Talanta 97 (2012) 349–354

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Ionic liquids with amino acids as cations: Novel chiral ligands in chiral ligand-exchange capillary electrophoresis

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ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form
12 April 2012
Accepted 21 April 2012
Available online 30 April 2012

Keywords:
Amino acid
Capillary electrophoresis
Enantiomer separation
Ionic liquid
Ligand exchange

ABSTRACT

Ionic liquids (ILs) with L-proline (L-Pro) as cations have been developed for the novel chiral ligands coordinated with Cu(II) in chiral ligand exchange capillary electrophoresis (CLE-CE). Four kinds of amino acid ionic liquids (AAILs), including [L-Pro][CF_3COO], [L-Pro][NO_3], [L-Pro][BF_4] and [L-Pro_2][SO_4], were successfully synthesized. Among them, [L-Pro][CF_3COO] was selected as the model ligand to optimize the separation conditions. The influences of AAIL concentration, pH, and methanol concentration on efficiency of chiral separation were investigated. Then it has been testified that the optimal buffer solution consisted of 25.0 mM $Cu(Ac)_2$, 50.0 mM AAIL and 20% (v/v) methanol at pH 4.0. The interesting thing is well enantioresolution could be observed with [L-Pro][CF_3COO] as the new chiral ligand and nine pairs of labeled D,L-AAs were successfully separated with the resolution ranging from 0.93 to 6.72. Meanwhile, the baseline separation of labeled D,L-AAs could be achieved with the other three kinds of AAILs as ligands. The results have demonstrated the good applicability of AAILs with AAs as cations for chiral separation in CLE-CE system. In addition, comparative study was also conducted for exploring the mechanism of the AAILs as new ligands in CLE-CE.

1. Introduction

Much attention has been focused on the avoidance of toxic and environmentally unfriendly organic solvents for further studies in chemistry. A good example is the increasing use of ionic liquids (ILs) in modern chemistry. ILs are a class of organic salts with a melting point close to ambient temperature [1–2]. They possess unique physicochemical properties, including a negligibly low vapor pressure, high conductivity, and high thermal stability [3–5]. Over the past years, ILs have been widely applied in organic synthesis [6–7] and analytical chemistry [8–10]. Since the properties of ILs are mainly influenced by both their cationic and anionic moieties [11], ILs are thus referred to as "tunable or tailor-made materials" [12–15]. Meanwhile, there are many chances to introduce novel functional groups and design functional ILs with controllable properties or desired functions by changing component ions. Among numerous functional ILs, chiral ILs have extraordinary potential in asymmetric synthesis [16] and separation science [2] owing to their unique properties and high-degree organization. Consequently, considerable efforts have been devoted to design and synthesis of new chiral ILs with requirement.

Since most of amino acids (AAs) have chiral carbon atoms, they are highly promising candidates for chiral ILs, and thus called amino acid ionic liquids (AAILs). In addition, AAs can be straightforwardly derived as chiral cations or chiral anions by protonation of the amino group or deprotonation of the carboxylic acid using a suitable Bronsted base or acid, respectively. Compared with other chiral ILs, these kinds of AAILs have many obvious advantages, including convenient synthesis, low cost and good biodegradability [3]. Ohno and co-workers [4] firstly reported a library of AAILs composed of imidazolium cations and amino acids anions, which showed diverse properties and potential of applications. Recently, this kind of AAILs has been extensively applied in heterogeneous catalysis [17] and chiral separation [2,18]. Although Kou and colleagues [19] had successfully synthesized a series of AAILs with AAs as cations by a simple atom-economic reaction, however, so far to our knowledge, the application of ILs with AAs as cations has not been explored. Because AAs could be protonated by the strong acids (HNO_3, HBF_4, CF_3COOH, H_2SO_4, etc.) to produce the corresponding AAILs structures, this novel generation of ILs is completely green including the synthesis processes and chiral

Abbreviations: ILs, ionic liquids; CLE-CE, chiral ligand exchange capillary electrophoresis; AAIL, amino acid ionic liquid; AA, amino acid; Dns-AA, dansyl amino acid; TFA, trifluoroacetic acid; Dns-Cl, dansyl chloride; L-Pro, L-proline; EOF, electroosmotic flow; Thr, threonine; Phe, phenylalanine; Ile, isoleucine; Ala, alanine; Asn, asparagine; Asp, aspartic acid; Met, methionine; Ser, serine; Tyr, tyrosine

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http://dx.doi.org/10.1016/j.talanta.2012.04.044

centers can be introduced easily. Thus, in consideration of the chiral structures of these ILs with AAs as cations, they are expected to be explored and used in chiral recognition.

