Influence of ionic liquids as electrolyte additives on chiral separation of dansylated amino acids by using Zn(II) complex mediated chiral ligand exchange capillary electrophoresis

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Abbreviations:

AAs	amino acids
CLE-CE	chiral ligand-exchange capillary electrophoresis
Dns-AAs	dansylated amino acids
Dns-Cl	dansyl chloride
ILs	ionic liquids
[BMIm]Cl	1-butyl-3-methylimidazolium chloride
[HMIm]Cl	1-hexyl-3-methylimidazolium chloride
[OMIm]Cl	1-octyl-3-methylimidazolium chloride
[BMIm]Br	1-butyl-3-methylimidazolium bromide
[BMIm]BF$_4$	1-butyl-3-methylimidazolium tetrafluoroborate
[BMIm]PF$_6$	1-butyl-3-methylimidazolium hexafluorophosphate
[BIm]Cl	N-butylimidazolium chloride
[BMMIm]Cl	1-butyl-2,3-dimethylimidazolium chloride

Key words: Chiral ligand exchange capillary electrophoresis, Dansylated amino acids, Ionic liquids

Abstract

In this work, investigation of the comparative influence of diverse ionic liquids as electrolyte additives on the chiral separation of dansylated amino acids by using Zn(II)-L-arginine complex mediated chiral ligand exchange capillary electrophoresis was conducted. It has been found that not only the varied substituted group number, but also the alkyl chain length of the substituted group on imidazole ring in the structure of ionic liquids show different influence on chiral separation of the analytes in the chiral ligand exchange capillary electrophoresis system, which could be understood by their direct influence on electroosmotic flow. Meanwhile, the variation of anion in the structure of ionic liquids displayed remarkably changed performance and the ionic liquids with Cl$^-$ showed the most obvious promoting effect on the chiral separation performance. Among the investigated seven ionic liquids, 1-butyl-3-methylimidazolium chloride was validated to be the proper electrolyte additive in the chiral ligand exchange capillary electrophoresis system. Further, it has been observed that 1-butyl-3-methylimidazolium chloride also has obviously promotive effect on the labeling performance. The results have demonstrated that the ionic liquids with different structures have important relation to their performance in chiral ligand exchange capillary electrophoresis and to their labeling efficiency in dansylation of the analytes.

1 Introduction

Because the chemical processes during different life phenomena are completed in highly asymmetric environment and most of basic biomolecules as well as some drugs possess chirality feature, chiral recognition has been one hot research point in the fields of analytical chemistry, pharmacy and life science during the last several decades, resulting in rapid development of chiral separation methods. Great effort has revealed that capillary electrophoresis (CE) technique can show excellent performance in chiral separation procedure based on its merit of high separation efficiency, small sample loading and short analysis time [1-10]. As one kind of chiral separation mode applied in CE, chiral ligand-exchange (CLE) mode is relied on the formation of diastereomeric ternary mixed metal complexes between chiral ligand and analytes to achieve chiral separation [11,12]. Till now, this CLE-CE method has been widely applied to the analysis of amino acids (AAs), hydroxyl acids, peptides and other bio-compounds because of its outstanding advantages of easy manipulation and controllable migration order [13]. However, depending on the CLE mechanism, effective chiral selecting systems applied to CLE-CE are quite limited, resulting in restricted numbers of separation objects and narrow applied range. Moreover, some of the chiral selectors used in CLE-CE method have just displayed unsatisfied chiral separation ability. For example, Cu(II)-D-quinic acid system was utilized for the chiral resolution of native D, L-tartaric acid with the best chiral resolution (Rs) of just approximately 1.30 under optimized condition [14].

Considering the unsatisfied R*s* might limit the application of some chiral selectors in life science area, hence searching for potent additives to improve the separation performance of reported chiral selectors is highly desired to solve the key problem in CLE-CE system.

In recent years, the emergence of ionic liquids (ILs) may offer a fresh insight in the exploration of effective electrolyte additive. ILs, which are a group of organic salts with melting points close to or below room temperature, possess unique chemical and physical properties, including air and moisture-stability, high solubility power, low vapor pressure and controllable chemical structures [15,16]. Therefore, ILs have received considerable attention in different areas ranging from material synthesis to separation science [17-19]. In the separation field, ILs have been successfully applied in solid phase extraction, electrochemistry, HPLC and CE based on their unique properties [20-23]. Increasing evidence has demonstrated that ILs could be utilized as main electrolyte solution, electrolyte additive or dynamic coating in various CE modes and their excellent performance should be ascribed to their high solubility, heat-stability and remarkable influence on the electroosmotic flow (EOF) [24-28]. Yanes and co-workers conducted a CE method by using an IL (a quaternary ammonium salt) as the main electrolyte solution for resolving phenolic compounds in grape seed extracts [24]. François and colleagues evaluated two chiral ILs (ethyl- and phenylcholine of bis(trifluoromethylsulfonyl)imide) as additives to cyclodextrins for enantiomeric separations of anti-inflammatory drugs 2-arylpropionic acids [29]. Although ILs were widely applied in CE separation, their performance in CLE-CE

mode was seldom reported till now and their influence on chiral separation result was still unknown.

