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# Preparation and characterization of a new open-tubular capillary column for enantioseparation by capillary electrochromatography

Yingjie Li | Yimin Tang | Shili Qin | Xue Li | Qiang Dai | Lidi Gao

College of Chemistry and Chemical Engineering, Qiqihar University, Qiqihar, PR China

**Correspondence**
Lidi Gao, University of Qiqihar, Building 6, Room D14, Qiqihar, Heilongjiang 161006, PR China.
Email: gaolidi@163.com

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**Abstract**

In order to use the enantioseparation capability of cationic cyclodextrin and to combine the advantages of capillary electrochromatography (CEC) with open-tubular (OT) column, in this study, a new OT-CEC, coated with cationic cyclodextrin (1-allylimidazolium-β-cyclodextrin [AI-β-CD]) as chiral stationary phase (CSP), was prepared and applied for enantioseparation. Synthesized AI-β-CD was characterized by infrared (IR) spectrometry and mass spectrometry (MS). The preparation conditions for the AI-β-CD-coated column were optimized with the orthogonal experiment design $L_9(3^4)$. The column prepared was characterized by scanning electron microscopy (SEM) and elemental analysis (EA). The results showed that the thickness of stationary phase in the inner surface of the AI-β-CD-coated columns was about 0.2 to 0.5 μm. The AI-β-CD content in stationary phase based on the EA was approximately 2.77 $mmol \cdot m^{-2}$. The AI-β-CD-coated columns could separate all 14 chiral compounds (histidine, lysine, arginine, glutamate, aspartic acid, cysteine, serine, valine, isoleucine, phenylalanine, salbutamol, atenolol, ibuprofen, and napropamide) successfully in the study and exhibit excellent reproducibility and stability. We propose that the column, coated with AI-β-CD, has a great potential for enantioseparation in OT-CEC.

**KEYWORDS**

cationic cyclodextrin, chiral separation, open-tubular capillary electrochromatography, thiol-ene click reaction

## 1 | INTRODUCTION

Chiral compounds have the same molecular formula and similar chemical and physical properties, but they are not the same completely because they have an opposite arrangement of molecules structure like a mirror image, so that they are very difficult to be distinguished. Currently, more than two thirds of the drugs are chiral. These enantiomers have different biological, physiological, and pharmacological behaviors on human body. One enantiomer may produce the expected pharmacological response whereas the opposite one may only show an antagonistic or a toxic response.[1-3] These important differences in pharmacological activities of enantiomers cause a rigorous policy of the regulatory authorities, which prescribed strict and specific guidelines for the commercialization of chiral drugs. The guidelines emphasize that approval will not be granted for a drug containing more than one isomer unless the pharmacokinetic and pharmacodynamic properties of each isomer are

described.[4,5] In the agricultural field, the ineffective configuration of chiral pesticides not only does not have the effect of weeding but also may cause environmental pollution and endanger human health. However, most of the pesticides currently on the market are still in the form of racemic forms.[6,7] Therefore, it is necessary for enantioseparation in the field of pharmaceutical and agricultural science. Various methods have been developed for enantioseparation, including high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE). In addition, a hybrid technique, such as capillary electrochromatography (CEC), has also been used for enantioseparations.[8-12]

CEC is a very powerful tool for enantioseparations because it combines the high sensitivity of HPLC with the high-separation efficiency of CE. In recent years, enantioseparations using CEC attract more and more attention because of the short analysis time and low sample and solvent consumption.[13-16] In CEC, the column is the most critical component. Depending on fabricating techniques, three types of columns have been employed including packed columns, monolithic columns, and OT columns. Open-tubular (OT)-CEC has some advantages in simple preparation process, infrequent bubble formation, low back pressure, and short analysis time, compared with packed and monolithic CEC.[17] However, because of the limited amount of stationary-phase coating and/or its weak bonding strength to the inner wall of columns, the OT-CEC also suffers some problems, such as relatively low sample capacity, short lifespan, and narrow pH range, which result in the low-separation capability and the limited practical application. Early in 1992, Mayer and Schurig separated enantiomers by OT-CEC using capillaries coated with a polydimethylsiloxane (PDMS) bound permethylated Chirasil-Dex (CD).[18] Then, Fang et al prepared a novel β-CD-GNPs-coated OT-CEC; three tested enantiomers of four pairs were baseline separated by this chiral column. Meanwhile, the columns exhibited good repeatability and stability for enantioseparation.[14] To prepare a high-performance and stable OT column, it is the crucial to find a suitable material as the stationary phase and immobilize it on the wall of capillary in OT-CEC for entioseparations.[19] Cyclodextrin (CD) and their derivatives (CDs) have been widely used as chiral stationary phases (CSPs), and the researches grow tremendously.[15] Cationic CDs become an important branch of functionalized CDs and have exhibited great potential in enantioseparations. For example, Yu et al prepared a new cationic β-CD derivative, mono-6-deoxy-6-piperdine- β-CD (PIP-β-CD), and successfully applied for the enantioseparation of meptazinol and its three intermediate enantiomers by CE.[20] Wang et al prepared eight single-isomer ammonium-β-CD derivatives with different side chains, and they were also applied successfully for enantioseparation in CE.[21] On the other hand, Yao et al prepared a novel cationic CD CSP by thiol-ene click chemistry and used to separate the dansyl amino acids, carboxylic aryl compounds, and flavonoids by HPLC.[22] Zhou et al prepared also a cationic CD CSP and successfully used it to separate several racemic enantiomers by HPLC.[23] However, most researches were focused on CE or HPLC; a few researches were reported to apply cationic CD to OT-CEC for enantioseparation.