As one of the important aspects in chiral recognition, chiral separation has attracted increasing attention since enantiomers indeed have different pharmacological and biological activities. In recent decades, numerous protocols following specific chiral recognition principles have been explored. In addition to high performance liquid chromatography (HPLC) and gas chromatography (GC), capillary electrophoresis (CE) is turned out to be a high-performance enantioseparation strategy. Generally, chiral recognition can be achieved with an enantioselective interaction between the analyte and the chiral selector. Among the various chiral selectors, chiral metal complex has been testified to be a powerful one in chiral ligand exchange capillary electrophoresis (CLE-CE), and it follows the principle of CLE which is based on the formation of diastereomeric ternary mixed metal complex between the chiral ligand and the analyte. The commonly used chiral ligands include AAs (L-arginine, L-lysine, etc.), AA ramifications (L-4-hydroxyproline, N-(2-hydroxyoctyl)-L-4-hydroxyproline, etc.) and other organic acids (L-tartrate, D-saccharic acid, etc.) [20–22]. Unfortunately, these available chiral ligands are limited and might not provide adequate enantioresolution, thus the limitation became the choke point for widening their applications. As a consequence, searching for new ligands becomes an important and imperative issue in CLE-CE system.

In this work, four kinds of AAILs with L-Proline (L-Pro) as cations have been explored as novel chiral ligands in CLE-CE system. [Pro][CF_3COO] coupled with Cu(II) was chosen as the model chiral selectors to evaluate the separation conditions when three pairs of labeled D,L-AAs were used as the model analytes. Meanwhile, the influences of AAIL concentration, buffer pH, and organic modifier on resolution were investigated in detail. Furthermore, the mechanism of the AAILs as new ligands in CLE-CE system was explored.

2. Experimental

2.1. Chemicals

All D- and L-AA standards, dansyl chloride (Dns-Cl) were purchased from Sigma-Aldrich Chemical Company (St. Louis, USA). Trifluoroacetic acid (TFA), nitric acid, fluoboric acid, hydrochloric acid, acetonitrile, methanol, and ethylamine were obtained from Aladdin Chemistry Company (Shanghai, China). Copper acetate monohydrate, sodium hydroxide, sulphuric acid, lithium carbonate and other reagents were all from Beijing Chemical Factory (Beijing, China). All reagents used in this work were analytical grade and used without further purification.

All aqueous solutions were prepared with triply distilled water and stored at 4 °C. Standard sample solutions were prepared by dissolving 2.0 mg/mL AAs in 40.0 mM lithium carbonate buffer (adjusted to pH 9.5 with 0.1 M HCl), then diluted to desired concentrations with the lithium carbonate solution by $10–10^4$ folds for further work. Peaks were identified by spiking relative standard AAs in samples, and the peaks with increased height were considered to be the targets. In addition, L-AAs were fortified to identify the migration order of enantiomers. All solutions were filtered through 0.45 μm membrane filter and degassed by the ultrasonicator before use.

2.2. Derivatization of AAs

Derivative solution was freshly prepared by dissolving 3.0 mg Dns-Cl in 2.0 mL acetone, and then AAs dansylation was conducted according to the previous literature with minor modification [23]. Briefly, an aliquot of 20 μL 40.0 mM lithium carbonate buffer, 20 μL AA solution (2.0 mg/mL), 20 μL Dns-Cl solution were mixed in a 200 μL vial and kept at room temperature in the dark for 30 min. The derivatization reaction was terminated by addition of 5 μL 2% ethylamine. All the dansyl amino acids (Dns-AAs) solutions were kept at 4 °C before injection.

2.3. Synthesis of AAILs

The AAILs were synthesized according to a previously described method [19]. Briefly, 5.76 g (0.05 mol) of L-Pro was dissolved in 10 mL water, and one mole equivalent of TFA was added dropwise. Then the mixture was heated to 60 °C and stirred for 24 h. Thereafter, the reacted solution was evaporated at 50 °C under vacuum to remove water. The solid was then collected and a solution of acetonitrile/ methanol (90/10, v/v) was added for precipitating excess AAs. After drying the filtrate in vacuum for 12 h at 60 °C, the final product of [L-Pro][CF_3COO] was obtained and the structure was confirmed by NMR (Bruker Avance 400, Switzerland). It should be noted that the NMR spectra were recorded in d_6-DMSO on a 400 MHz instrument with TMS as internal standard.

