules, which directly influence the solubility of CILs in different solvents. Related investigation for above solubility behaviors can lay the foundation for their application and recovery.

3.2 | Resolution of racemic amino acids

3.2.1 | Screening on CILs

Four kinds of amino acid anionic ionic liquids with different cationic structure are compared in equal mole, including straight chain-type ($[BuEt_3N]^+$), cyclic alkane-type ($[BuPyro]^+$), cyclic aromatic type ($[BuIm]^+$), and polycyclic type ($[BuTr]^+$). Actually, the formation process of coordination unit between metal ions and other ligands besides water in aqueous solution should be considered as the process that the water molecules around the metal ions of Cu^{2+} are substituted by other ligands. As long as the alkyl chain on the cations of ionic liquids reaches a certain length, the resulting hydrophobic region will be strong enough around the ligands which can hinder the formation of hydrated copper ions. It is beneficial to reduce the competition between water molecules and ionic liquids and improve the stability of ternary complex. In the ternary complex (L-Pro-Cu (II)-D/L-Phe), copper ion containing space orbitals play the role of central atom, CIL and phenylalanine with coordination atoms (oxygen atom and nitrogen atom) can provide the lone pair electrons as ligands. So the butyl-substituted chain was finally selected on four kinds of cations through preliminary experiments. As a result, the e.e. values of D- and L-Phe in solid and liquid

TABLE 1 Solubility properties of four ILs in common solvents at room temperature[a]

Solvent	[$BuEt_3N$][L-Pro]	[BuPyro][L-Pro]	[BuIm][L-Pro]	[BuTr][L-Pro]
Water	Very easily soluble	Very easily soluble	Easily soluble	Very easily soluble
Ethanol	Very easily soluble	Very easily soluble	Easily soluble	Very easily soluble
Methanol	Easily soluble	Freely soluble	Easily soluble	Very soluble
Acetonitrile	Easily soluble	Freely soluble	Easily soluble	Soluble
Acetone	Soluble	Soluble	Soluble	Soluble
Chloroform	Soluble	Soluble	Insoluble	Soluble
Ethyl acetate	Sparingly soluble	Sparingly soluble	Insoluble	Slightly soluble
Dichloromethane	Sparingly soluble	Slightly soluble	Insoluble	Slightly soluble
Ether	Slightly soluble	Slightly soluble	Insoluble	Slightly soluble
Toluene	Very slightly soluble	Very slightly soluble	Insoluble	Very slightly soluble
Hexane	Insoluble	Insoluble	Insoluble	Insoluble
Petroleum	Insoluble	Insoluble	Insoluble	Insoluble

[a]According to Chinese pharmacopoeia, basic solubility of substance is defined as followings: very easily soluble: 1 g solute can be dissolved in less than 1 mL solvent, easily soluble: 1 g solute can be dissolved in 1 to 10 mL solvent, soluble: 1 g solute can be dissolved in 10 to 30 mL solvent, sparingly soluble: 1 g solute can be dissolved in 30 to 100 mL solvent, slightly soluble: 1 g solute can be dissolved in 100 to 1000 mL solvent, poor soluble: 1 g solute can be dissolved in 1000 to 10 000 mL solvent, insoluble: 1 g solute cannot be dissolved in 10 000 mL solvent.