Encouraged by the enantioseparation capability of cationic CD and to combine the advantages of CEC with OT column, in this study, a novel type of chiral OT-CEC column was prepared with cationic CD, AI-β-CD, as the stationary phase and then used it to separate chiral analytes, such as amino acids, drugs, and pesticide. Meanwhile, the effects on enantioseparation by the interactions between the analytes and CSPs were explored. Finally, the repeatability and stability regarding the AI-β-CD-coated OT column were studied.

## 2 | MATERIALS AND METHODS

### 2.1 | Reagents and materials

All reagents used were of analytical grade unless otherwise stated. β-CD, anhydrous n,n-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were ordered from Kermel Chemical Reagent Co (Tianjin, China). γ-Mercaptopropyltrimethoxy-silane (γ-MPTS), azobisisobutyronitrile (AIBN), 1-allylimidazole, and 4-toluenesulfonylchloride (TsCl) were purchased from Acros Chemical Co (NJ, USA). All the amino acids racemates were obtained from Sinopharm Chemical Reagent Co (Shanghai, China). Ibuprofen, atenolol, and salbutamol were purchased from Pharmaceutical and Biological Products Inspection (Beijing, China). Napropamide was obtained from Aladdin Industrial Co (Shanghai, China). All solvents used in CEC were of chromatographic grade. Fused silica capillaries (50-μm i. d. and 375-μm o.d.) were purchased from Yongnian Optical Fiber Factory (Hebei, China).

### 2.2 | Instrumentation

All the CEC experiments were performed on an Agilent 7100CE system (Agilent Technologies, Waldbronn, Germany) equipped with diode-array detector. A syringe pump P200 (Elite, China) was employed to inject the mixtures into the fused silica capillary. Mass spectra were

recorded on 6230TOFMS mass spectrometer (CA, USA). Scanning electron microscopy (SEM) images were obtained by an S-4300 (Hitachi, Japan) SEM. The Fourier-transform infrared (FTIR) spectra were measured with SpectrumOne FTIR spectrometer (PE Co, USA). EA was performed on PE2400 organic element analyzer (PE Co, USA).

## 2.3 | Synthesis of AI-β-CD

The preparation pathway of AI-β-CD was reported previously, and it was diagramed in Figure 1.[22,24]

## 2.4 | Preparation of the AI-β-CD-coated OT-CEC columns

Briefly, the bare capillary was pretreated.[25] Then, the capillary was rinsed with 80% (*v*/*v*) ethanol solution of γ-MPTS for 30 minutes, both ends sealed and treated in water bath for 24 hours at 40°C. In the process, γ-MPTS was bonded on the inner wall of bare capillary by dehydration-condensation reaction. After that, the capillary was washed with methanol to remove the residual reagents and dried by a nitrogen stream. At this point, the inner wall of the capillary was modified by a layer of γ-MPTS with free —SH groups. Next, AI-β-CD, AIBN, and DMSO were mixed and sonicated for 20 minutes; the mixture was poured into the modified capillary. With both ends sealed, the capillary was heated in water bath to couple the AI-β-CD with the layer of γ-MPTS by thiol-ene click chemistry reaction.[26] Finally, the AI-β-CD-coated columns were washed with methanol thoroughly to remove the residual of reactants, then flushed with the running buffer for 10 minutes and filled with ultrapure water and stored until use. The AI-β-CD-coated column was characterized by SEM and EA. The whole process for the preparation was diagramed in Figure 1.

## 2.5 | CEC process

The AI-β-CD-coated column was installed on the CE instrument; detecting wavelength of analytes was set at 200 nm except for napropamide (220 nm). Before started, the AI-β-CD-coated column was rinsed with ultrapure water for 5 minutes and running buffer for another 5 minutes. The sample was injected into the AI-β-CD-coated column by 50 mbar × 3 seconds and separated immediately by applying a running voltage of 15 kV. The CEC was performed with 20-mM acetate buffer (pH 3.0-10.0) at room temperature. All the solutions including the test samples were filtered through a 0.22-μm pore-size PES membrane.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Characterization of AI-β-CD

FTIR analysis were carried out to verify the functioning groups on AI-β-CD (Figure 2). The β-CD had the characteristic absorptions at 3400 $cm^{-1}$($\nu_{O—H}$), 2927 $cm^{-1}$ ($\nu_{—CH2—CH2—}$), 1640 $cm^{-1}$($\nu_{C—H}$), and 1028 $cm^{-1}$($\nu_{C—O}$). The 6-OTs-β-CD had the characteristic absorptions at 1365 and 843 $cm^{-1}$, which represented the S=O and C—H of benzene rings (Figure 2B), respectively. The AI-β-CD had the characteristic absorptions at 1566 and 1660 $cm^{-1}$, which represented the C=C in imidazole rings and C=C in allyl group, as shown in Figure 2C. Additionally, mass spectrometry (MS) was applied to confirm further AI-β-CD structure (Figure 3). In mass spectra of AI-β-CD, $m/z = 1225.49708$ was assigned to the ($M^+$) peak in AI-β-CD. These results showed that allylimidazolyl has been successfully introduced into AI-β-CD.