Other AAILs were prepared similarly except that TFA was replaced by equal mole of nitric acid, fluoboric acid and sulphuric acid.

2.4. CE analysis

The CE instrumental setup involves a 1229 HPLC high voltage power supply (Beijing institute of new technology and application, Beijing, China), a UV detector (Rilips photoelectricity factory, Beijing, China) and a HW-2000 chromatography workstation (Qianpu software, Nanjing, China). The separations were performed in fused-silica capillaries of 60 cm (effective length 45 cm) × 75 μm i.d. (Yongnian optical fiber factory, Hebei, China). The UV detection was set at the cathodic end of capillary and accomplished at 254 nm.

Before use, capillaries were sequentially washed with 0.1 M NaOH and water for 30 min. And between injections they were flushed with 0.1 M HNO_3, water, 0.1 M NaOH, water and running electrolyte for 2 min, respectively. AA samples were siphoned to the capillary for 8 s at 15 cm height and separated at +20 kV. All the separations were carried out at room temperature. The running buffer, unless stated otherwise, was consisted of 25.0 mM $Cu(Ac)_2$, 50.0 mM AAIL and 20% (v/v) methanol, adjusted to pH 4.0 with 1.0 M NaOH. Resolution (R_s) of Dns-AA enantimers was calculated by using the following equation:

$$R_s = 2(t_2 - t_1)/(w_1 + w_2),$$

where t_1, t_2 represent the migration time of D and L-analytes, respectively; and w_1, w_2 are the baseline peak width of corresponding enantiomers.

3. Results and discussion

L-Pro has been proved to be a good chiral ligand in HPLC [24] and CLE-CE [25] system. Herein in this work, the AAILs with L-Pro as the cations were synthesized for chiral separation and characterized by NMR.

3.1. NMR characterization of AAILs

The obtained product of [L-Pro][CF_3COO] was straw yellow crystal, and its NMR results are shown as follows:

^{1}H NMR: δ=8.77–9.76 (s, 2H, NH_2), 4.27 (m, 1H, CH), 3.21 (m, 2H, CH_2), 2.19–2.28 (m, 1H, CH_2), 1.80–2.01 (m, 3H, CH_2, CH_2) ppm.

^{13}C NMR: δ=171.04, 159.28 (4), 115.86 (4), 59.45, 46.67, 28.60, 23.61 ppm.

The results of NMR analysis, which was comparable with the values as mentioned in literature [19], indicated that AAIL [L-Pro][CF_3COO] was successfully synthesized. In addition, the NMR spectra of other three AAILs ([L-Pro][NO_3], [L-Pro][SO_4], [L-Pro][BF_4]) were quite similar with that of [L-Pro][CF_3COO] and published data [19].

3.2. Optimization of separation conditions

To optimize the chiral separation condition, [L-Pro][CF_3COO] was chosen as the chiral ligand for chiral separation in CLE-CE system. By using Dns-D,L-threonine (Dns-D,L-Thr), Dns-D,L-phenylalanine (Dns-D,L-Phe) and Dns-D,L-isoleucine (Dns-D,L-Ile) as the model analytes, the effects of important parameters, such as AAILs concentration, buffer pH and methanol concentration on separation behaviors have been investigated.

3.2.1. Effect of AAIL concentration

Since Cu(II)–AAIL complexes could work as the chiral selectors, the enatiorecongnition ability mostly depends on the complexes formation. To optimize the selector concentration, the influence of Cu(II)–AAIL complex concentration was investigated in the range from 20.0 mM to 70.0 mM when the molar ratio of Cu(II) to chelating AAIL was kept at 1:2 [2,26–28]. As shown in Fig. 1, the R_s increased with the increase of AAIL concentration until the concentration reached 50.0 mM. The results could be explained that in general, high Cu(II)–AAIL complex concentrations will increase the adsorption onto the capillary wall. Then the double-layer thickness and subsequently the zeta potential reduced to yield the decreased EOF, finally resulting in the prolongation of migration time and the improvement of separation resolution [27]. However, obvious peak tailing was observed that compromised the selectivity resulting in the decline of Rs when the concentration was higher than 50.0 mM. As a result, 50.0 mM AAIL ([L-Pro][CF_3COO]) was finally chosen for further study.