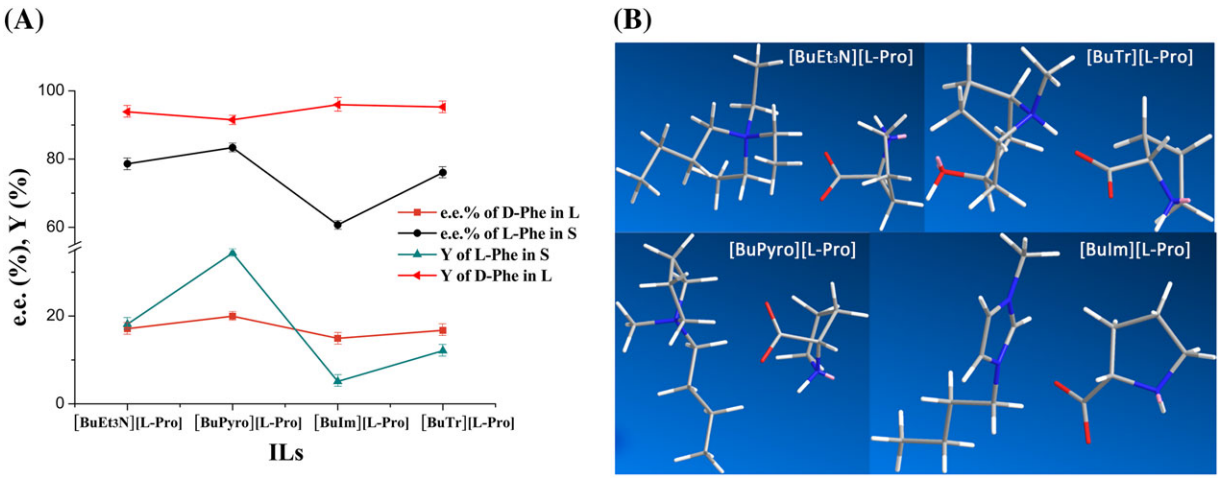

FIGURE 2 Comparison of various CILs on e.e. value (%) and yield

phases in resolution of phenylalanine by four CILs are shown in Figure 2A. Related experimental conditions were as follows: 6.0×10^{-4} mol ionic liquid,7.2×10^{-4} mol Cu $(Ac)_2$, 1 mL deionized water, 10 mg D,L-Phe and constant temperature at 5°C. It can be seen from Figure 2A that e.e. value of enantiomer in solid phase is higher than that in liquid. The precipitated particles could be regularly arranged as blue needle crystals, and the generated solid phase is more ordered than the liquid phase, so the entropy is reduced after precipitation. Consequently, the isomer which can form the more stable complex is preferred to be precipitated. On the other hand, there is an obvious difference using different cationic CILs to resolute racemic amino acid, the result shows [BuPyro][L-Pro] has the best resolution result, so the following scale-up and recovery experiment selected [BuPyro][L-Pro] as object to carry out our research. It can indicate that this kind of ionic liquid has the strongest complexation with copper ion and L-Phe, so the hydrophobic ternary complex is easy to form. If the Van der Waals volume without solvent sphere of interacting groups is large or there is great steric hindrance near the coordination atoms on multidentate ligand, they can interfere with the chelating formation, or/and reduce the stability of ternary complex, thus affecting the separation performance. L-proline is the anion of the CILs, and it is also the origin of the chirality of ionic liquids. The coordination atoms in ligands directly provide the lone pair electrons to form the coordination bonds with the central atom (copper ion), which are oxygen atom of carbonyl-group and nitrogen atom of amino-group in the proline-based anion of CILs. Furthermore, the steric hindrance of the cation moiety influences the interaction of coordination atoms and copper ions, and then results in the different resolution performance of CILs. Besides that, the cation moiety can also interact with these chelating groups on the anion. If their interaction is stronger, it is less conducive to the chelation between the anion and other complex parts. In order to explore the interaction between the anion and cation, Gaussian 09 (Gaussian, Inc., Wallingford CT) was used to obtain the energy-optimized structures of four CILs, which are shown in Figure 2B. The method selected was Ground State at the level of HF/6-31G(d). As a result, the calculated distance between the anion and cation center is accorded with the order of [BuPyro][L-Pro] > [$BuEt_3N$][L-Pro] > [BuTr][L-Pro] > [BuIm][L-Pro], which can prove above effect resulting from the cationic structure.

3.2.2 | Effects of various conditions on enantioseparation results

In the process of using CIL [BuPyro][L-Pro] to separate target racemic phenylalanine, the involved experimental conditions were investigated systematically, which included the types of metal salts, the molar ratio of Cu (II) to CILs, pH value, the amount of racemic phenylalanine, and temperature. The detailed influence of different factors on the e.e. value and yield are shown in Figure 3A-D.

Types of metal salts

According to our preliminary experiment and literature,[37] the combination of L-proline as the chiral ligand and Cu (II) as the central atom has strong enantiomeric recognition ability. Hence, a series of copper salts were firstly selected for further study. In order to make comprehensive comparison and then obtain the ideal resolution effect, other transition metals containing space orbitals such as $Fe^{3+}/Co^{2+}/Cr^{6+}/Zn^{2+}$ were also considered. As the result, eight kinds of salts including Cu $(Ac)_2$, $CuSO_4$, $CuCl_2$, $CuBr_2$, Zn $(Ac)_2$, $CoCl_2$, $CrCl_6$, and $FeCl_3$ were studied in this experiment under the following