In this study, by using dansylated amino acids (Dns-AAs) as test samples and Zn(II)-L-arginine (Zn(II)-L-Arg) as chiral selector, we have applied seven kinds of ILs (**Table 1**) as electrolyte additives and investigated their different influence on the chiral separation results in CLE-CE system in detail. Firstly, we compared the influence of diverse ILs on the labeling of AAs to obtain good dansylation results. Then, we added these ILs in CLE-CE electrolyte system respectively and studied the relationship between their structures and their performance in CLE-CE system. Meanwhile, the variation of EOF with the addition of ILs was examined to explore the chiral separation mechanism.

2 Materials and Methods

2.1 Chemicals

All D-, L-AAs and dansyl chloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All ILs, including 1-butyl-3-methylimidazolium chloride ([BMIm]Cl, 99%, m/m), 1-hexyl-3-methylimidazolium chloride ([HMIm]Cl, 99%, m/m), 1-octyl-3-methylimidazolium chloride ([OMIm]Cl, 99%, m/m), 1-butyl-3-methylimidazolium bromide ([BMIm]Br, 99%, m/m), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm]BF$_4$, 99%, m/m),

1-butyl-3-methylimidazolium hexafluorophosphate ([BMIm]PF$_6$, 99%, m/m),

N-butylimidazolium chloride ([BIm]Cl, 99%, m/m) and

1-butyl-2,3-dimethylimidazolium chloride ([BMMIm]Cl, 99%, m/m) were purchased

from Lanzhou Institute of Chemical Physics (Lanzhou Greenchem ILS, LICP, CAS,

China). Zinc sulfate, boric acid, sodium hydroxide, tris(hydroxymethyl)aminomethane

(Tris), ammonium acetate, hydrochloric acid, acetone and other chemicals were all of

analytical reagent grade from Beijing Chemical Factory (Beijing, China).

2.2 Sample preparation

All aqueous solutions were prepared with triply distilled water and stored at 4°C for

further analysis. 2.0 mg mL^{-1} D- and L-AAs standard stock solutions were prepared

in 50.0 mM Tris-HCl buffer (50.0 mM Tris was titrated by HCl to pH 8.2) and diluted to

desired concentrations with Tris-HCl buffer by 10-10^4 folds to get work solutions.

Derivative solution was freshly prepared by dissolving 3.0 mg dansyl chloride

(Dns-Cl) in 2.0 mL acetone. 200.0 mM ILs stock solutions were prepared with triply

distilled water and then diluted to desired concentrations.

Dansylation of AAs was conducted as following (without ILs): an aliquot of 30.0

μL AAs, 30.0 μL Tris-HCl buffer and 30.0 μL Dns-Cl solution were all mixed to

experience the derivatization process in dark at room temperature for 30 min. Then,

5.0 μL 2% (v/v) ethylamine was added to terminate the reaction. To observe the

influence of different ILs on the dansylation process, 30.0 μL Tris-HCl buffer used in

the normal dansylation was replaced by 30.0 μL 5.0 mM ILs solution. The other

procedures were kept unchangeable. Lastly, the reacted solution was either directly

injected for CE separation or kept at 4 ^{o}C for future analysis.

2.3 CE system

The CE system consisted of a 1229 HPCE 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 (Nanjing Qianpu Software, China). All separation processes were carried out in fused silica capillaries (50 µm id) with a total length of 65.0 cm and an effective length of 50.0 cm obtained from Yongnian Optical Fiber Factory (Hebei, China). Before sample introduction, bare fused-silica capillary was sequentially washed with 0.1 M HNO_3, water, 0.1 M NaOH, water and running electrolyte for 2 min, respectively.