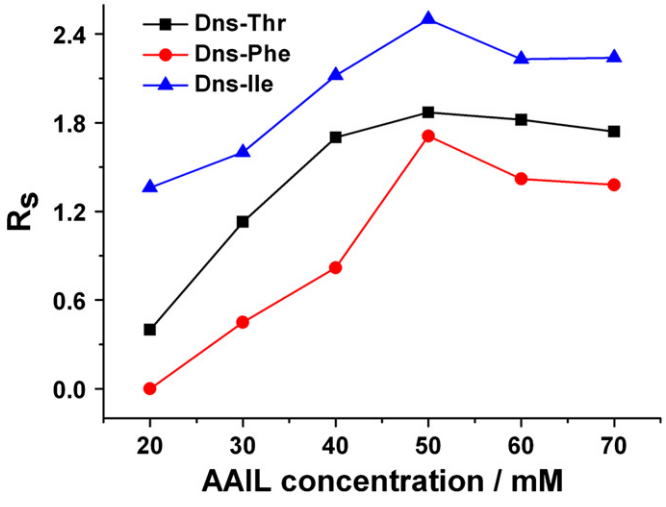

Fig. 1. Influence of AAIL concentration on resolution. The concentration of $Cu(Ac)_2$ was kept at half of the AAIL concentration. Capillary: 75 μm i.d. × 60 cm length (45 cm effective); injection: siphoned for 8 s at 15 cm; voltage: +20 kV; UV detection: 254 nm; room temperature.

3.2.2. Effect of pH on separation

Considering the fact that the complexation among the central ions, analytes and chiral ligands mostly depends on pH, the buffer pH thus has a significant impact on the chiral separation behaviors of CE [29]. In this work, the influence of buffer pH on the separation was investigated in the range from pH 3.8 to 4.6. As displayed in Fig. 2, it has been found that the resolution increased with the increase of pH, but then decreased at the pH higher than 4.0. This phenomenon could be explained by the fact that the stability of formed Cu(II)–AAIL complexes would be enhanced with the pH increase, thus the chiral resolution was increased. However, further increasing of pH would cause high stability of the complexes, which might make it difficult for analytes to replace the ligands from the complexes [28]. Therefore, buffer pH at 4.0 was selected for further analysis.

3.2.3. Effect of methanol concentration

Previous studies [24,30] reported that organic solvents could improve the enantioselectivity of chiral selectors, thus the dependence of resolution of Dns-D,L-AAs on the organic modifier (methanol)

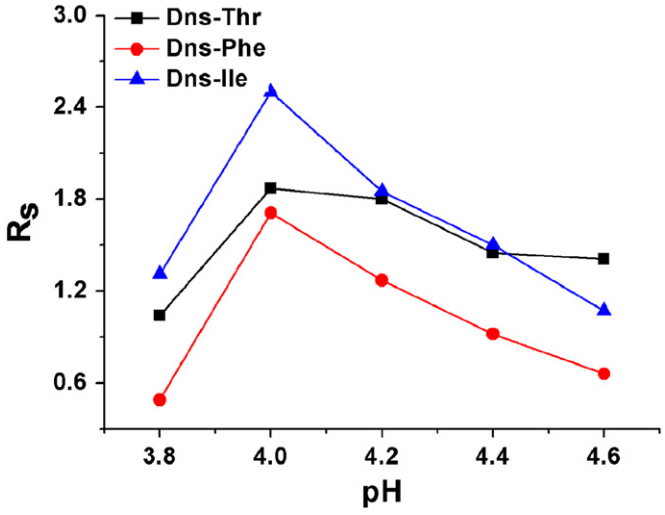

Fig. 2. Effect of buffer pH on the resolution. Buffer conditions: 25.0 mM $Cu(Ac)_2$, 50.0 mM AAIL, 20% (v/v) methanol adjusted to different pHs. Other conditions are the same as indicated in Fig. 1.

