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ARTICLE TYPE

Development of a capillary electrophoresis system with Mn(II) complexes and β-cyclodextrin as the dual chiral selectors for enantioseparation of dansyl amino acids and its application in screening enzyme inhibitors

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L-alanine (L-Ala) derived amino acid ionic liquids (AAILs) were synthesized successfully and applied as new chiral ligands in chiral ligand exchange capillary electrophoresis (CLE-CE) system. With Mn(II)-AAIL complexes and β-cyclodextrin (β-CD) as the dual chiral selectors, a CLE-CE system was further developed for enantioseparation of dansyl D,L-amino acids (Dns-D,L-AAs). The influence of different separation parameters in CLE-CE, including the different kinds of AAILs, pH of the running buffer, the ratio of Mn(II) to AAILs, the concentration of complexes and β-CD were investigated and the optimum buffer was obtained: 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), and 5.0 mM [1-butyl-3-methylimidazolium][L-Ala] at pH 8.3. Under the optimum conditions, twelve pairs of Dns-D,L-AAs could be baseline separated and six pairs were partly separated. Additionally, a control study between the performance of Mn(II)-L-Ala and Mn(II)-[1-butyl-3-methylimidazolium][L-Ala] complexes was conducted to prove the unique behavior of AAILs in CLE-CE. Moreover, the proposed CLE-CE method was applied in screening tyrosinase inhibitors with benzoic acid and its derivatives as the typical compounds. The results illustrated that the CDs acting synergistically with the CLE-CE system could produce better performance on enantioseparation, and the novel CLE-CE method has great application potential in enzyme analysis.

1. Introduction

Recently, chiral separation has gained great research interest in the fields of analytical chemistry, pharmaceuticals, environmental and life sciences, causing the speedy development of enantioseparation techniques [1-5]. Capillary electrophoresis (CE) has been shown to be a premier analytical tool for the chiral resolution over the last decades owing to its merits such as simplicity, minimum samples and solvents consumption, high separation efficiency, short analysis times and economical equipment [6-10]. As one of the chiral separation mode in CE, the chiral ligand exchange capillary electrophoresis (CLE-CE) technique, which was first introduced by Zare and co-workers in 1985 for the chiral separation of dansyl D,L-amino acids (Dns-D,L-AAs), has been gained increasing attention[11]. The separation mechanism of CLE is relied on the formation of diastereomeric ternary mixed metal complexes between the chiral selector ligand and the analytes, and the enantioseparation was realized on account of the different thermodynamic stability of the mixed complexes between the selector and both enantiomers [12-17]. Although the CLE-CE method has been widely employed for the analysis of chiral biomolecules [18, 19], it still faces some fundamental challenges. Importantly, the species of available central ions and chiral ligands were quite limited, leading to restricted application [5, 18-20].

During the past years, a series of CLE-CE systems that employed Cu(II) or Zn(II) as the central ion have been successfully explored [21-27]. It should be noted that up to now, only a few papers have been published with using other metal ions, such as Co(II) or Ni(II), as the central ions in the CLE-CE systems [23, 28, 29], however, baseline enantioseparation could not be obtained in most cases. Therefore, inquiring into more kinds of the central ions and development of effective CLE-CE systems are of great significance. Importantly, it is necessary to explore more and new chiral ligands for building available CLE-CE system. In recent years, amino acid ionic liquids (AAILs) have attracted great attention as its unique advantages, such as convenient synthesis, low cost, good biodegradability, excellent chiral stability, reduced toxicity and high biocompatibility [30-32]. Although several CLE-CE systems with AAILs as the chiral ligands have been probed [5, 18, 19, 33-42], it is still crucial to search novel AAILs ligands and construct effective CLE-CE systems with new central metal ions in chiral separation.