Samples were siphoned to the capillary for 10 s at 15.0 cm height and separated at -20 kV. The buffer solution in CE for this study was composed of 5.0 mM ammonium acetate, 100.0 mM boric acid, 3.0 mM $ZnSO_4$, 6.0 mM L-arginine and 20.0 mM ILs (**Table 1**), adjusted to pH 8.2 with solid Tris. Before use, all the running buffer were filtered through a membrane filter with 0.45 µm pores and degassed by sonication for 2 min. UV detection was set at the anodic end of capillary with the wavelength of 254 nm for Dns-AAs determination. To mark the EOF, acetone was used as the neutral sample and determined at +20 kV. All process was conducted at room temperature. Peaks were identified by spiking relative standard chiral AAs in sample solutions. The peaks with increased height were considered to be the targets.

2.4 Calculation

Electroosmotic mobility (μ_{eo}) was determined by measuring the migration time of

acetone (t_{eo}) according to the following expression:

$$\mu_{eo} = (L_t \cdot L_d) / (V \cdot t_{eo}) \qquad\qquad (1)$$

Where L_t is the total capillary length, L_d is the effective capillary length (from the

injection inlet to the detector) and V is the applied voltage.

Resolution of each Dns-AA enantiomers was calculated by measuring the Rs

value, which is defined by the following equation:

$$Rs = 2(t_2-t_1) / (w_1+w_2) \qquad\qquad (2)$$

Where t_1 and t_2 are the migration times, w_1 and w_2 are the peak widths at the baseline

of the enantiomers, respectively.

3. Results and discussion

3.1 The effect of ILs on the dansylation of AAs

Besides their great potential application in separation science, ILs have been widely

utilized as green solvents or catalysts in organic chemistry to promote chemical

reaction and obtained high reaction yield. Here, because of the absence of

chromophore in most AAs structures, the labeling process by using special derivative

agents was needed to improve detecting sensitivity. In this experiment, Dns-Cl was

adopted as a typical labeling agent to study the effect of different ILs on the

derivatization of AAs based on the advantages of fast dansylation process and high

labeling efficiency. The reaction between AAs and Dns-Cl was shown in **Fig. 1** [30, 31].

According to previous studies [30, 31], higher yield of this reaction could be obtained in basic environment, thus Tris-HCl buffer (pH 8.2) was chosen as the basal reaction solution for AAs labeling. By using D,L-alanine (D,L-Ala) as the test sample, the effect of seven different ILs (**Table 1**) on the derivatization of AA was examined by CLE-CE method. With the addition of 5.0 mM [BMIm]Cl in Dns-Cl mediated labeling solution, the obtained peak height of Dns-D,L-Ala in electropherogram was almost two times higher than that obtained under normal dansylation condition. Although the addition of 5.0 mM [HMIm]Cl also resulted in higher peaks of Dns-D,L-Ala, the changed peak height is obviously lower than that obtained in [BMIm]Cl system. Moreover, the statistical results was displayed in **Fig. 2** to demonstrate the effect of seven different ILs on the derivatization of D,L-Ala. The addition of [BMIm]Cl and [HMIm]Cl could result in 2.75 and 1.94 times increase in peak height of Dns-D,L-Ala respectively, indicating the rise in reaction yield between D,L-Ala and Dns-Cl. The other five ILs didn't shown obviously improved effect on this derivative reaction. Thus, [BMIm]Cl was validated to be the most effective IL to improve the labeling process of AAs mediated by Dns-Cl while [HMIm]Cl was the second effective agent.

Diverse improved effect of the seven ILs could be understood from the difference between their structures. **Fig. 2** indicated that the change of substituted group number on the imidazole ring, varied alkyl chain length of substituted group and different anions were all responsible for their different performance in the dansylation process. Longer alkyl chain on imidazole ring resulted in poor improvement of the reaction yield. ILs with Cl$^-$ as anion displayed better improvement

to the reaction compared with ILs with other anions. Based on comprehensive understanding of the reaction between AAs and Dns-Cl, we presumed that the acidity-basicity properties of ILs might be responsible for their improved effect on the labeling process. The most basic IL displayed the best improved effect on the reaction between AAs and Dns-Cl. [BMIm]Cl was demonstrated to be the most basic IL among these ILs [32], thus it improved the labeling process at the maximum degree.

3.2 The effect of ILs on the chiral separation of AAs in CLE-CE

To study the effect of different ILs on the chiral separation of AAs by using CLE-CE method, Dns-D,L-serine (Dns-D,L-Ser), Dns-D,L-methionine (Dns-D,L-Met) and Dns-D,L-isoleucine (Dns-D,L-Ile) were selected as the test samples. The seven ILs (**Table 1**) were divided into three groups according to their structure features (the number of substituted group on imidazole ring, the alkyl chain length of substituted group and different anions) and applied as the electrolyte additives respectively to examine their performance in CLE-CE system.