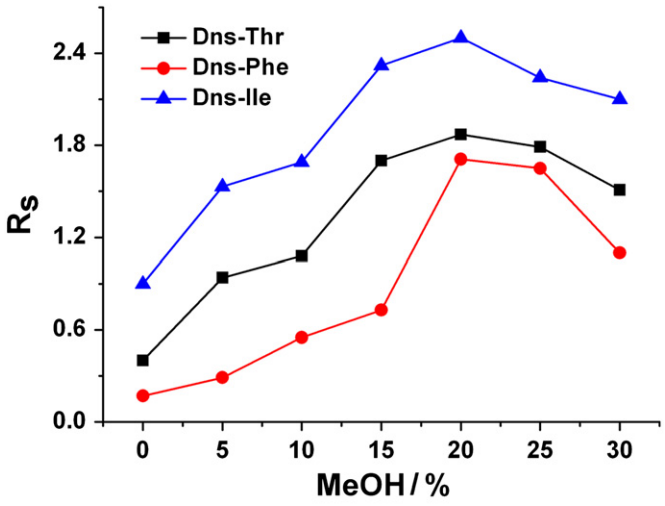

Fig. 3. Effect of methanol concentration on resolution. Buffer conditions: 25.0 mM $Cu(Ac)_2$ and 50.0 mM AAIL at pH 4.0 with different concentration of methanol ranging from 0–30% (v/v). Other conditions are the same as indicated in Fig. 1.

in the running electrolyte, which contained 25.0 mM $Cu(Ac)_2$ and 50.0 mM [L-Pro][CF_3COO] at pH 4.0, was studied in the range of 0%–30% (v/v). As displayed in Fig. 3, the results showed that the enantioselectivity was indeed improved by addition of methanol. It is speculated that because the organic modifier can influence the polarity and viscosity of the buffer solution, and subsequently the ligand exchange reaction can be modulated, finally resulting in the increase of resolution. Meanwhile, it has been found that the retention times were prolonged with the increasing of methanol concentration. For achieving well resolution in short time, 20% methanol was thus selected as the optimum condition for chiral separation in CLE-CE.

Under the optimal condition, the well-separated electropherogram of three model analytes was shown in Fig. 4. Meanwhile, the separation of other AA enantiomers was investigated, as demonstrated in Table 1, seven pairs of Dns-D,L-AAs showed baseline separation and two pairs were partially resolved. Furthermore, the results indicated that the L-enantiomers invariably ran faster than the corresponding D-enantiomers. It implied that the interaction of Dns-D-AA with Cu(II)–AAIL was stronger than that of Dns-L-AA with Cu(II)–AAIL.