## 3.2 | Optimization of preparation conditions of AI-β-CD-coated columns

In order to optimize the preparation conditions of AI-β-CD-coated columns, reaction temperature, reaction

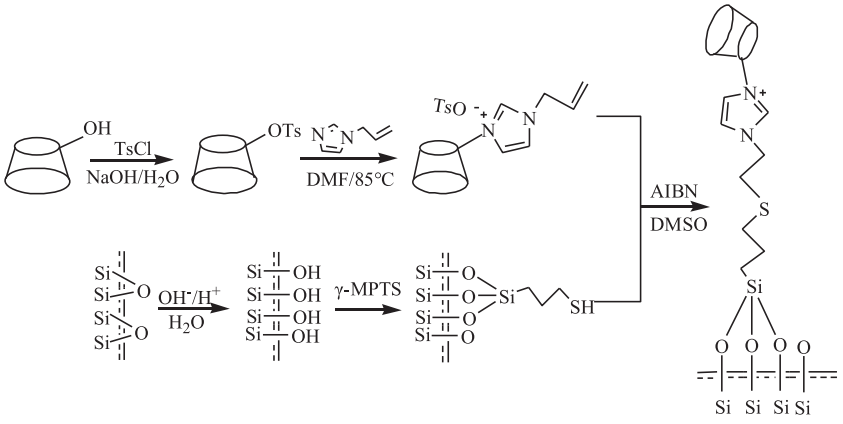



**FIGURE 1** Synthesis pathway of the AI-β-CD-coated capillaries

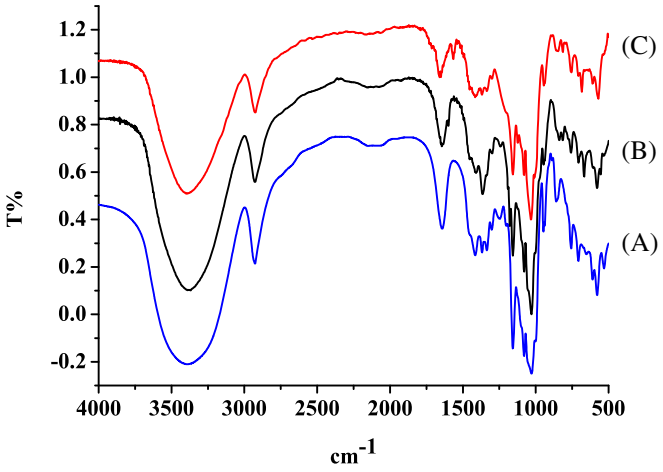



**FIGURE 2** Fourier-transform infrared (FTIR) spectra (A) β-CD, (B) 6-OTs-β-CD, and (C) AI-β-CD

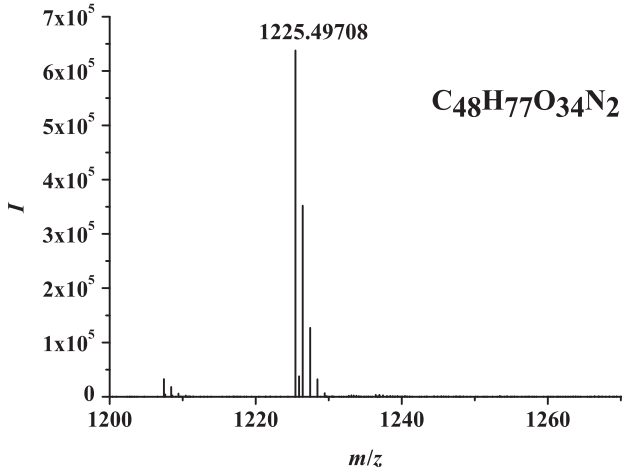



**FIGURE 3** Mass spectra for AI-β-CD

time, and AI-β-CD concentration were investigated. The orthogonal experiment design $L_9(3^4)$ was applied for the optimization. The main significant factors and levels can be identified by the analysis of variance as shown in Table 1, where the resolution (*Rs*) of histidine enantiomers was used to weigh the performance of AI-β-CD-coated columns under different conditions.

According to the analyses, the performance of AI-β-CD-coated columns was influenced by the three factors (Table 1), and the influence intensity was in the order of AI-β-CD concentration > reaction temperature > reaction time. On the basis of the analysis results, AI-β-CD solution with 0.3 g/mL, which is close to saturation, was optimal one ($T_{3C} > T_{2C} > T_{1C}$). Grafting of the AI-β-CD to capillary columns by thiol-ene reaction can be realized at mild reaction temperature that is benefit to long-term stability.[25,27] The maximum separation efficiency was achieved at 70°C ($T_{2A} > T_{3A} > T_{1A}$). In addition, the reaction time can also affect the immobilization of AI-β-CD. The reaction time of 24 hours was not long enough to immobilize sufficient AI-β-CD for the enantioseparation; meanwhile, the reaction time of 48 hours did not make the resolution better. Therefore, 36 hours was chosen for the preparation of the AI-β-CD-coated columns ($T_{2B} > T_{3B} > T_{1B}$).