3.2.1 The performance of ILs with different substituted group number on imidazole ring

Fig. 3 showed the varied performance of three ILs with different substituted group number (1, 2, 3) on the imidazole ring in CLE-CE system. The addition of three different ILs in background electrolyte led to diverse chiral separation results of Dns-AAs. The three test samples were well separated in 45 min without the addition of ILs. However, the addition of [BIm]Cl resulted in severe unstability of baseline and distortion of peaks in electropherogram. When [BMIm]Cl was added into Zn(II)-L-Arg

system, the migration times of Dns-D,L-Met and Dns-D,L-Ile were obviously prolonged and Rs were remarkably improved. But the prolonged migration time of Dns-D,L-Ser here (62.0 min) led to evident peak tailing and unsatisfied Rs. Compared to [BIm]Cl and [BMIm]Cl, the addition of [BMMIm]Cl showed no influence on the chiral separation in CLE-CE system. The migration times of the three test samples were almost the same as that obtained in CLE-CE system without ILs while Rs decreased a little bit. As a result, the three ILs with different substituted group number (1, 2, 3) on the imidazole ring had totally different performance in CLE-CE system with Zn(II)-L-Arg as the chiral selector.

Considering the fact that nitrogen atoms on imidazole ring could form chelated bond with Zn(II) [33, 34], different performance of the three ILs was easily understood from the insight of their special structures. When [BIm]Cl was added into Zn(II)-L-Arg system, nitrogen atom on imidazole ring could seize Zn(II) to form chelated bond and interfere the formation of Zn(II)-L-Arg complex, resulting in bad influence on the chiral selecting agent formation and then disturbing the peak shapes. However, the peak distortion problem did not exist in [BMIm]Cl and [BMMIm]Cl system, because the presence of alkyl chain connected to two nitrogen atoms on imidazole ring prevented effective coordination between Zn(II) and imidazolium cation. The prolonged migration times in [BMIm]Cl system could be explained by its remarkable effect on EOF. Additionally, the performance of [BMMIm]Cl could be understood by the suppose that the presence of the third alkyl chain may generate steric hindrance and prevent its interaction with the silanol group on the inner wall of capillary, then have little influence on EOF and chiral separation. Thus, the ILs with two substituted groups on imidazole ring were chosen for further study.

3.2.2 The performance of ILs with varied alkyl chain length of substituted group on imidazole ring

ILs with varied alkyl chain length of substituted group on imidazole ring also showed different performance in Zn(II)-L-Arg mediated CLE-CE system. As shown in **Fig 4**, with the increase of alkyl chain length, the migration times of the three test samples decreased and R*s* also decreased. Taking Dns-D,L-Met as an example, its R*s* was 6.44 in [BMIm]Cl system, 4.61 in [HMIm]Cl system and 3.21 in [OMIm]Cl system. Moreover, the migration time of Dns-D-Met was 42.5 min in [BMIm]Cl system, 37.4 min in [HMIm]Cl system and 34.4 min in [OMIm]Cl system. Compared with the separation results obtained in CLE-CE system without ILs, the utilization of [BMIm]Cl caused the increase of migration times and the improved R*s*. However, the application of [HMIm]Cl and [OMIm]Cl led to obvious peak tailing and little change in the migration times of the three test samples, as well as decreased R*s.*

Different performance of the three ILs with varied alkyl chain on imidazole ring could be explained by the change of EOF. By using acetone as neutral marker, we have studied the change of EOF in detail when the three ILs were added into electrolyte respectively (**Table 2**). According to our previous study, EOF is a special resistance of sample migration in CLE-CE system with Zn(II) as central ion while the value of EOF mobility was minus in this experiment. As shown in **Table 2**, with the increment of alkyl chain length, μ_{eo} increased strikingly, indicating weaker resistance and fast migration rate. From the insight of ILs structures, the presence of ILs with varied alkyl chain length might influence the interaction between imidazolium cation and silanol group on the inner wall of capillary, thus leading to different effect on EOF. Therefore, the three ILs with varied alkyl chain on imidazole ring showed diverse

effect on EOF [28, 35, 36], resulting in different performance in Rs and the migration times of the three test samples.