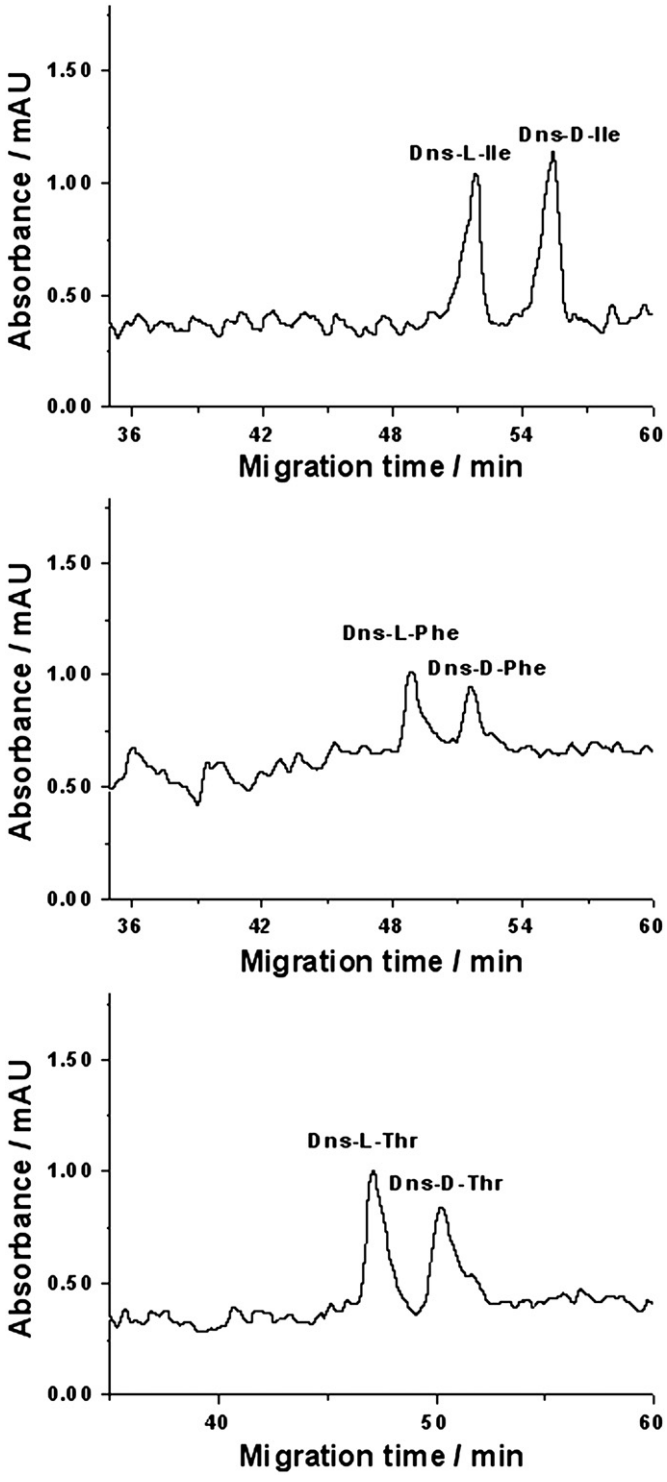

Fig. 4. Enantioseparation of Dns-D,L-Ile, Dns-D,L-Phe, Dns-D,L-Thr using [Pro-CF_3COO] as the chiral ligand. Buffer condition: 25.0 mM $Cu(Ac)_2$, 50.0 mM [Pro-CF_3COO] and 20% (v/v) methanol at pH 4.0. Other conditions are the same as indicated in Fig. 1.

3.3. Separation performed by other chiral ligands

To extend the range of ligand alternatives, the separation was conducted by using other three kinds of synthesized AAILs with different anions ([L-Pro][NO_3], [L-Pro][SO_4], [L-Pro][BF_4]) as the ligands and Cu(II) as the central ion. As shown in Fig. 5, the effective separation could be achieved by using these kinds of AAILs, indicating that AAILs derived from AAs as the cations with different anions have the potential to be explored as the effective ligands. The results demonstrated the wide availability of this Cu(II)–AAIL system for chiral separation in CLE-CE.

3.4. Mechanism exploration

To explore the separation mechanism of AAILs, which are favorable alternatives as chiral ligands coordinating with metal ions for chiral separation in the CLE-CE system, comparative experiments with L-Pro as a chiral ligand were conducted under the same condition as displayed in Fig. 6. Notably, AAILs showed a significant superiority over the conventional AA ligands. Furthermore, the control experiment was performed by using L-Pro (50.0 mM) and TFA (50.0 mM) as the binary additives. Interestingly, the enantioseparation performance was better than that of L-Pro system, but inferior to that of AAIL system, indicating that the use of AAILs is not simply equal to mixing of AAs and corresponding anions as the binary additives. The results illuminated that AAILs played a special role in this enantiorecognition system.

According to the descriptions in literatures [31,32], most of ILs tend to be ionized in aqueous solution. Therefore, the performance of [L-Pro][CF_3COO] could be discussed from the influences of $[CF_3COO]^-$ and $[L\text{-Pro}]^+$, respectively.

It is reported that the counter-ions of Cu(II) in buffer solutions has a crucial effect in the kinetics of metal complex formation in a CLE-CE environment, thus enantioseparation effeciency is strictly related to the counter-ions [33]. Based on our experiment results, the combined use of L-Pro and TFA as the binary additives

Table 1
Enantioseparation of Dns-D,L-AAs under optimum conditions.

Dns-D,L-AAs	R_s	t_L (min)	t_D (min)
Dns-D,L-Ala	1.14	57.91	58.89
Dns-D,L-Asn	3.44	30.92	33.85
Dns-D,L-Asp	6.72	64.73	75.20
Dns-D,L-Ile	2.50	51.83	55.43
Dns-D,L-Met	1.55	58.18	60.53
Dns-D,L-Ser	0.93	45.23	46.17
Dns-D,L-Phe	2.25	48.45	51.23
Dns-D,L-Thr	1.87	46.56	49.75
Dns-D,L-Tyr	1.82	39.48	40.95

R_s: the resolution between Dns-L-AA and Dns-D-AA. t_L: the migration time of Dns-L-AAs. t_D: the migration time of Dns-D-AAs.