**TABLE 1** The experimental design based on the Taguchi's $L_9(3^4)$ orthogonal array and the results for the optimization on preparation conditions of AI-β-CD-coated column

| Factor<br>Experiment No. | Reaction Temperature (°C)<br>A | Reaction Time (h)<br>B | $C_{AI\text{-}\beta\text{-}CD}$ (g/mL)<br>C | Index<br>*Rs* |
|---|---|---|---|---|
| 1 | 60 | 24 | 0.20 | 0.82 |
| 2 | 60 | 36 | 0.25 | 1.11 |
| 3 | 60 | 48 | 0.30 | 1.42 |
| 4 | 70 | 24 | 0.25 | 1.22 |
| 5 | 70 | 36 | 0.30 | 1.78 |
| 6 | 70 | 48 | 0.20 | 1.03 |
| 7 | 80 | 24 | 0.30 | 1.50 |
| 8 | 80 | 36 | 0.20 | 0.98 |
| 9 | 80 | 48 | 0.25 | 1.13 |
| $T_{1j}$ | 3.35 | 3.54 | 2.83 | |
| $T_{2j}$ | 4.03 | 3.87 | 3.46 | |
| $T_{3j}$ | 3.61 | 3.58 | 4.70 | |
| $R_j$ | 0.68 | 0.33 | 1.87 | |

DL-histidine was used as a model analyte. The other experimental conditions: capillary column: 40-cm total length (26.5-cm effective length), 50-μm i.d.; 20-mM acetate buffer solution; pH: 5.0; separation voltage: 15 kV; injection: 50 mbar × 3 seconds; detection wavelength: 200 nm. *T* was the sum of indexes of the same level, *R* was the difference between the maximum index and the minimum index from the same factor and level, and j represented the different factors.

### 3.3 | Characterization of the AI-β-CD-coated columns

In this work, AI-β-CD was readily immobilized on the inner wall of a capillary column by thiol-ene click chemistry reaction and served as an OT-CEC stationary phase. The morphological structure and thickness of the inner surface were characterized by SEM. As shown in Figure 4A, the inner surface of a bare capillary was very smooth; after coating, it became much rough (Figure 4 B,C). It was clear that some aggregates were tightly coated onto the inner wall of the AI-β-CD-coated columns and the thickness was about 0.2 to 0.5 μm, which indicated that a solid phase was made successfully on the inner wall of the capillary column.

Simultaneously, elemental analysis was conducted and confirmed that the elements of C, H, and N exist on the surface of the bare capillary column and the AI-β-CD-coated column, respectively. It could be found that the difference between the nitrogen content of the bare capillary column and the AI-β-CD-coated column was significant; they were 0.70% ± 0.05% versus 0.85% ± 0.06%, respectively. The difference between the carbon content of the bare capillary column and the AI-β-CD-coated column was also significant; they were 8.32% ± 0.08% to 11.47% ± 0.06%, respectively. Meanwhile, the hydrogen content increased from 0.25% ± 0.05% of the bare capillary column to 0.66% ± 0.04% of the AI-β-CD-coated column. The elemental analysis results demonstrated that the coating procedure was successful. The content of the AI-β-CD precursor was calculated from elemental analysis, and it was approximately 2.77 mmol·m$^{-2}$.

The electroosmotic flow (EOF) is the key factor in OT-CEC, and it is highly affected by buffer pH. In the present study, we investigated the EOF values on both the bare capillary column and the AI-β-CD-coated column, with varied pH of 3.0 to 10.0. As shown in Figure 5, the EOF increased gradually regardless of the bare capillary or the AI-β-CD-coated column. The AI-β-CD-coated column had lower EOF than the bare capillary column had due to blocking by silanol. The EOF reached 1.22 × 10$^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ for the coated column at pH 8.0, compared with 3.23 × 10$^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ for the bare one. Additionally, unlike that of the bare capillary column, EOF of the AI-β-CD-coated column was relatively stable in the studied pH range (Figure 5). The relative standard deviation (RSD) of the EOF on the AI-β-CD-coated column was 1.42% at pH 8.0, which was lower than that on the bare one (RSD = 3.29%, n = 3). This result further indicates that the coated surface is stable and reproducible, which may facilitate the applications of AI-β-CD on CEC.