3.2.3 The performance of ILs with different anions

Because ILs were a group of organic salts consisting of different kinds of cations and anions, the change of anions had remarkable effect on their properties. With [BMIm] as cation, we have observed the performance of the three ILs with different anions in Zn(II)-L-Arg mediated CLE-CE system. As shown in **Table 3**, [BMIm]Cl displayed striking influence on Rs and the migration times of the three test samples. Meanwhile, the addition of [BMIm]Br and [BMIm]BF$_4$ only showed relatively little influence on the migration times of the three test samples, which were almost the same as that obtained in CLE-CE system without ILs. In addition, Rs of the three samples became worse in both [BMIm]Br and [BMIm]BF$_4$ system. Thus the anion had important influence on the performance of ILs in CLE-CE system and ILs with Cl$^-$ as anion were validated to be effective electrolyte additives.

Because all of the employed ILs could be considered as completely dissociated in carrier electrolyte, the observed different performance of the three ILs with varied anions in CLE-CE system has been strictly related to the presence of different anion in electrolyte. As shown in **Table 3**, in Zn(II)-L-Arg mediated CLE-CE system, Cl$^-$ displayed the most obvious influence while Br$^-$ and BF$_4^-$ showed neglectful influence. It has been reported [37] that by varying the physico-chemical nature of Cu(II) anion in a CLE chromatography system, a pronounced impact upon the resolution factor could be observed and the enantioseparation factor values also could undergo variation. To explain these interesting phenomenon, a competitive behavior by the

copper(II) counter-ion over each enantiomer toward the mixed ternary complex

formation ([selector-Cu(II)-analyte]) was plausibly envisaged. In this study, the

presence of different anions might influence the effective formation of ternary

complex [L-Arg-Zn(II)-analyte], thus the chiral selectors displayed distinct effect on

chiral separation of the test examples.

Generally, 20.0 mM [BMIm]Cl was proved to be the most effective electrolyte

additive in CLE-CE system with Zn(II)-L-Arg as chiral selecting agent. Although the

migration time of the three test samples prolonged under this condition, Rs of

Dns-D,L-Ile and Dns-D,L-Met had appropriate improvement.

4 Concluding remarks

The unique properties of ILs guaranteed their great potential application in organic

chemistry and separation science. For exploring the performance of different ILs in

CLE-CE method for the chiral separation of Dns-AAs, the influence of seven ILs with

different structures on the labeling of AAs with Dns-Cl was firstly examined. [BMIm]Cl

was approved as proper agent to improve the derivative reaction with the increment

of 275%. Then, the ILs were applied as electrolyte additives and their influence on

chiral separation result in Zn(II)-L-Arg mediated CLE-CE system was investigated in

detail. Generally, the relationship between the structures of ILs (the number of

substituted group on imidazole ring, the alkyl chain length of substituted group and

different anions) and their performance in CLE-CE (the influence on Rs and migration

times of the three test samples) was observed and rationally explained from a

structural insight. With the addition of different ILs in electrolyte, their unique structure

properties could cause the variation of EOF and interfering effect on the formation of

chiral selecting complex, thus resulting in diverse influence on the chiral separation

efficiency in Zn(II)-L-Arg mediated CLE-CE system. From a comparative study,

[BMIm]Cl was validated to be the most effective electrolyte additive to improve Rs of

Dns-D,L-AAs. The results demonstrated here were believed to give new insight into

the application of ILs in separation science.

The authors have declared no conflict of interest.

Reference

[1] Ward, T. J., Baker, B. A., *Anal. Chem.* 2008, 80, 4363-4372.

[2] Ward, T. J., Ward, K. D., *Anal. Chem.* 2010, 82, 4712-4722.

[3] Chankvetadze, B., *J. Chromatogr. A* 2007, 1168, 45-70.

[4] Gübitz, G., Schmid, M. G., *J. Chromatogr. A* 2008, 1204, 140-156.

[5] Scriba, G. K. E., *J. Sep. Sci.* 2008, 31, 1991-2011.

[6] Mikuš, P., Maráková, K., *Electrophoresis* 2009, 30, 2773-2802.

[7] Preinerstorfer, B., Lämmerhofer, M., Lindner, W., *Electrophoresis*, 2009, 30, 100-132.

[8] Fanali, S., *Electrophoresis*, 2009, 30, S203-S210.

[9] Chankvetadze, B., *Electrophoresis*, 2009, 30, S211-S221.

[10] Scriba, G. K. E., *J. Pharm. Biomed. Anal.* 2011, 55, 688-701.

[11] Davankov, V. A., *J. Chromatogr. A* 2003, 1000, 891-915.

[12] Maccarrone, G, Contino, A., Cucinotta. V., *Trends Anal.Chem.* 2012, 32, 133-153.

[13] Schmid, M.G., Gübitz, G., *Anal. Bioanal. Chem.* 2011, 400, 2305-2316.