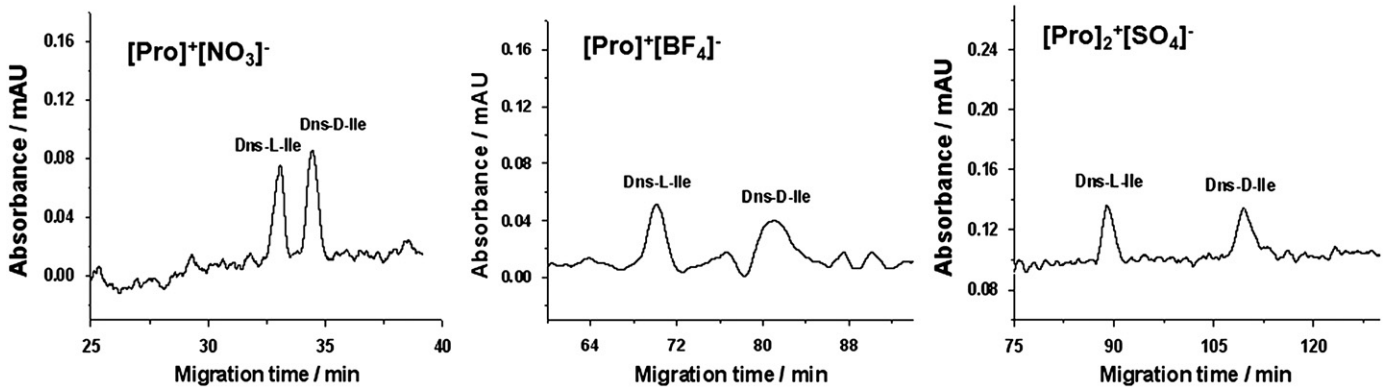

Fig. 5. Enantioseparation of Dns-D,L-Ile by using L-Pro derived AAILs with different anions as the chiral selectors. Buffer conditions: 25.0 mM $Cu(Ac)_2$, 50.0 mM AAILs and 20% (v/v) methanol at pH 4.0. Other conditions are the same as indicated in Fig. 1.

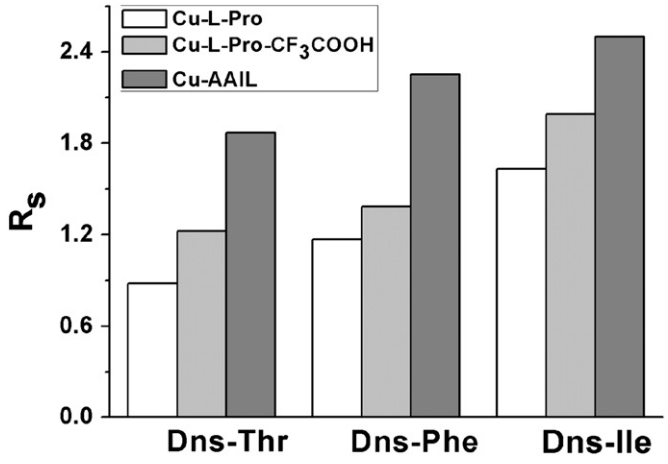

Fig. 6. Comparative enantioseparation with different chiral selectors. L-Pro, L-Pro + CF_3COOH, and L-Pro derived ionic liquid [Pro-CF_3COO] were used as the chiral selectors, respectively. Buffer conditions: 25.0 mM $Cu(Ac)_2$, 20% (v/v) methanol at pH 4.0, and 50.0 mM L-Pro for Cu-L-Pro system, 50.0 mM L-Pro and 50.0 mM CF_3COOH for Cu-L-Pro-CF_3COOH system, or 50.0 mM [Pro-CF_3COO] for Cu-AAIL system. Other conditions are the same as indicated in Fig. 1.