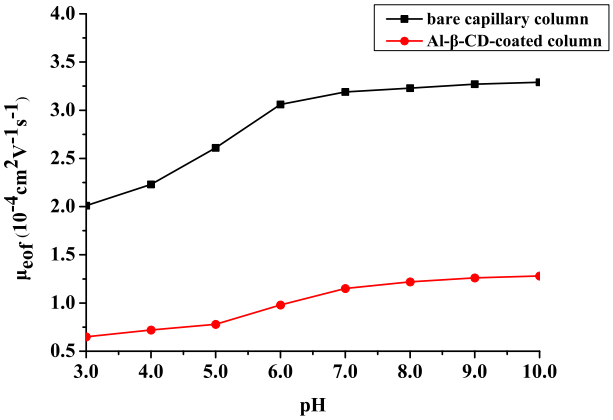



**FIGURE 5** Effect of eluent pH on the electroosmotic mobility. The experimental conditions: sample: thiourea; 20-mM acetate buffer solution (pH 3.0-10.0); separation voltage: 15 kV; injection: 50 mbar × 3 seconds; detection wavelength: 200 nm

### 3.4 | Enantioseparation performance of AI-β-CD-coated columns

The enantiomers, including 10 amino acids (histidine, lysine, arginine, glutamate, aspartic acid, cysteine, serine, valine, isoleucine, phenylalanine), three drugs (salbutamol, atenolol, and ibuprofen), and one pesticide (napropamide), were separated on the AI-β-CD-coated column by CEC. As shown in Table 2 and Figure 6, most enantiomers could be separated successfully by the

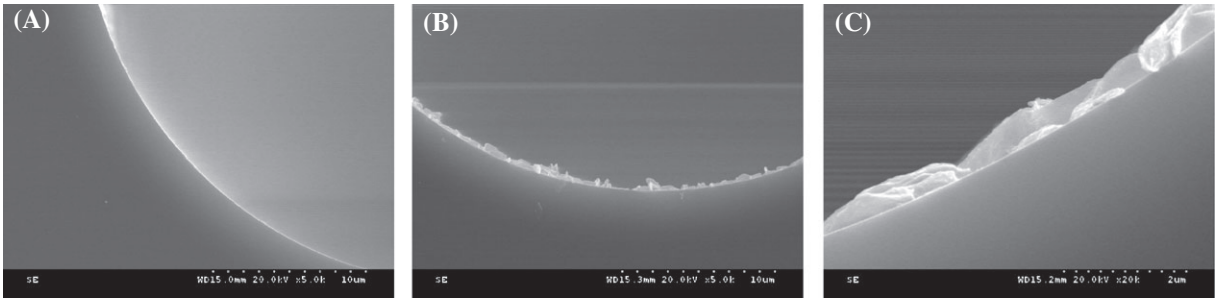
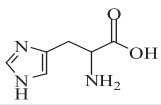
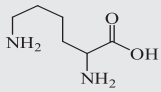
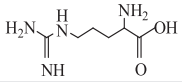
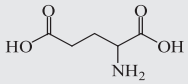
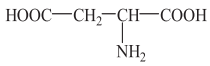
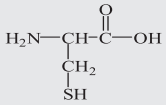
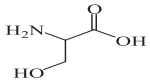
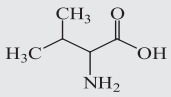
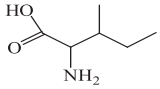
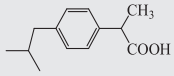
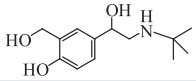
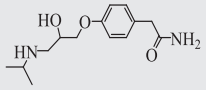
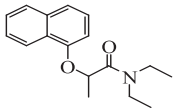
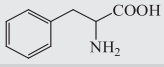



**FIGURE 4** Scanning electron microscopy (SEM) images for the inner surface of (A) bare capillary column (×5.0 k), (B) AI-β-CD-coated column (×5.0 k), and (C) AI-β-CD-coated column (×20 k)

**TABLE 2** The optimum pH for the separation of each analyte

| Type | Analytes | Structure | PKa (PKa1/PKa2) | pI | Optimum pH | *Rs* | *α* |
|---|---|---|---|---|---|---|---|
| Alkaline amino acids | Histidine |  | 1.82/9.17 | 7.59 | 5 | 1.78 | 1.87 |
| | Lysine |  | 2.18/8.95 | 9.74 | 5 | 1.04 | 1.22 |
| | Arginine |  | 2.17/9.04 | 10.76 | 8 | 2.47 | 2.29 |
| Acidic amino acids | Glutamate |  | 2.19/9.67 | 3.22 | 9 | 0.71 | 1.21 |
| | Aspartic acid |  | 2.09/9.82 | 2.97 | 8 | 0.74 | 1.26 |
| | Cysteine |  | 1.71/8.33 | 5.02 | 8 | 4.14 | 1.66 |
| | Serine |  | 2.21/9.15 | 5.68 | 8 | 2.25 | 1.35 |
| | Valine |  | 2.32/9.62 | 5.91 | 8 | 1.68 | 1.26 |
| | Isoleucine |  | 2.36/9.68 | 6.02 | 8 | 0.89 | 1.13 |
| | Phenylalanine |  | 1.83/9.13 | 5.48 | 8 | 1.56 | 1.32 |
| Chiral compounds | Salbutamol |  | 10.12 | - | 8 | 2.64 | 2.43 |
| | Atenolol |  | - | - | 6 | 3.47 | 3.14 |
| | Napropamide |  | - | - | 3 | 1.51 | 1.22 |
| | Ibuprofen |  | 4.55 | - | 7 | 3.99 | 2.10 |