[14] Kodama, S., Yamamoto, A., Matsunaga, A., Hayakawa, K., *J. Chromatogr. A* 2001, 932, 139-143.

[15] Huddleston, J. G., Visser, A. E., Reichert, W. M., Willauer, H. D., Broker, G. A., Rogers, R. D., *Green Chem.* 2001, 3, 156-164.

[16] Welton, T., *Chem. Rev.* 1999, 99, 2071-2083.

[17] Bhargava, B. L., Balasubramanian, S., Klein, M. L., *Chem. Commun.* 2008, 3339-3351.

[18] Ludwig, R., Kragl, U., *Angew. Chem. Int. Ed.* 2007, 46, 6582-6584.

[19] Sun, P., Armstrong, D. W., *Anal. Chim. Acta* 2010, 661, 1-16.

[20] Buzzeo, M. C., Evans, R. G., Compton, R. G., *Chem. Phys. Chem.* 2004, 5, 1106-1120.

[21] Liu, J. F., Jönsson, J. A., Jiang, G. B., *Trac-Trend. Anal. Chem.* 2005, 24, 20-27.

[22] Baker, G. A., Baker, S. N., Pandey, S., Bright, F. V., *Analyst* 2005, 130, 800-808.

[23] Pandey, S., *Anal. Chim. Acta* 2006, 556, 38-45.

[24] Yanes, E. G., Gratz, S. R., Stalcup, A. M., *Analyst* 2000, 125, 1919-1923.

[25] Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robison, S. E., Stalcup, A. M., *Anal. Chem.* 2001, 73, 3838-3844.

[26] Laamanen, P. L., Busi, S., Lahtinen, M., Matilainen, R., *J. Chromatogr. A* 2005, 1095, 164-171.

[27] Yu, L. J., Qin, W. D., Li, S. F. Y., *Anal. Chim. Acta* 2005, 547, 165-172.

[28] Jiang, T. F., Gu, Y. L., Liang, B., Li, J. B., Shi, Y. P., Ou, Q. Y., *Anal. Chim. Acta* 2003, 479, 249-254.

[29] Francois, Y., Varenne, A., Juillerat, E., Villemin, D., Gareil, P., *J. Chromatogr. A* 2007, 1155, 134-141.

[30] Gozel, P., Gassmann, E., Michelsen, H., Zare, R. N., *Anal. Chem.* 1987, 59, 44-49.

[31] Tapuhi, Y., Schmidt, D. E., Lindler, W., Karger, B. L., *Anal. Biochem.* 1981, 115, 123-129.

[32] Bi, W., Tian, M., Row K. H., *Analyst* 2011, 136, 379-387.

[33] Zhao C.C., Ni W.W., Yu M.M., Cui, A. L., Kou, H. Z., *Transit. Metal. Chem.* 2008, 33, 861-866.

[34] Mroué, K. H., Power, W. P., *J. Phys. Chem. A* 2010, 114, 324-335.

[35] Liu, Q., Wu, K., Tang, T., Yao, L., Yang, F., Nie, Z., Yao,S., *Chem. Eur. J.* 2009, 15, 9889-9896.

[36] Li, J., Han, H., Wang, Q., Liu, X., Jiang, S. X., *Anal. Chim. Acta* 2010, 674, 243-248.

[37] Natalini, B., Sardella, R., Carbone, G., *Anal. Chim. Acta* 2009, 638, 225-233.

Figure captions

Figure 1. The reaction between amino acids (AAs) and dansyl chloride (Dns-Cl).
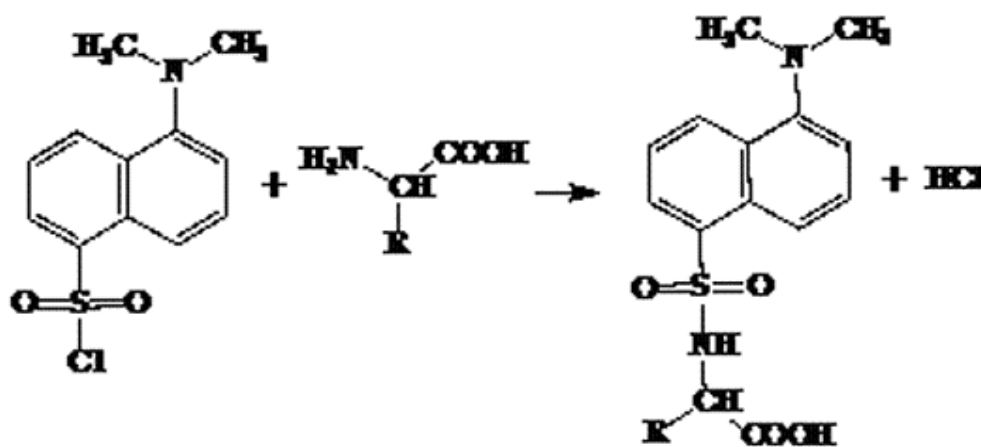
Figure 2. Effect of different ILs on the dansylation efficiency of D,L-Ala. Separation condition: 5.0 mM ammonium acetate, 100.0 mM boric acid, 3.0 mM $ZnSO_4$, 6.0 mM L-arginine and 20.0 mM different ILs, adjusted to pH 8.2 with solid Tris.

