

## RESEARCH ARTICLE

# Synthesis of $\beta$ -Cyclodextrin–Calix[4]arene Polymer-Based Chiral Stationary Phase for Chromatographic Separation of Enantiomers

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**Keywords:**  $\beta$ -cyclodextrin | calix[4]arene | chiral stationary phase

## ABSTRACT

In this work, a new strategy was proposed for the preparation of a chiral stationary phase based on  $\beta$ -cyclodextrin and calix[4]arene polymer. The chiral stationary phase was characterized by scanning electron microscopy, energy dispersive x-ray spectroscopy, Fourier transform infrared spectroscopy, and thermogravimetric analysis. The introduction of calix[4]arene polymer increased chirality recognition by increasing recognition sites and interactions, including hydrogen bonding,  $\pi$ – $\pi$  interaction and synergistic inclusion effect. Compared with the home-made  $\beta$ -cyclodextrin-based chiral column, the prepared  $\beta$ -cyclodextrin–calix[4]arene polymer-based chiral column showed superior enantioseparation performance toward 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol. These chiral compounds can be separated within 10 min under reversed-phase mode with resolutions ranging from 1.62 to 1.80. These findings will provide an important reference for developing novel supramolecule-based chiral stationary phases.

## 1 | Introduction

Separation and analysis of chiral enantiomers is of great importance in the fields of pharmaceutical analysis, food additives, agriculture, materials science, and environmental science [1–4]. Among various chiral separation techniques, high-performance liquid chromatography (HPLC) based on chiral stationary phases (CSPs) has emerged as one of the most useful methods for enantioseparation for analytical and preparative purposes [5–9]. As the core of chiral separation, the design and synthesis

of novel CSPs with high enantioselectivity are crucial in advancing the developments in the field of chiral chromatography.

Over the past few decades, many materials have been developed as the CSPs, such as cyclodextrins (CDs) and their derivatives, chiral covalent organic frameworks (COFs), chiral metal–organic frameworks (MOFs), polysaccharides derivatives, and chiral crown ethers [7, 10–14]. Among these materials, CDs and their derivatives showed extensive applications in chiral separation due to their unique structures, which can provide

hydrogen bonding interactions, electrostatic interactions, and steric repulsive effects with chiral molecules [15–18]. In 1984, Armstrong's group [19] synthesized and commercialized the first CD-CSP for chiral separation. During the decades of the 1980s–2000s, their research group has developed many kinds of CDs and their derivatives as CSPs for the chiral separation of thousands of compounds [20–27]. The derivatization of CDs' rims can alter their enantioselectivities toward different analytes. Their research confirmed the significant effects of CDs and their derivatives in chiral separation, laying an outstanding contribution to subsequent research on CDs. At present, the development and application of novel CDs-based CSPs still draw great concern [10, 16, 28–31]. Armstrong's research group [28] developed a new method for separating both cobalt bis (dicarbollide) and *nido*-7,8- $C_2B_9H_{12}(1-)$  derivatives by commercially available chiral columns. They found that the most potent column was hydroxypropyl  $\beta$ -CD chiral column. Furthermore, they broadened the application of a 2-hydroxypropyl- $\beta$ -CD chiral column to nonaqueous chromatographic modes for separating 1,4-dihydropyridines [29]. Additionally, Chen et al. [10] synthesized the porous methylated CDs-containing polymers (MP-CDPs) for chiral adsorption and HPLC chiral stationary phase. Li's team [16] developed an ethylenediamine dicarboxyethyl diamido-bridged bis( $\beta$ -cyclodextrin)-bonded chiral stationary phase (EBCDP), which demonstrated excellent performance in resolving 28 different racemic compounds in the reversed-phase or polar organic mode. Wang's group [30] constructed a series of allylimidazole CD derivatives and coated them onto silica gel by the thiol-ene click reaction. The enantioseparation capabilities of the prepared CSPs were tested with 35 chiral analytes.

Indeed, there have been many reports on the preparation and application of CDs-based CSPs; however, many CDs-based CSPs generally involve multistep derivation or modification processes of CDs (e.g., long preparation time, purifying products by column chromatography) and irritant chemicals (e.g., pyridine, sodium hydride). Therefore, there is still a need to build new CDs-based CSPs with simpler preparation processes and shorter analysis time.