In addition, it has been reported that the dual chiral selectors systems based on the synergistic effect can improve the enantioseparation efficiency in CLE-CE systems [23, 43]. As it well known, cyclodextrins (CDs) are the most common chiral selectors as an additive in chiral separation system due to its outstanding advantages, such as good complexation ability, high water solubility, weak UV cut-off, low cost, good stability and excellent chiral discrimination. Meanwhile, the enantioseparation efficiency also can be improved after adding CDs into the buffer solution in CLE-CE systems [44-46]. Consequently, development of dual chiral selectors systems based on the synergistic effect of

CDs should be an effective method for improving the enantioseparation efficiency of D,L-AAs and expected to expand the application of CLE-CE technique in the fields of analytical chemistry and life sciences.

Tyrosinase is a multifunctional copper-containing enzyme, which has an important effect on the melanin forming and is connected with the hyper pigmentation [47, 48]. Since tyrosinase has been applied in the fields of agriculture, food, medicine and cosmetics, so the development and screening of potent inhibitors is significant [49, 50]. Many efforts have been made to search for effective tyrosinase inhibitors. Benzoic acid and its derivatives have been reported as the potential inhibitors of tyrosinase recently [51, 52]. During past years, many methods have been probed for screening the inhibitors of tyrosinase, such as electrophoretically mediated microanalysis, UV spectrophotometry, high performance liquid chromatography and gas chromatography-mass spectrometry [48, 49, 53-56]. However, these protocols require complex sample processing, high sample consumption, and are susceptible to disturbance [48, 49, 53]. Hence, it is need to develop a new method for efficient screening tyrosinase inhibitors. Moreover, our previous work proved the CLE-CE technique was a valid method for screening the inhibitors of tyrosinase [27].

In this work, three kinds of AAILs with L-Alanine (L-Ala) as cation ([1-ethyl-3-methylimidazolium][L-Ala], [1-butyl-3-methylimidazolium][L-Ala], [1-hexyl-3-methylimidazolium][L-Ala]) have been synthesized and employed as the novel chiral ligands in the CLE-CE system when Mn(II) was selected as the central ion. To improve the enantioseparation efficiency, β-CD was used as the additional chiral selector due to its synergistic effect. Further, the new CLE-CE system with Mn(II)-[1-butyl-3-methylimidazolium][L-Ala] complex and β-CD as the dual chiral selectors was successfully constructed for enantioseparation of Dns-D,L-AAs and applied in screening the tyrosinase inhibitors.

2. Materials and methods

2.1 Reagents and Chemicals

1-Butyl-3-methylimidazolium bromide ([BMIm]Br), 1-ethyl-3-methylimidazolium bromide ([EMIm]Br) and 1-hexyl-3-methylimidazolium ([HMIm]Br) were from Lanzhou Institute of Chemical Physics (Lanzhou Greenchem ILS, LICP, CAS, China). All D,L-AAs and dansyl chloride (Dns-Cl) were bought from Sigma-Aldrich Chemical Company (St. Louis, USA). Boric acid, ammonium acetate, manganese sulfate, sodium hydroxide, lithium carbonate, tris (hydroxymethyl) aminomethane (Tris), acetone, β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), α-cyclodextrin (α-CD) and other reagents were purchased from Beijing Chemical Factory (Beijing, China). Benzoic acid, 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, 2-hydroxybenzoic acid and 4-aminobenzoic acid were obtained from Aladdin Chemistry Company (Shanghai, P.R. China). Tyrosinase (source: mushrooms) was got from Express Technology Co., Ltd. (565 U mg^{-1}, Beijing, China).

2.2 Instruments and separation conditions

All separations were carried out with the CE apparatus which included a 1229-High Performance Capillary Electrophoresis (HPCE) analyzer (Beijing Institute of New Technology and Application, Beijing, China), a UV detector (Rilips Photo electricity Factory, Beijing, China), a bare fused-silica capillary of 60 cm (effective length 45 cm) × 75 mm id (Yongnian Optical Fiber Factory, Hebei, China) and a HW-2000 chromatography workstation (Qianpu Software, Nanjing, China).