Identification: 1. No ILs; 2. [BMIm]Cl; 3. [BMIm]Br; 4. [BMIm]BF4; 5. [HMIm]Cl; 6. [OMIm]Cl; 7. [BIm]Cl; 8. [BMMIm]Cl. (The asterisks above column 2 and column 5 represent two most effective ILs with improved effect on the labeling process between AAs and Dns-Cl.)
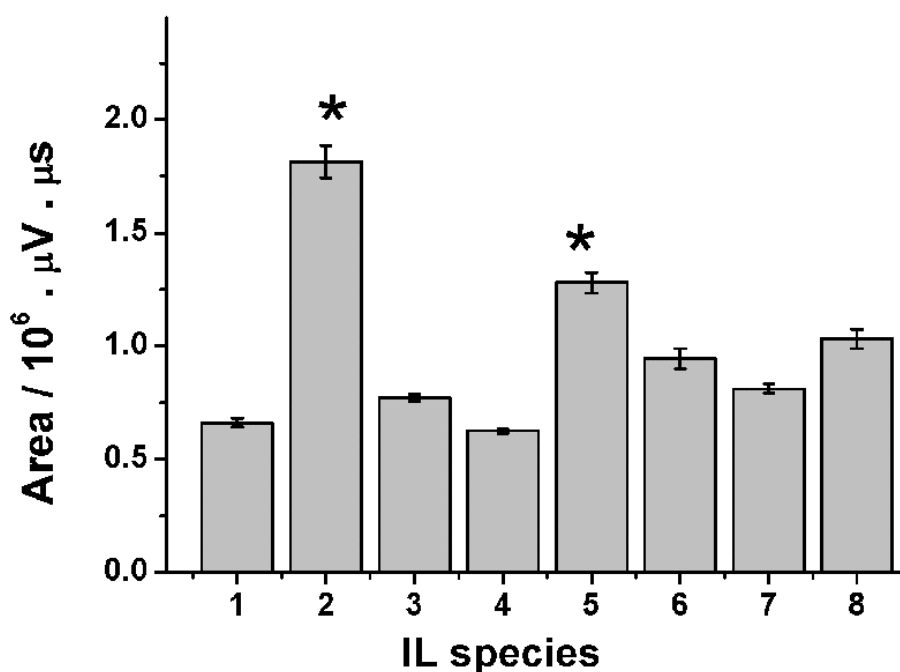
Figure 3. Effect of ILs with different substituted group number on imidazole ring on the chiral sepration results. Except 20.0 mM ILs ([BIm]Cl, [BMIm]Cl and [BMMIm]Cl), other separation conditions were the same as that in **Figure 2**.