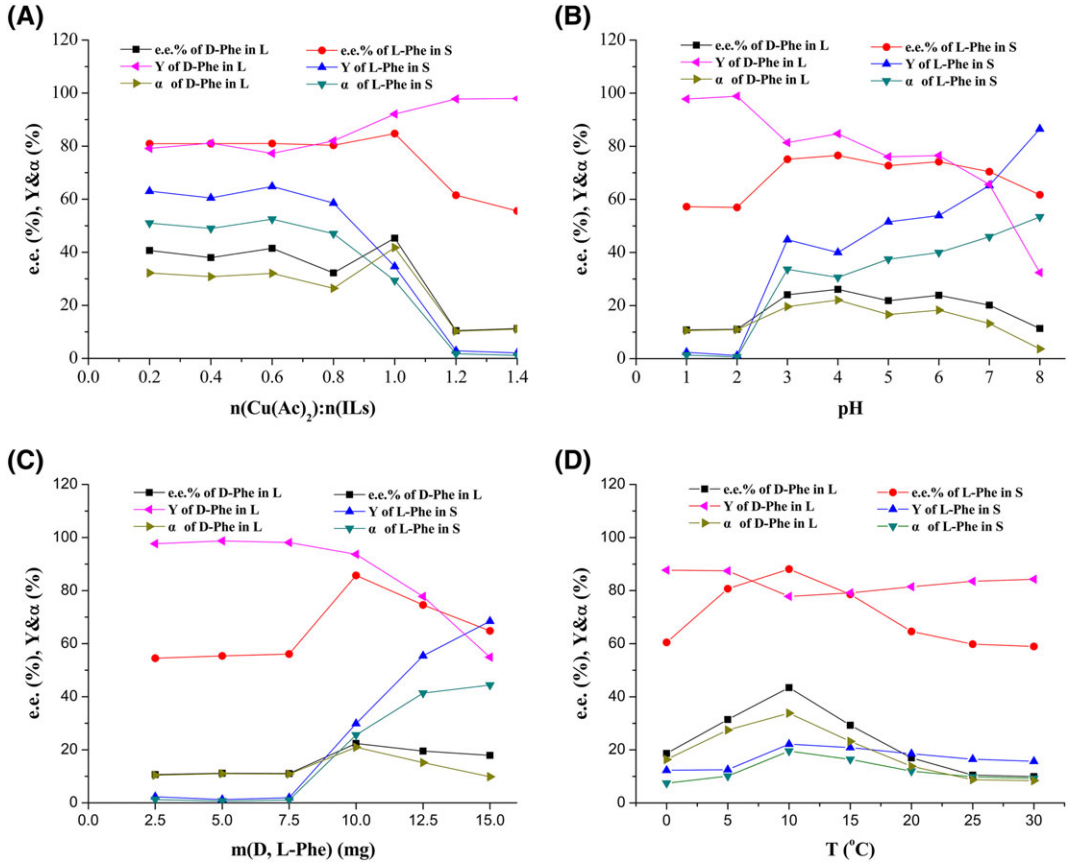

FIGURE 3 Effects of various factors on the enantioseparation results

conditions: 6.0×10^{-4} mol [BuPyro][L-Pro], metal ion-to-IL molar ratio = 1.2, 10 mg D,L-Phe, 1 mL deionized water, 5°C. It can be seen from Table 2 that only metal salts of Cu $(Ac)_2$, Zn $(Ac)_2$, $CoCl_2$, and $CrCl_6$ can precipitate complexes solid in the same conditions. Among them, the solid complex precipitated by salt Cu $(Ac)_2$ has the highest e.e. value. The less hydrophilic anion will promote the precipitation of ternary complex to a certain extent. On the contrary, the anion with strong hydrophilicity cannot only form ion pairs with copper ions but also form hydrogen bonds with the water molecules, so the solubility of the complexes in water will increase, which is not beneficial for the improvement of their yield.