The new uncoated fused-silica capillary was initially conditioned with 0.1 M NaOH and water for 30.0 min in turn. Prior to sample injection, the capillary was washed with 0.1 M HCl, 0.1 M NaOH, water and running buffer for 2.0 min, respectively. Samples were siphoned to the capillary for 8.0 s at 15.0 cm height and separated at –23.0 kV. All operations were conducted at 25^0C. Unless mentioned otherwise, the running buffer system was consisted of 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM manganese sulfate, 5.0 mM [BMIm][L-Ala] and 5.0 mM β-CD, and pH of the solution was adjusted to 8.3 with Tris. All of CE operations were operated for multiple measurements (n=3).

2.3 Sample preparation

All aqueous solutions were prepared with triply distilled water and stored at 4 ^{0}C. D-, and L-AAs standard solutions of 2.0 mg / mL were prepared in 40.0 mM lithium carbonate buffer (adjusted to pH 9.5 with 0.1 M HCl), then diluted to the required concentration with lithium carbonate solution by 10-10^4-fold.

The D,L-AAs were derived according to the previous literature [49]. Briefly, 20.0 μL D,L-AAs, 20.0 μL 40.0 mM lithium carbonate and 20.0 μL Dns-Cl (1.5 mg mL^{-1} in acetone) were mixed in a 200 μL vial and kept at room temperature for 30 min. Then 5 μL 2% ethylamine was added to terminate the reaction. All Dns-D,L-AAs samples were stored at 4^0C before use.

2.4 Preparation of AAILs

All AAILs were synthesized according to the reported literatures with minor revisions [5, 18]. The synthesis of [BMIm][L-Ala] was shown as follows: [BMIm]OH aqueous solution was obtained from [BMIm]Br by using anion exchange resin. Then this solution reacted with a slightly excess equimolar L-Ala aqueous solution at 25 ^{0}C for 24 h under vigorous agitation and followed by evaporated at 55 ^{0}C in vacuum. To remove the excess L-Ala, a solution of acetonitrile / methanol (9/1, v/v) was added to the mixture solution followed by filtration. The filtrate was dried under vacuum at 60 ^{0}C to obtain the final product [BMIm][L-Ala]. Other AAILs were synthesized as [BMIm][L-Ala] besides [BMIm]Br was displaced by equal mole of [EMIm]Br and [HMIm]Br. The structure of AAILs was examined by nuclear magnetic resonance (NMR) (Bruker Avance 400, Switzerland), and the NMR spectra were recorded in D_2O on a 400 MHz instrument with TMS as the internal standard.

2.5 Enzyme incubation

All enzymatic reactions were performed at 25 ^{0}C. Standard enzyme solution was prepared by dissolving 2.0 mg of tyrosinase

in 100 mM phosphate buffer (pH 6.8). The enzymatic hydrolysis experiments were conducted as follows: 40 μL of different concentration L-Tyr and 40 uL phosphate buffer were incubated with 40 μL tyrosinase (565 U mg^{-1}) at 25 ^{0}C for 15 min, then, the reactions were terminated by heating in boiling water for 10 min, followed by centrifugation for 10 min at 10,000 rpm. Thereafter, the supernatant were gathered and derived by Dns-Cl according to the same steps as standard AAs.

In the inhibitor screening experiments, five kinds of inhibitors (benzoic acid, 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, 2-hydroxybenzoic acid and 4-aminobenzoic acid) were incubated with tyrosinase (2.0 mg / mL) and L-Tyr (333 uM) solution. All other procedures were the same as enzymatic hydrolysis experiments.

3. Results and discussion

3.1 Synthesis and characterization of AAILs

In this study, three kinds of L-Ala derived AAILs ([EMIm][L-Ala], [BMIm][L-Ala], [HMIm][L-Ala]) have been synthesized and the structure of AAILs was examined by NMR. The results were displayed in supporting information, indicating the successful preparation of AAILs.