Assume that all the separation curves in this experiment satisfy the Gaussian curve. *Rs*: the resolution, when the baseline separation is achieved; the *Rs* can be calculated by the formula, $Rs = 2\,(t_2 - t_1)/(W_1 + W_2)$; if the baseline separation has not been achieved, Rs can be calculated by the formula, $Rs = 1.18\,(t_2 - t_1)/(Wh_1 + Wh_2)$, where *W* is the peak width (minute), *Wh* is the half width (minute), *t* is the the migration time (minute), and *α* is the the selectivity factor, $\alpha = t_2/t_1$.

coated column, and the *Rs* and the selectivity factors (*α*) varied from 0.71 to 4.14 and from 1.13 to 3.14, respectively, for different enantiomers. All of the racemates were completely separated with $\alpha > 1.05$ under the stated conditions.[28] Meanwhile, the enantiomers of histidine, arginine, cysteine, serine, valine, phenylalanine, salbutamol, atenolol, napropamide, and ibuprofen reached very good baseline separation with *Rs* less than 1.5. In this work, the influence of buffer pH on the enantioseparation was evaluated from pH 3.0 to 10.0 for alkaline amino acids (Figure 7A). When the pH is in the range of 3.0 to 10.0, the AI-β-CD-coated column shows a more stable cathodic EOF. Typically, when histidine is used for enantioseparation, since histidine is an alkaline amino acid, if the pH is less than pI and greater than pKa1, histidine will have a positive charge; its

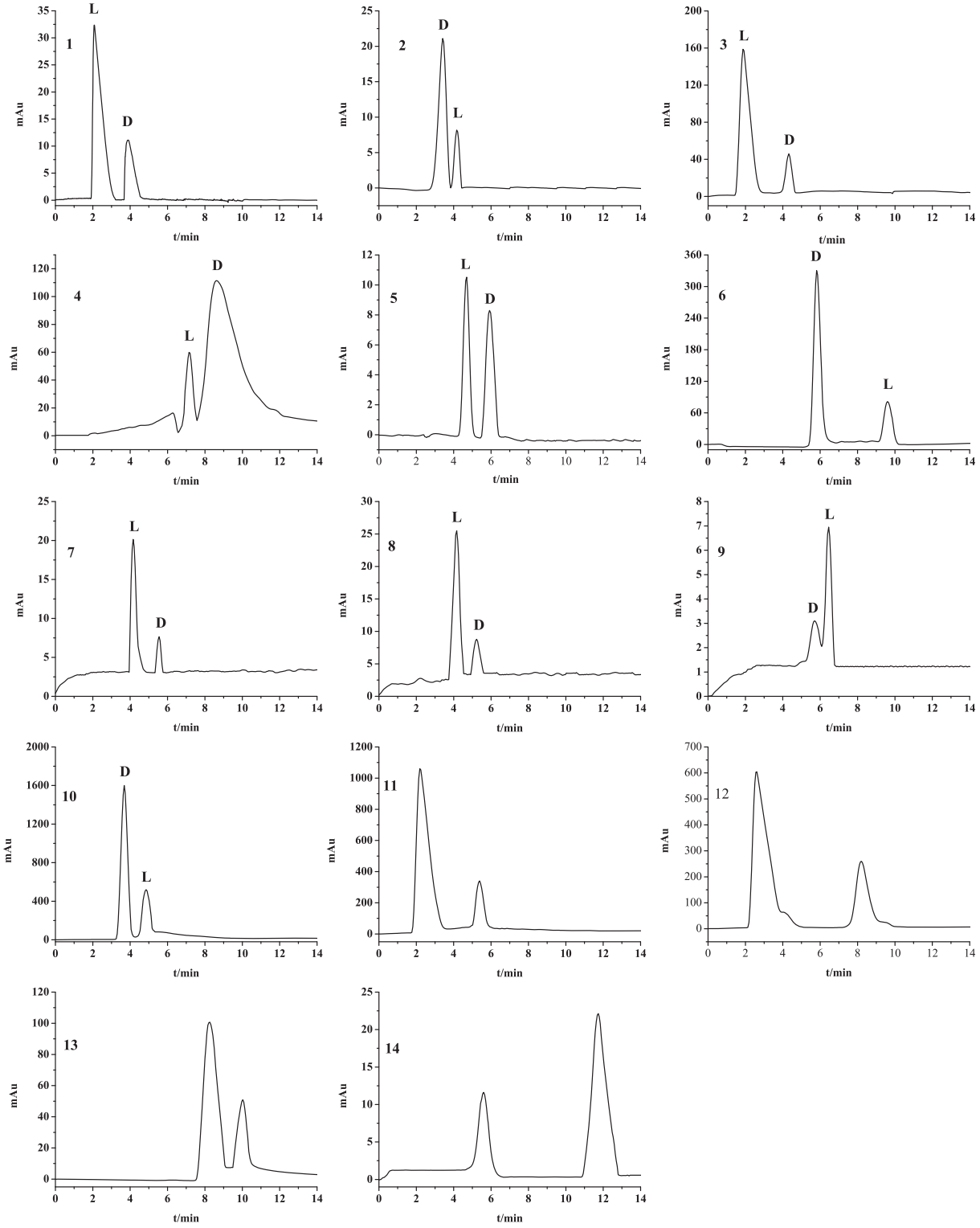



**FIGURE 6** Chiral separation chromatograms of analytes. 1. Histidine, 2. lysine, 3. arginine, 4. glutamate, 5. aspartic acid, 6. cysteine, 7. serine, 8. valine, 9. isoleucine, 10. phenylalanine, 11. salbutamol, 12. atenolol, 13. napropamide, and 14. ibuprofen. The experimental conditions: capillary column: 40-cm total length (26.5-cm effective length), 50-μm i.d.; 20-mM acetate buffer solution; separation voltage: 15 kV; injection: 50 mbar × 3 seconds; detection wavelength: 200 nm except for napropamide (220 nm); optimum pH of 14 analytes is shown in Table 2