TABLE 2 Effect of salt types on the separation results

Salts	If There is Any Complex	e.e.% of L-Phe in Solid Phase
Cu $(Ac)_2$	Y	75.3%
$CuSO_4$	N	-
$CuCl_2$	N	-
$CuBr_2$	N	-
Zn $(Ac)_2$	Y	3.4%
$CoCl_2$	Y	10.2%
$CrCl_6$	Y	10.9%
$FeCl_3$	N	-

Cu (II)-to-IL molar ratio

Effect of Cu (II)-to-IL molar ratio was explored under the following conditions: 6.0×10^{-4} mol [BuPyro][L-Pro], 10 mg D,L-Phe, 1 mL deionized water, 5°C. The results of investigating the ratio of copper salt to ionic liquid [BuPyro][L-Pro] are illustrated in Figure 3A. When the molar ratio of Cu $(Ac)_2$ to CIL is 1, the e.e. value of L-Phe in solid phase reaches its maximum (80.99%). Meanwhile, when the molar ratio of Cu $(Ac)_2$ to ILs is 0.6, the yield of L-Phe peaks at 60.59%. According to previous studies, the activity coefficient of ions will decrease with the increase of ionic strength within a certain range.[38] The resolution mechanism in this paper is due to the different stability of the ternary complex formed by racemic amino acids in water. When the Cu (II)-to-IL molar ratio changes, the varied ionic strength of the solution will affect the activity coefficient of all ions participating in the coordination reaction, thus affecting the stability of complexes with different configuration. When the ionic strength is low, the solubility of complex formed by L-Phe is lower than that formed by D-Phe, and the solubility of both the two complexes will decreases with the increase

of ionic strength in a certain range. According to the experimental results, it is found: (1) When the molar ratio of Cu $(Ac)_2$ to CIL is increased from 0.2 to 1, the e.e. value of L-AA complex in solid phase will become higher, and the solubility of L-AA complex will decrease with the increase of ionic strength. In this range of molar ratio, the L-AA complex can become more and more stable in solution, which is also more stable than D-AA complex; (2) when the molar ratio is increased from 0.2 to 0.6, the e.e. value of L-Phe in the solid phase will rise and the yield is increased. If the molar ratio is further increased from 0.6 to 1.4, the ligand of ionic liquid will be used up. So L-Phe has to combine with the large amount of free Cu^{2+} ions to form water-soluble complex, which can make the precipitation amount less and reduce the separation efficiency. As the result, the e.e. value of L-Phe in the liquid phase will decrease with the increase of Cu (II)-to-IL molar ratio, while the percentage of D-Phe in the solid phase is also increased, resulting in the rapid decrease of e.e. value in solid phase under high salt concentration.

pH value

The change of hydrogen ion concentration in the solution often affects the coordination equilibrium through the protonation with AAs or their ions. Here sodium acetate and acetic acid were used to adjust pH value in the following system at 5°C: 6.0×10^{-4} mol [BuPyro][L-pro], Cu (II)-to-IL molar ratio = 1, 10 mg D,L-Phe, 1 mL deionized water with different pH values. Because the ligand in ternary complex is amino acidic ion rather than strongly acidic ion, H^+can combine with it to regenerate amino acid when the concentration of hydrogen ions is increased in aqueous solution or the value of pH becomes lower. According to above mechanism, the concentration of hydrogen ions in solution can affect the coordination equilibrium and the stability of complex. As can be seen from Figure 3B, when the pH value is varied from 3 to 7, the e.e. value of the solid phase is less affected; and when the pH value is 4, the e.e. value reaches its maximum (81.08%). It can be concluded that strong acidic or alkaline environment is not conducive to the separation of racemic amino acids. N atom in phenylalanine can bind with proton in strong acidic conditions to form quaternary ammonium ions and its lone electron pair will be occupied,[39] which cannot make it coordinate with Cu^{2+} ion to form a complex. When the pH value reaches 8, it can be observed that the complex amount is more than that obtained in acidic condition, but the e.e. value decreases. More importantly, there is the blue cotton-like sediment appearing in the solution. It is because the copper ions can be hydrolyzed to become copper hydroxide precipitation when the pH value is 8. Therefore, the alkaline environment should be avoided for efficient resolution.

Amino acid amount

Effect of amino acid amount was explored under the following conditions: 6.0×10^{-4} mol [BuPyro][L-Pro], 6.0×10^{-4} mol Cu $(Ac)_2$, 1 mL deionized water (pH = 4), 5°C. As can be seen from Figure 3C, if the concentration of amino acids is low, the resulting complex is less likely to be agglomerated and the resolution is less selective. As the amount of amino acid increases, the e.e. value in the solid phase rises rapidly from the lower level and then decreases. When the amount of racemic amino acid is 10 mg, the e.e. value reaches its maximum (81.49%). In summary, the dosage of D,L-Phe is closely related with the concentration of ternary complexs and then has an obvious effect on solid precipitation and separation selectivity. The increase of amino acid is beneficial for more complex and higher e.e. value in a certain degree, but its limited solubility should be fully considered for the possible precipitation together with the complex during centrifuge.