3.2 Optimization of separation condition

A novel CLE-CE system with Mn(II)-[BMIm][L-Ala] complex as the chiral selector coordinating β-CD was developed in this work. The principle of CLE-CE is based on the difference of the thermodynamic stability of the mixed complexes between the selector and both enantiomers. In order to enhance the separation effect, β-CD was selected as the chiral additive and added into the buffer solution in the CLE-CE system. In order to better understand the separation performance of this novel dual chiral selectors system, a control experiment between the performance of single chiral selector and dual chiral selectors as the chiral ligands were performed. The results were displayed in Table S1 and Table 1. It could be observed that the three target analytes were baseline separated in the dual chiral selectors system. But there was no resolution or just poor resolution when either β-CD or Mn(II)-[BMIm][L-Ala] complexes worked alone in the running electrolyte. The control experiment results indicated that the dual chiral selectors system exhibited better resolution effect than the single chiral selector did. Therefore, several key parameters included AAILs, pH, the concentration ratio of central ion to ligand, the complex concentration and β-CD in the dual chiral selectors system were investigated to obtain the optimal separation conditions.

3.2.1 Effect of CDs

CDs are the most common chiral selectors as an additive in chiral separation system [57-62]. In this study, the separation effects of three different kinds of CDs (α-CD, β-CD, γ-CD) were investigated and the data was exhibited in **Table S2**. We found that γ-CD and β-CD displayed good enantioseparation ability (separation factor (α) and resolution (Rs)) for the test analytes, while α-CD showed poorer resolution abilities. In order to obtain the best enantioseparation results within the short migration time, β-CD was finally chosen for further study.

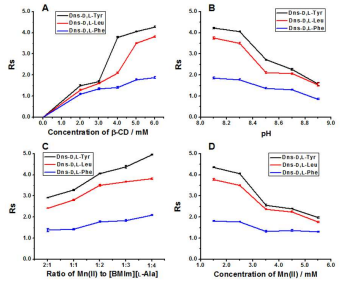

Fig. 1. Influence of the concentrations of β-CD (A), pH (B), concentrations of Mn(II) to [BMIm][L-Ala] (C) and complex concentrations (D) on Rs. Buffer conditions: (A) 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and various concentrations of β-CD at pH 8.3; (B) 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0 mM β-CD at different pH; (C) 100.0 mM boric acid, 5.0 mM ammonium acetate, 5.0 mM β-CD at pH 8.3, and the concentrations ratio of Mn(II) to [BMIm][L-Ala] was 2:1 to 1:4 with Mn(II) kept at 2.5 mM; (D) 100.0 mM boric acid, 5.0 mM ammonium acetate, 5.0 mM β-CD at pH 8.3, and the concentrations of Mn(II) was ranged from 0.5 to 5.5 mM with the concentrations ratio of Mn(II) to [BMIm][L-Ala] kept at 1:2. Capillary: 75 μm i.d. 60 cm length (45 cm effective); injection: siphoned for 8s at 15 cm high; voltage: -23 kV; UV detection: 254 nm; 25 ^{0}C.

It was displayed that β-CD could enhance the chiral resolution in the CLE-CE system [63, 64]. Then, the effect of the concentration of β-CD was investigated. The results were shown in **Fig. 1A** and **Table S3**. Obviously, it demonstrated that both of α and Rs increased after adding β-CD in the buffer solution. In addition, the Rs of the three target analytes could reach to 1.5 when the concentration of β-CD was 5.0 mM. Meanwhile, the migration times of the test analytes were also increased with increasing concentration of β-CD (**Fig. S1A**). Taking α, Rs and migration times into the consideration, 5.0 mM β-CD was finally adopted for further analysis.