Peak identification: 1, Dns-D-Ile, 1', Dns-L-Ile; 2, Dns-D-Met, 2', Dns-L-Met; 3, Dns-D-Ser, 3', Dns-L-Ser.
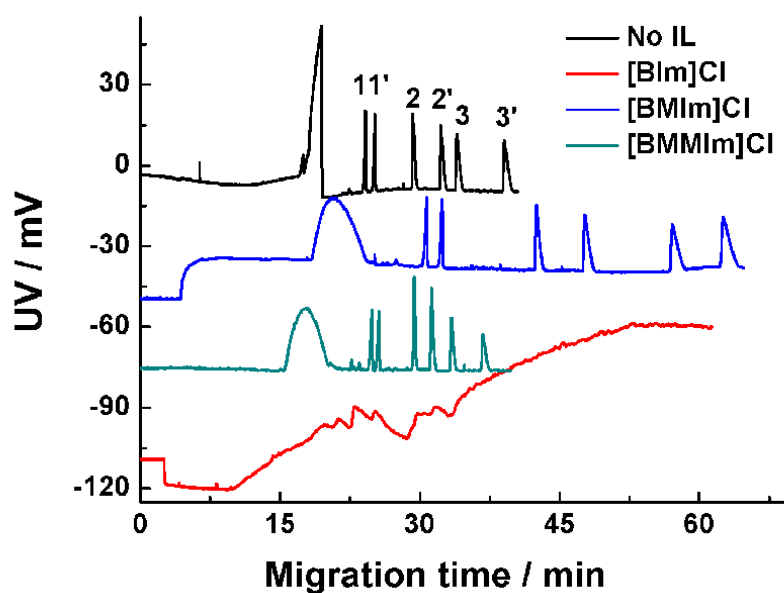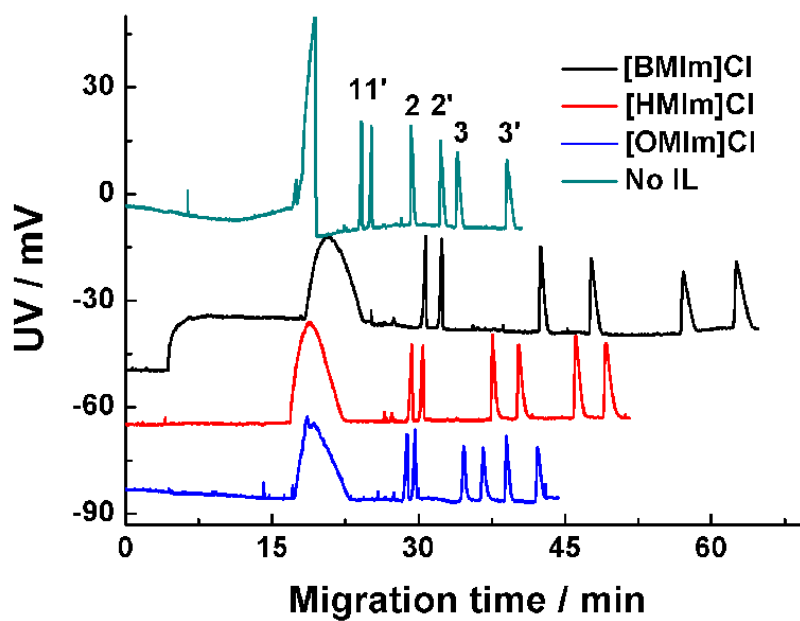
Figure 4. Effect of ILs with varied alkyl chain length on imidazole ring on the chiral sepration results in CLE-CE system. ILs used here were [BMIm]Cl, [HMIm]Cl and [OMIm]Cl. The other separation conditions and peak identification were the same as that in **Figure 3**.

Tables

Table 1 Abbreviations and structures of seven ILs investigated in this study
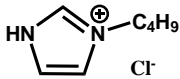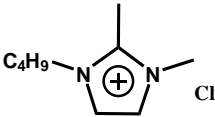
No.	Abbreviation	Structure	No.	Abbreviation	Structure
1	[BMIm]Cl		5	[BMIm]BF₄	
2	[HMIm]Cl		6	[BIm]Cl	
3	[OMIm]Cl		7	[BMMIm]Cl	
4	[BMIm]Br				

Table 2 Effect of ILs with varied alkyl chain length on R*s* and μ_{eo} [a]

ILs	R*s* (Dns-D,L-Ile)	R*s* (Dns-D,L-Met)	R*s* (Dns-D,L-Ser)	μ_{eo} ($10^{-8} \cdot m^2 /V \cdot s$)
[BMIm]Cl	4.18	6.44	4.18	-1.87
[HMIm]Cl	2.43	4.61	3.32	-1.43
[OMIm]Cl	2.19	3.21	4.59	-1.31

[a] Experimental conditions were the same as that in **Figure 2**.

Table 3 Effect of ILs with different anions on the migration times and Rs [a]

Additives	Dns-D,L-Ile			Dns-D,L-Met			Dns-D,L-Ser		
	t_D(min)	t_L(min)	Rs	t_D(min)	t_L(min)	Rs	t_D(min)	t_L(min)	Rs
No IL	24.17	25.16	3.81	29.27	32.23	6.21	33.95	39.05	7.41
[BMIm]Cl	30.71	32.35	4.18	42.50	47.70	6.44	57.09	62.56	4.18
[BMIm]Br	29.39	30.15	1.78	34.15	35.86	4.11	38.23	41.12	5.75
[BMIm]BF$_4$	26.90	27.50	1.31	30.88	32.35	3.62	34.52	37.06	5.18

[a] Experimental conditions were the same as that in **Figure 2** except 20.0 mM ILs ([BMIm]Cl, [BMIm]Br and [BMIm]BF$_4$).