moving direction is consistent with the direction of EOF. Herein, as the pH ranges from 3.0 to 6.0, the migration time of histidine enantiomers shortens. However, the resolution increases when pH ranges from 3.0 to 5.0 and then decreases if pH increases up to 6.0. This might be due to the shortening of the interaction time and the

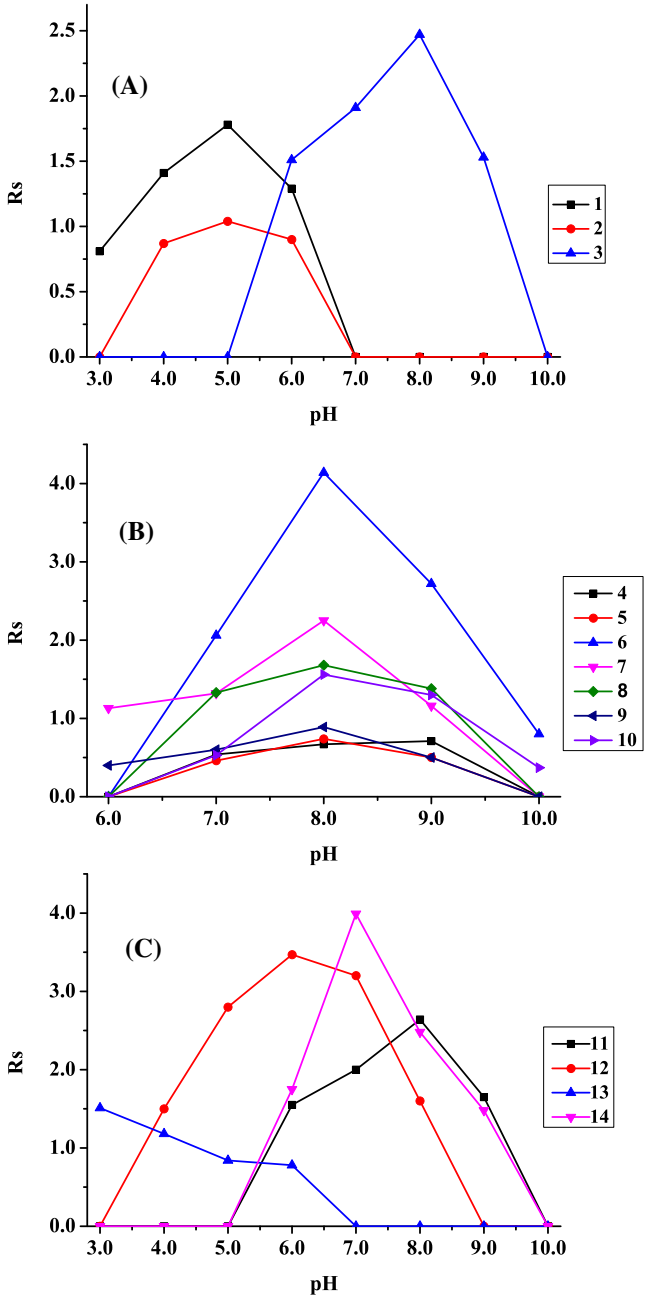



**FIGURE 7** Effect of pH on the resolution of the analytes. (A) 1. Histidine, 2. lysine, and 3. arginine, (B) 4. glutamate, 5. aspartic acid, 6. cysteine, 7. serine, 8. valine, 9. isoleucine, and 10. phenylalanine, (C) 11. salbutamol, 12. atenolol, 13. napropamide, and 14. ibuprofen

weakening of the interaction force between the analytes and the CSPs. Thus, pH 5.0 was chosen for the chiral separation of histidine enantiomers based on the resolution and migration time (Table 3). Since alkaline amino acids have a group of —$NH_2$ or —NH and easily bind to $H^+$ to form —$NH_3^+$ and —$NH_2^+$ when pH is less than pI value, they produce a hydrogen bond with hydroxyl group on the surfaces of β-CD, so that the intermolecular forces between the AI-β-CD and the enantiomers are enhanced,

**TABLE 3** Effect of pH

| Sample | pH | $t_L$ (min) | $t_D$ (min) | *Rs* | α |
|---|---|---|---|---|---|
| Histidine | 3.0 | 3.56 | 4.56 | 0.76 | 1.28 |
| | 4.0 | 2.53 | 3.91 | 1.42 | 1.55 |
| | 5.0 | 2.08 | 3.89 | 1.78 | 1.87 |
| | 6.0 | 1.22 | 2.24 | 1.30 | 1.84 |