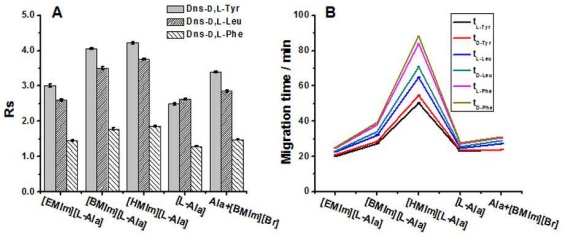

Fig. 2. Influence of various AAILs on Rs (A) and migration times (B). Buffer conditions: 100.0 mM boric acid, 5.0mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0 mM β-CD at pH 8.3. Other conditions are the same as **Fig. 1**.

3.2.2 Effect of different AAILs

In this study, L-Ala derived AAILs were used instead of L-Ala as the chiral ligand for enantioseparation in the CLE-CE system. Firstly, the comparative experiments were conducted with L-Ala as the chiral ligand under the same running buffer conditions to investigate the separation effect of L-Ala derived

AAILs. The results were shown in **Fig. 2** and **Table S3**. Obviously, AAILs ligands displayed good resolution ability over the AA ligands (Rs and α). Then the control experiment was performed by using L-Ala and [BMIm][Br] (the concentration ratio was 1:1) mixture as the ligand, and the results demonstrated that its chiral separation ability was better than that of pure L-Ala ligand system, but not as good as that of AAILs ligands system (**Fig. 2** and **Table S3**). All these data indicated that the AAILs as the chiral ligands showed the best separation effect in the CLE-CE system.

Furthermore, the influence of different cation in L-Ala-derived AAILs was studied in detail to investigate the enantioseparation performance of AAILs acted as the chiral ligands in CLE-CE system. In this work, three different kinds of L-Ala derived AAILs which have different alkyl chain length in the imidazolium cation were synthesized. The enantioseparation results were displayed in **Fig. 2** and **Table S3**. It could be observed that both α and Rs of the three test analytes increased when the alkyl chain length of the AAILs became longer. Additionally, the migration times also were prolonged with longer alkyl chain of AAILs. Based on the CLE-CE principle of short migration time and high resolution, the synthesized [BMIm][L-Ala] was finally chosen as the optimal chiral ligand in the further work.

3.2.3 Effect of pH

The pH value mainly affect the complex formation process between central ions and chiral ligands, the separation of analytes and the dissociation of silanol groups on the inner surface of capillary [18], resulting in the influence on the CLE-CE separation system. Therefore, the pH value of the running buffer was studied ranging from 8.1 to 8.9. As shown in **Fig. 1B**, **Fig. S1B** and **Table S3**, both α and Rs of the three test analytes showed a decreasing tendency with pH increasing. Meanwhile, the migration times of the three test analytes also displayed the same tendency as the Rs did. Based on a compromise of α, Rs and migration time, the buffer solution at pH 8.3 was chosen for the further investigation.

3.2.4 Effect of metal complexes

In this study, Mn(II)-[BMIm][L-Ala] complexes work as the chiral selector, influencing the chiral separation. Thus the ratio of Mn(II) to [BMIm][L-Ala] and the complex concentration were two critical factor in the complexes formation process and should be investigated in detail. Mn(II) concentration was kept at 2.5 mM while the concentrations of [BMIm][L-Ala] changed from 1.25 to 10.0 mM with the ratio of Mn(II)/ [BMIm][L-Ala] at 2:1, 1:1, 1:2, 1:3 and 1:4. The results were displayed in **Fig. 1C** and **Table S3**. Notably, both α and Rs of the three test analytes increased from the ratio of 2:1 to 1:4. Meanwhile, the incremental ratio of Mn(II)/ [BMIm][L-Ala] resulted in an obvious raise in the migration times of the three test analytes **(Fig. S1C**). In order to gain desirable α and Rs in short migration time, the ratio of Mn(II)/ [BMIm][L-Ala] at 1:2 was thus selected for further research

Further, the concentrations of Mn(II)-[BMIm][L-Ala] complexes was also studied ranged from 1.5 to 5.5 mM with the ratio kept at 1:2. As shown in **Fig. 1D** and **Table S3**, it has been found that both α and Rs decreased with the increasing concentrations of Mn(II). Meanwhile, we observed that the migration times of the three test analytes also decreased as the Rs did (**Fig. S1D**). As a result, 2.5 mM Mn(II), 5.0. mM [BMIm][L-Ala] at pH 8.3 was selected as the final complexes concentration in this work.