Resolution temperature

Effect of temperature was explored under the following conditions: 6.0×10^{-4} mol [BuPyro][L-Pro], 6.0×10^{-4} mol Cu $(Ac)_2$, 1 mL deionized water, 10 mg D,L-Phe. According to the previous experiments, it was found that the solid precipitation of the ternary complex was very rapid, so the investigation in this section was carried out by controlling the temperature of adding deionized water. As shown in Figure 3D, the e.e. value of L-Phe in the solid phase increases continuously with the increase of temperature. When the temperature rises to 10°C, the e.e. value reaches its maximum as same as the yield of L-Phe. If the resolution temperature is further increased, the thermal motion of molecules will be accelerated and the racemization of the amino acids can be enhanced, so the e.e. value begins to decrease. Excessive temperature is also not beneficial for the yield of L-Phe, because it will improve the solubility of the ternary complex in water and hinder the precipitation process. On the other hand, it was found the temperature below 10°C could accelerate precipitation of solid phase significantly. Under these circumstances, the effect of adsorption and occlusion was more obvious, so the coprecipitation of different enantiomers occurred more easily and then e.e. value would decrease. The whole precipitation process did not need too low temperature, which was also not beneficial for efficient resolution.

As a result, the highest e.e. value 85.72% of L-Phe in solid phase was finally obtained in single separation when the resolution system was composed of 6.0×10^{-4} mol CIL [BuPyro][L-Pro], 6.0×10^{-4} mol Cu $(Ac)_2$, 1 mL deionized water and 10 mg D,L-Phe,

meanwhile 10°C was chosen as the resolution temperature and the pH value was 4.

3.2.3 | Comparison with the separation results of nonaromatic AAs

In order to find more rules in separation of racemic amino acids with the developed method, D,L-serine and valine are selected as the representatives of nonaromatic AAs and their resolution results are shown in Table 3. It shows that the resolution of D,L-valine was better than that of D,L-serine, because the water solubility and stability of the complexes formed by different amino acids and chiral ligands are different. However, the enantioseparation result of D,L-Phe is better than theirs, and it is obvious the difference of the coordination between D/L-AA and Cu (II) will be more significant if there is a group with larger Van der Waals volume in the structure of AA, which can result in enough steric hindrance for effective chiral recognization.[40]

3.3 | Scale-up and posttreatment research for the resolution of amino acids

In order to further investigate the multiplication performance of above method for enantioseparation of amino acids, it was designed about a tenfold scale-up experiment based on the screened experimental conditions (0.6 mol/L [BuPyro][L-Pro] and Cu $(Ac)_2$ aqueous solution, 0.1 g D, L-Phe, 10°C). Furthermore, in the previous studies of similar resolution, the posttreatment of obtaining a single configuration of the target amino acids from complexes was rarely mentioned, so it is difficult to be applied in the actual preparation and the recovery of ionic liquids has not been effectively realized, which will cause the waste of expensive CILs and increase the operation cost. It also cannot meet the requirements of green chemistry. In this section, an ionic liquid recovery strategy and actual procedure to obtain target amino acids with single configuration are proposed as following. After the resolution, the system was centrifugally filtrated to remove solid phase, and the liquid phase flowed through 201 × 7 type anionic exchange resin and then the alkaline solution containing Na^+ and $[Bupyro]^+$ was obtained. Then the solution was neutralized with L-proline ethanol solution after concentration, and sodium salt was filtered and the solvent was removed by vacuum distillation to acquire the CIL. As for the solid phase resulting from complex-precipitation, 0.1 M HCl aqueous solution was used to dissolve it and adjust the pH to 7, and L-proline anions and phenylalanine were transformed into free amino acids which could be precipitated and removed by 10 000 r/min centrifuge for 5 minutes. Based on the solubility difference of L-phenylalanine and L-proline, the former was obtained by washing with water for three times.

TABLE 3 The properties of nonaromatic AAs and their enantioseparation results[a]

AAs	pI	S/g ($100\ g^{-1}$)	M (mg)	e.e. value of L-Phe in solid phase
D,L-serine	5.68	38	30	55.55%
D,L-valine	5.68	4.50	20	63.50%

[a]S is the solubility of the amino acids in water at 25°C; M is the amount of the amino acid.