The experimental conditions: capillary column: 40-cm total length (26.5-cm effective length), 50-μm i.d.; sample: histidine; 20-mM acetate buffer solution; pH: 3.0 to 6.0; separation voltage: 15 kV; injection: 50 mbar × 3 seconds; detection wavelength: 200 nm.

and the chiral separation was achieved successfully. If the amino acids are acidic and the buffer pH is greater than pI and less than pKa2, the amino acid will have the negative charge, which will result in repulsion between the negatively charged amino acid and the cathode. When the optimum pH for isolating these amino acids was larger than their pI, such as cysteine, serine, valine, isoleucine, and phenylalanine, these amino acids can interact with the cationic AI-β-CD by electrostatic interaction and finally result in the separation for the enantiomers (Figure 7B). However, for glutamate and aspartic acids, they have a long retention time and low resolution. As reported by Yao et al, the long retention may be because of the stronger electrostatic interaction generated by the second —$COO^-$ moiety. [22] Besides, the competition of the two —$COO^-$ moieties with the cationic sites on CSPs may weaken the "three-point" interaction; that is, three side groups of the chiral center have to be held in place by specific interactions with its environment, either attractive or repulsive, [29] hence reduce the enantioselectivity. Additionally, salbutamol, atenolol, napropamide, and ibuprofen were separated successfully based on their alkalinity or acidity; the optimum pH was 8.0, 6.0, 3.0, and 7.0, respectively (Figure 7C). It needs to emphasize that the hydrophobic interaction between hydrophobic groups (such as benzene rings) and the hydrophobic cavity of β-CD also plays an important role in the separation. The hydrophobic, hydrogen bonding, π-π, and electrostatic interactions between the analytes and the CSPs all contribute to chiral recognition process. These results show that the AI-β-CD-coated column has excellent performance on enantioseparation. In addition, the separation method used in this study has the advantages of short time and low consumption.

## 3.5 | Reproducibility and stability

The reproducibility and stability about the AI-β-CD-coated columns were evaluated in terms of the RSDs on the retention time and resolution using histidine enantiomers as the testing sample, and the results were exhibited

**TABLE 4** The reproducibility of the column for the enantioseparation

| Type and Numbers (n) of Experiments | *Rs* (% RSD) | $t_L$ (% RSD) | $t_D$ (% RSD) |
|---|---|---|---|
| Run to run (n = 6) | 1.72 | 1.20 | 0.39 |
| Day to day (n = 6) | 2.35 | 2.23 | 1.79 |
| Column to column (n = 6) | 3.13 | 3.04 | 2.43 |

Abbreviation: RSD, relative standard deviation. The experimental conditions: capillary column: 40-cm total length (26.5-cm effective length), 50-μm i.d.; sample: histidine; 20-mM acetate buffer solution; pH: 5.0; separation voltage: 15 kV; injection: 50 mbar × 3 seconds; detection wavelength: 200 nm.

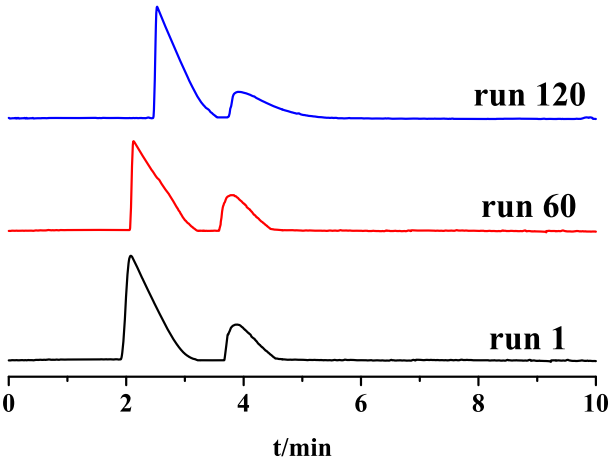



**FIGURE 8** The chromatogram of histidine enantiomers with different runs. The experimental conditions were described as in Table 1

in Table 4. All experiments were repeated for three times. As shown in Table 4, the RSDs for run to run, day to day, and column to column were less than 4%. Moreover, the separation efficiency of the AI-β-CD-coated columns did not decrease obviously over 120 runs (Figure 8). These results demonstrate that the AI-β-CD-coated columns prepared have excellent reliability and reproducibility.

Although the AI-β-CD-coated column was flushed by large amount of buffers varying pH from 3.0 to 10.0, the retention time and chiral selectivity are reproducible and indicate that the stability of the cationic CSP is great. This may be attributed to the strong thiolether linkages and stable imidazolium moiety, which are able to withstand various operating conditions.

## 4 | CONCLUSIONS

The preparation and charaterization of the AI-β-CD-coated OT-CEC column for enantioseparation were described. The AI-β-CD-coated column possesses relatively stable EOF at varying pH values and better chiral separation performance for 14 chiral compounds (histidine, lysine, arginine, glutamate, aspartic acid, cysteine, serine, valine, isoleucine, phenylalanine, salbutamol, atenolol, ibuprofen, and napropamide). Moreover, the AI-β-CD-coated column exhibits an excellent reproducibility and stability. These results indicate that the OT-CEC column coated with AI-β-CD has great potential for enantioseparation.

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## ORCID

*Yimin Tang* https://orcid.org/0000-0003-1271-9592

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