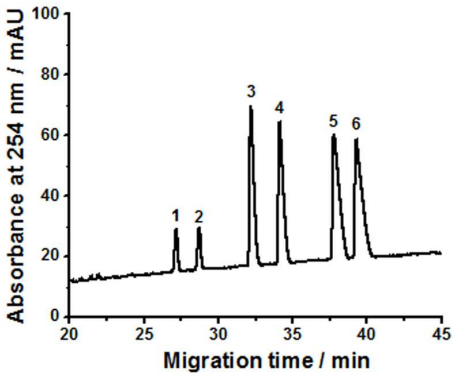

Fig. 3. Enantioseparation of Dns-D,L-Tyr, Dns-D,L-Leu, Dns-D,L-Phe. Buffer conditions: 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0mM β-CD at pH 8.3. Other conditions are the same as **Fig. 1**. Peaks: **1**. Dns-L-Tyr, **2**. Dns-D-Tyr, **3**. Dns-L-Leu, **4**. Dns-D-Leu, **5**. Dns-L-Phe, **6**. Dns-D-Phe.

3.3 Enantioseparation of Dns-D,L-AAs

On account of the above experiments results, the optimized buffer solution were obtained: 100 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala]. The electropherogram of the three target analytes (Dns-D,L-Tyr, Dns-D,L-Leu and Dns-D,L-Phe) was showed in **Fig 3**. As exhibited in **Table 1**, it could be found that twelve pairs of Dns-D,L-AAs were baseline separated and six pairs of Dns-D,L-AAs were partly separated under the optimized condition. In addition, the enantiomer migration orders were examined by injecting a sample solution enriched with the L-amino acid. The results demonstrated the L-AAs generally migrated faster than the D-AAs (**Fig. S2**.).

It should be noted that based on the above experiments, we could assume that the chiral recognition of the CLE-CE system was indeed attributed to the ligand exchange interaction of the analytes with Mn(II)-[BMIm][L-Ala] complexes in combination with a host–guest interaction of the ternary complexes with β-CD.

3.5 Application

3.5.1 Quantitative analysis of L-Tyr

To extent the application of the proposed method in screening enzyme inhibitors, it is necessary to study the quantitative analysis of L-Tyr which was the substrate of tyrosinase. In this work, a series of standard solutions of Dns-D,L-Tyr ranging from

6.47 to 413.3 μM were examined and the linearity was obtained by plotting the peak area versus analyte concentration. The equation of linearity regression of L-Tyr was displayed as follows: y=192.3x + 7096.3 (R^2=0.993, LOD=3.23 μM).The obtained quantitative analysis results demonstrated the proposed CLE-CE method has good availability for the application in monitoring the enzymatic activity of tyrosinase and further screening its inhibitors.

Table 1 Enantioseparation of Dns-D,L-AAs[a]

Dns-D,L-AAs	Rs	t_L/min	t_D/min
Dns-D,L- Thr	3.33	24.93	25.98
Dns-D,L-Val	3.25	24.42	25.33
Dns-D,L-Tyr	4.06	27.18	28.71
Dns-D,L-Leu	3.50	32.19	34.13
Dns-D,L-Ile	2.10	26.36	27.13
Dns-D,L-Pro	2.03	27.87	28.47
Dns-D,L-Met	1.99	24.90	25.48
Dns-D,L-Ser	1.83	23.57	24.08
Dns-D,L-His	1.76	33.23	34.07
Dns-D,L-Phe	1.78	37.77	39.28
Dns-D,L-Ala	1.58	29.01	29.54
Dns-D,L-Asn	1.51	22.76	23.21
Dns-D,L-Trp	1.39	28.20	28.76
Dns-D,L-Gln	1.23	30.94	31.83
Dns-D,L-Orn	1.22	57.30	58.63
Dns-D,L-Glu	1.03	15.37	15.51
Dns-D,L-Arg	0.75	23.29	23.33
Dns-D,L-Asp	0.73	15.23	15.38
Dns-D,L-Cys	0.00	20.54	20.54
Dns-D,L-Lys	0.00	19.52	19.52