In order to further investigate the reliability and practicability of above separation conditions, a scale-up experiment for separating the D- and L-Phe using ionic liquid [BuPyro][L-Pro] was conducted. Both of the volume of [BuPyro][L-Pro] and Cu $(Ac)_2$ aqueous solution and D,L-phenylalanine amount were magnified tenfold. It was found the e.e. value of solid phase of L-Phe in the amplified experiment was 80.5% in single resolution, which was slightly reduced compared to the e.e. value (85.7%) of the solid phase in the small-scale test before. However, there is no obvious change of the e.e. value of liquid phase. The overall resolution effect is basically satisfied, and it was helpful to obtain the higher e.e. value of solid phase by reducing the amount of amino acid in the scale-up system. L-phenylalanine could be recovered in the solid phase according to above posttreatment procedures, and the supernatant (containing $[BuPyro]^+$ and Cl^-) could be directly used as the raw material and react with L-pro through the anion exchange resin to reacquire the CIL of [BuPyro][L-Pro].

3.4 | Characterization of ternary complex and resolution mechanism

In aqueous solution, the copper ions can be coordinated with adjacent water molecules as ligand to form hydrated ions. So it is known that the so-called free metal ions in water solution are actually hydrated metal ions. Therefore, the formation process of coordination unit between metal ions and other ligands besides water in aqueous solution should be considered as the process that the water molecules around the metal ions of Cu^{2+} are substituted by other ligands.

According to ligand-field theory, Cu^{2+} belongs to the $3d^9$ electronic configuration. In the electrostatic field of asymmetric ligand, the energy improvement of the five degenerated orbits (d_{xy}, d_{xz}, d_{yz}, d_z^2, $d_{x^2-y^2}$) is different, and the splitting of energy levels will occur which leads

to the generation of new orbits. During the coordination process, the six ligands are distributed along six directions of *x*, *y* and *z* axes and approach towards *d* orbital of Cu^{2+} ions to form octahedron. As the result, the electric field forces vary in different orbits, d_z^2 and $d_{x^2-y^2}$ are affected apparently by electrostatic repulsion and their energy rise remarkably, then the double-fold degenerate orbit of d_γ or e_g forms. On the other hand, d_{xy}, d_{xz}, and d_{yz} result in threefold degenerate orbit of d_ε or t_{2g}, which have lower energy. Among them, t_{2g} and e_g orbits will split into low-energy and high-energy π orbitals when coordinate bonds form; the energy improvement of the former is less, the distance between Cu^{2+} and ligand is longer and interaction is weaker; the latter is completely opposite. As for the coordination process involving CIL with L-proline anion, D,L-Phe and Cu^{2+}, the former two have O and N atoms as ligand teeth in their structures which can result in stronger coordination than CH_3COO^- and H_2O. The ligands surrounding Cu^{2+} ions are substituted by nucleophilic reaction. In the formation of complexes, ligand exchange can be used to explain the separation mechanism (see Equation (5)). The stability constant of the coordination ions is the same as all other equilibrium constants, which can be very different under external conditions. In the formation of complex, all the factors mentioned in Section 3.2 will affect the coordination equilibrium.

$$\begin{aligned} &Cu^{2+}([BuPyro][L{-}Pro])_x(H_2O)_y(Ac)_z + L{-}Phe \rightarrow Cu^{2+} \\ &\quad ([BuPyro][L{-}Pro])_x(L{-}Phe)_y(Ac)_z\downarrow + H_2O \\ &Cu^{2+}([BuPyro][L{-}Pro])_x(H_2O)_y(Ac)_z + D{-}Phe \rightarrow Cu^{2+} \\ &\quad ([BuPyro][L{-}Pro])_x(D{-}Phe)_y(Ac)_z + H_2O. \end{aligned} \tag{5}$$

As mentioned above, the amino acid ions (both proline and phenylalanine) have two coordination atoms of O and N, which often combine with the central atom to form chelating individual. They are located on the same plane as copper (II) and the same chemical atoms are in the para-position; thus, the system has the lowest energy and is stable. Consequently, α- and β-amino acids used to form the five- and six-membered chelating ring, respectively; and the coordination ratio is usually 1:1. The TG diagram of precipitated complex is shown in Figure 4A. In the temperature range of 30 to 160°C, the first stage of weightlessness (1.95%) occurs for the loss of bound water in the complex. During 160 to 250°C, the second stage of weightlessness (53.98%) is mainly related with the decomposition of the ionic liquid and phenylalanine in the precipitates. During 280 to 320°C, the third stage of weightlessness (11.78%) accords with the decomposition of the acetate radical according to the following