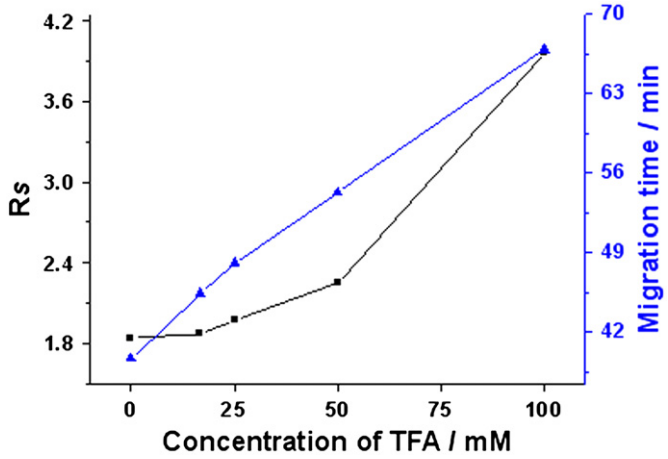

Fig. 7. Dependence of migration time (▲) and enantioresolution (■) of Dns-D,L-Ile on the TFA/L-Pro molar ratios.

displayed better enantioselectivity than that of L-Pro system. Further, we investigated the influence of different TFA/L-Pro molar ratios on separations in detail, and the results were shown in Fig. 7. It has been found that TFA was indeed responsible for improvement of the chiral resolution, but the migration time was prolonged. Moreover, Dalluge and colleagues reported that the addition of TFA could not only improve the chiral separation efficiency, but also successfully eliminate the peak tailing problem [34], which was well agreed with our results.

As a ligand, it is commonly considered that the L-Pro interacts with Cu(II) ion via its nitrogen atom in the ring and oxygen atom from carboxyl in complexation reactions [26,35]. In principle, the existing form of L-Pro in solution of AAIL system or combined system should be as same as in aqueous solution. However, our experimental data displayed that the formed metal- L-Pro complexes were different with the AAIL system or the combined system: the electroosmotic flow (EOF) of Cu(II)–AAIL system and Cu(II)-L-Pro system were 0.58×10^{-4} $cm^2/(v.s)$ and 0.37×10^{-4} $cm^2/(v.s)$, respectively; however, the migration time of analytes in Cu(II)–AAIL system was much longer than that in Cu(II)-L-Pro system, for example, the migration time of Dns-L-Ile was 51.83 min in AAIL system while it was 39.69 min in L-Pro system. It is well known that EOF is one of the most important features for separating analytes in CE and the EOF flow is typically attracted to the cathode in a bare capillary [36]. In CLE-CE system, different ternary mixed metal complexes are also driven by EOF and the separation is generally achieved based on their different electrophoretic behaviors. In this work, the experimental results showed that the EOF value of Cu(II)–AAIL system was higher than that of Cu(II)-L-Pro system, and consequently the migration time should be shorter. However, the opposite phenomenon was observed in AAILs systems, which could be explained by the speculation that AAILs may only be partly ionized. Thus the metal complexes in AAILs system were in larger size which may block the ligands exchanging with the analytes, resulting in longer migration time. Moreover, AAILs might form more complicated complexes with Cu(II) ion in the buffer solution.

It should be mentioned that many factors could affect the complex formation in CLE-CE system, but till now the mechanism of the complex formation in CLE-CE system has not been explored completely and clearly. The results obtained in this work indicated that it is of great significance to study on the specific structure for exploring the behaviors of AAILs in chiral recognition. As a novel kind of chiral ligand, the complex structures of metal ions and ILs should be further studied, and it would be helpful for understanding the role of ILs for chiral recognition in CLE-CE system. Meanwhile, further exploration on the CLE-CE mechanism also should be conducted in the future.

4. Conclusions

In this work, the application of ILs derived from AAs as cations in chiral separation of Dns-D,L-AAs based on the CLE-CE principle

has been demonstrated. Then, the procedure has been optimized in terms of AAIL concentration, pH, and methanol concentration. Under the optimal separation conditions, well enantioseparation of nine pairs of Dns-D,L-AAs was achieved. For further probing into the chiral separation mechanism of AAILs as ligands, comparative studies of the AAILs and AAs in coordination behaviors with Cu(II) ion have been performed. Impressively, AAILs could be obtained without sophisticated synthesis, and the chiral separation efficiency of AAILs in CLE-CE system was better than that of conventional AAs. This study will make an inspiration to explore more effective chiral separation CLE-CE systems and extend the application of ILs in separation science.

Acknowledgments

We gratefully acknowledge the financial support from National Natural Science Foundation of China (Nos. 21175138, 20935005), Ministry of Science and Technology of China (No. 2007CB714504), and Chinese Academy of Sciences. Also, we have appreciated Guanghui Ouyang for his kind help for NMR results.

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