[a] Rs: the resolution between Dns-L-enantiomer and Dns-D-enantiomer; tD: the migration time of Dns-D-enantiomer; tL: the migration time of Dns-L-enantiomer; running buffer: 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0mM β-CD at pH 8.3.

3.5.2 Kinetics study of tyrosinase

Then the enzyme kinetics was evaluated by the Michaelis-Menten equation as shown following:

$$V_0 = V_{max}[S]/(K_m + [S])$$

Where V_0 is the initial rate of enzyme reaction, [S] is the substrate concentration, V_{max} is the maximum rate, and Km is the Michaelis constant [51]. In this work, V_0 was determined after a short time, so the incubation time of 15 min at 25 ^{0}C was selected. As demonstrated in **Fig. S3**, the Line-weaver Burk plot was obtained and the linearity is good (R^2 =0.987). It was calculated that the values of K_m and V_{max} were 690 uM and 178 μmole/min/mg, respectively. The results compared well with the previous literature data [51], suggesting the novel CLE-CE technique could be applied in enzyme research and inhibitor screening.

3.5.3 Inhibitors screening

The above experimental results indicated the proposed CLE-CE method could be applied in investigating the inhibition efficiency of the tyrosinase inhibitors. In addition, the benzoic acid and its derivatives showed potential inhibition effects on tyrosinase [54-56]. Therefore, the experiment of inhibition efficiency was conducted to screening the inhibitors of tyrosinase. The results were shown in **Table 2** and **Fig. S4**. The value of IC_{50} was smaller, the inhibition efficiency was better. Therefore, the 4-amino benzoic acid showed the best inhibition efficiency according to the experimental data. In addition, the inhibition efficiency order of the selected inhibitors was displayed as follows: 4-amino benzoic acid > benzoic acid > 4-hydroxyl benzoic acid > 3-hydroxyl benzoic acid > 2-hydroxyl benzoic acid. Moreover, the results illustrated that the novel CLE-CE system with dual chiral selectors based on synergistic effect of β-CD is available for screening the inhibitors of tyrosinase.

Table 2 IC_{50} of the five inhibitors of tyrosinase[a]

Inhibitions	IC_{50} / mM
4- Amino benzoic acid	0.51
Benzoic acid	0.58
4- Hydroxyl benzoic acid	0.64
3- Hydroxyl benzoic acid	0.80
2- Hydroxyl benzoic acid	1.24

a Incubation conditions: 333 mM L-Tyr as the substrate and different inhibitors were incubated with tyrosinase for 15.0 min at 25 ^{0}C.

4. Conclusion

In this work, we successfully synthesized three kinds of L-Ala derived AAILs. The prepared AAILs were further employed as the novel chiral ligands in CLE-CE system. With the introduction of the β-CD, a dual chiral selectors CLE-CE system was developed, and applied in enantioseparation of Dns-D,L-AAs. Under the optimum condition, twelve pairs of AAs could be baseline-separated. The proposed CLE-CE method showed satisfactory separation performance compared with the literatures. Meanwhile, the enantioseparation mechanism was predicted to be the synergistic effect between the metal complexes and β-CD. Moreover, the contracted CLE-CE method was applied in screening tyrosinase inhibitors. Our results indicated that the novel CLE-CE system which used dual chiral selectors exhibited better performance than the single chiral selector on enantioseparation of Dns-D,L-AAs and therefore could provide a new strategy in the development of CLE-CE technique.

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Notes and references

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