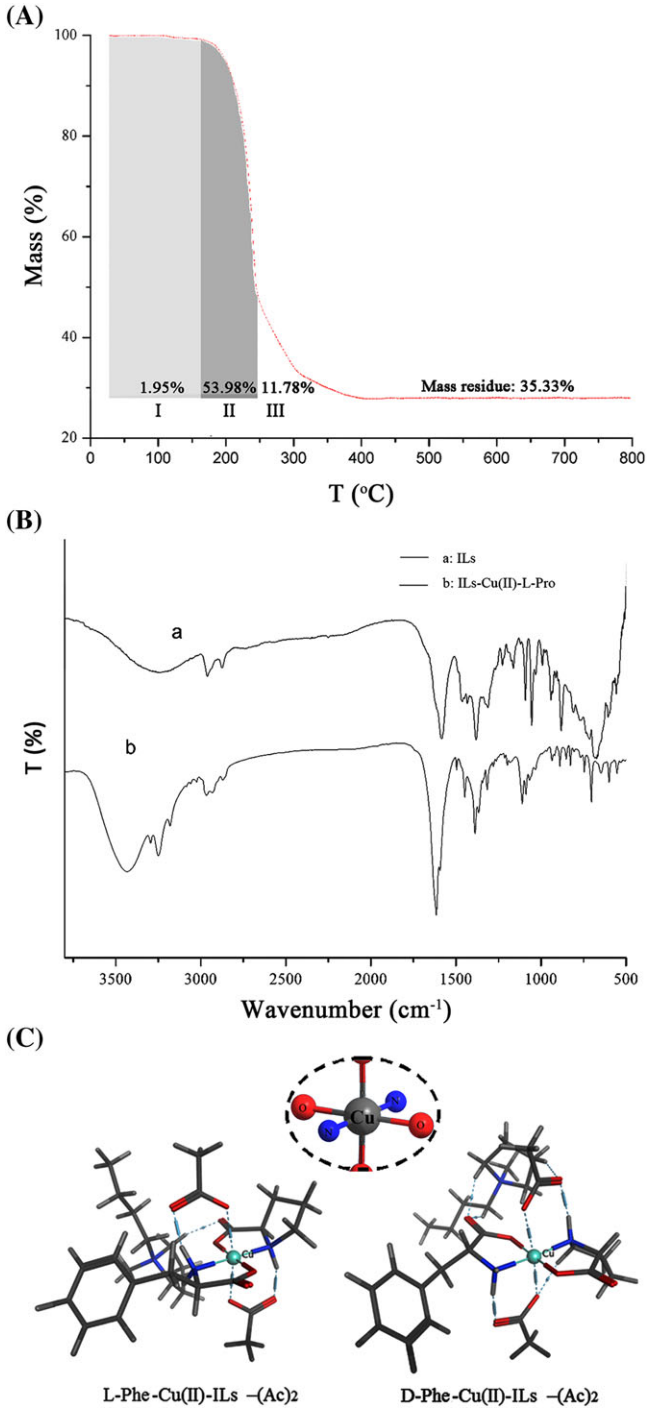

FIGURE 4 A, Thermogravimetric analysis and B, FT-IR of precipitated complex together with C, molecular modeling of ternary complex

equation:$2Cu(CH_3COO)_2 \rightarrow Cu_2O + C_2H_6 + CH_4 + CO_2 + 4CO + H_2O$. Finally, the residue of Cu_2O accounted for 35.33% of the total mass. Based on these thermogravimetric data and the contents of H, C, and N element measured by EA3000 elemental analyzer (Euro vector S.P.A, USA), it can be calculated and proved that CIL to L-Phe to Cu $(Ac)_2$ approximates to 1:1:1 in the

composition of blue complex, which can prove that Cu (II) is coordinated with six ligands and octahedral geometric configuration in the complex.

For further structural analysis, the infrared spectrum of the ternary complex is shown in Figure 4B. The main IR data of the ternary complex are as follows: IR (KBr, cm^{-1}): 3435, 3295.27, 3249, 3180, 2966.07, 1615.51, 1496.09, 1448.24, 1387.86, 1366.92, 1316.3, 1396, 1198.29, 1110.75, 1088.34, 935.94, 889.09, 851.92, 827.04, 745.5, 704.22, 647.31, 600, and 553.29. In which, the peak at 3435 cm^{-1} accords with the absorbance of residual water, two peaks at 3295 and 3249 cm^{-1} belong to ν_{as} and ν_s of primary amine group in L-Phe. The absorbance of secondary amine group in the anion of ionic liquid is weak which is covered by the adjacent peaks. The signal at 3180 cm^{-1} is related with ν_{as} and ν_s of N^+CH_3 and Ar-H, and the peak slightly below 3000 cm^{-1} can be ascribed to the absorbance of stretching vibration of $-CH_3$ and $-CH_2-$. Because of the oxygen atom of carbonyl group interacts with copper ion to form coordination bond, which results in redshift of absorbance peaks. So 1615.51 cm^{-1} is lower than the wavenumber of $\nu_{as\ C=O(COOH)}$ in uncoordinated CIL. Meanwhile, the absorbance at 1387 cm^{-1} belongs to $\nu_{s\ C=O(COOH)}$ of CIL anion in the ternary complex. Moreover, because of the nitrogen atom of amino acid can also coordinate with copper ion, the C-N vibration is red-shifted to 1400 to 1350 cm^{-1}. The peaks in the range of 1200 to 1000 cm^{-1} accords with the absorbance of stretching vibration of C-O and C-C, and the signals at 745 and 704 cm^{-1} can be ascribed to the out-of-plane bending of Ar-H in L-Phe.

In the following calculation research, molecular structures were firstly developed in Gauss-View 5.0, and then they were optimized at the level of HF/6-31G(d) (Method: Ground State) with Guassian 09 software (Gaussian, Inc, Wallingford Connecticut) to obtain the configurations with lowest energy. In the quantitative calculation for coordination, the part of L-Pro-Cu (II)-D/L-Phe was selected as host (main body) and $[BuPyro]^+$ was treated as guest. The binding energy ($\triangle G$, kcal/mol) can be obtained according to Equation (6).

$$\triangle G_{bind} = G_{complex} - \left(G_{host} + G_{guest}\right), \quad (6)$$

where $G_{complex}$ is Gibbs free energy of the complex, G_{host} is Gibbs free energy of L-Pro-Cu (II)-D/L-Phe, G_{guest} is Gibbs free energy of $[BuPyro]^+$. If the value of binding energy of the complex is negative, it indicates that the coordination compound can be formed between the host and guest, and the lower the binding energy, the easier to form a stable complex. The Gauss-View software was also used to depict the spatial structure and the interaction difference between D,L-AA complexes. The results are shown in Figure 4C and Table 4, which prove that the enthalpy is negative. So the coordination is a spontaneous process, and the binding energy of L-Phe-Cu (II)-CILs is less than that of D-Phe-Cu (II)-CILs, indicating the L-complex is more stable, consistent with the experimental results. The results of energy optimization and spatial structure of D,L-complexes also prove that the main part of ionic liquids participating in the formation of complexes is the anion of L-proline. When N atom in the cationic nucleus becomes the quaternary ammonium form, it will also form an atypical hydrogen bond with the coordination center of Cu (II).

TABLE 4 The calculated energy results of complex (D,L-Phe-Cu (II)-CILs)

Configuration of Complex	$\triangle H$ (kcal/mol)	$\triangle G$ (kcal/mol)
D-Phe-Cu (II)-CILs	−64.48	−53.12
L-Phe-Cu (II)-CILs	−64.89	−54.25

4 | CONCLUSION

In this study, a series of CILs with L-proline anion were synthesized by using triethylamine, N-methyl pyrrolidine, N-methylimidazole, and tropine as cationic nuclear, and their specific optical rotation and solubility in different common solvents were determined after structural identification. Moreover, the properties of CILs separating D,L-Phe by complex-precipitation method were investigated. The relationship between cationic structure of CILs and their performance was discussed, and the effects of main factors on the separation results of racemic phenylalanine using these ionic liquids were studied in detail. The suitable conditions for resolution D,L-Phe in this experiment are 6.0×10^{-4} mol CIL [BuPyro][L-pro], 6.0×10^{-4} mol Cu $(Ac)_2$, 1 mL deionized water (pH = 7), 10 mg D,L-Phe and constant temperature at 10°C. As the result, 85.7% e.e. value of L-phenylalanine in solid phase could be achieved. After tenfold scale-up of the resolution system, it still showed an ideal resolution performance. Finally, the study of resolution mechanism indicated that the electron pairs of CILs anion and spatial configuration of CILs played important role in the resolution process.

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