Calix[n]arene is the third-generation compound of supramolecules following CDs and crown ethers [32]. Compared with native CDs, there are large amounts of phenyl groups in calix[n]arene structures, which can provide  $\pi$ - $\pi$  interactions and stronger hydrophobic interactions. Benefiting from its cup-shaped structure and easily functionalized property, calix[n]arene can be applied in drug delivery, molecular sensing, and separation [33–36]. So far, a few calix[n]arene materials have been investigated as chiral CSPs in chiral chromatographic separations [37–40]. For example, Gong's group [37] prepared the bromoacetate-substituted [3-(2-O- $\beta$ -cyclodextrin)-2-hydroxypropoxy]propylsilyl-appended silica particles and then modified them with calix[4]arene to obtain calix[4]arene-capped  $\beta$ -CD CSP for HPLC. After that, they synthesized the 4-isopropylcalix[4]arene-capped  $\beta$ -CD CSP through a similar method [38]. Their results showed that the combination of calix[4]arene (or 4-isopropylcalix[4]arene) and  $\beta$ -CD moieties can facilitate the selectivity for separating aromatic positional isomers and chiral aromatic analytes. Based on the above-mentioned examples, we speculated that the functionalization of calix[n]arene derivatives onto silica gel surface might enhance the CSP chiral resolution ability. However, the

reported calix[n]arene-based CSPs have some limitations such as high preparation costs, complicated and cumbersome synthesis processes. Therefore, developing a facile and efficient strategy to design a novel chiral CSP that combines the good features of CDs and calix[n]arene is crucial for the application of CDs-calix[n]arene-based CSPs. Previously, we synthesized a calix[4]arene-based polymer with good adsorption capacity for six cationic organic dyes and two antihistamines through a simple one-step method. The (phenolic) hydroxyl groups and benzene groups in the porous structure facilitated the recognition and adsorption of analytes [41]. Inspired by this, it is desirable to further explore the separation ability of calix[4]arene-based polymer when employed as CSP material. However, the study of calix[n]arene-based polymer as CSPs has not been reported yet. Therefore, CDs and calix[n]arene-based polymer are being examined for the first time as surface modification materials for silica gel in order to investigate their potential in chiral HPLC.

In this work, the calix[4]arene-based polymer was synthesized and bonded onto  $\beta$ -CD functionalized silica gel through a simple and facile strategy to prepare a  $\beta$ -CD-calix[4]arene polymer (CD-C4AP) based CSP. The chemical structures of CD-C4AP-based CSP were characterized by scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDS), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). A series of chiral analytes (including 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol) and polycyclic aromatic hydrocarbons (including naphthalene, anthracene, and pyrene) were used to evaluate the separation performance of the developed CSP under RPLC mode. Compared with the home-made  $\beta$ -CD-based CSP, the CD-C4AP-based CSP showed a superior separation performance for the studied chiral analytes. In a word, the CD-C4AP-based CSP has potential in separating chiral compounds.

## 2 | Materials and Methods

### 2.1 | Materials and Measurements

Calix[4]arene (C4A), 1-phenylethanol, 3-isocyanatopropyltriethoxysilane (IPTS),  $\beta$ -CD, tetrafluoroterephthalonitrile (TFTPN), and epichlorohydrin (EPI) were purchased from Energy Chemical (Shanghai, China). Sodium hydroxide (NaOH), N,N-Dimethylformamide (DMF), ethanol (EtOH), hydrochloric acid (HCl) and acetone of analytical grade and acetonitrile (ACN), and methanol (MeOH) of HPLC grade were obtained from Shandong Yuwang Industrial Co. Ltd. (Shandong, China). The spherical silica (5  $\mu$ m, 120 Å, 300 m<sup>2</sup>/g) was purchased from Greenherbs Science and Technology (Beijing, China). HPLC grade water was obtained from Jilin Wahaha Foods Co. Ltd. (Jilin, China). Prothioconazole (98.7%) was purchased from the Shanghai Pesticide Research Institute (Shanghai, China). Naphthalene (99.7%), anthracene (99.5%), pyrene (99.5%), promethazine (98%), propranolol (98%), and bisoprolol (98%) were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). Scanning electron microscope images were recorded from the Zeiss Gemini SEM 300 field emission scanning electron microscope. The EDS analysis was recorded on an Ultim Max Oxford instrument (Shanghai, China). Thermogravimetric data were determined by a STA200 thermogravimetric analyzer (HITACHI Corporation,

Japan). Infrared absorption spectra were obtained from a Nicolet iS20 Fourier transform infrared spectrometer (Thermo Fisher, USA). HPLC analysis was performed on an LC-16 instrument (Shimadzu, Kyoto, Japan).

## 2.2 | Synthesis of CD-C4AP-Based Chiral Stationary Phase

The synthetic scheme of CD-C4AP column is shown in Scheme 1.

### 2.2.1 | Synthesis of $\beta$ -CD-Functionalized Silica Gel

$\beta$ -CD (4g) was mixed with anhydrous DMF (50mL) with the addition of 0.87mL IPTS; the mixture was reacted for 12h at 80°C under a nitrogen atmosphere. Subsequently, the activated silica gel was added to the above solution and the reaction was carried out at 110°C for 24h. Finally, the products were washed with anhydrous DMF, EtOH, and acetone and then dried under vacuum at 60°C.

### 2.2.2 | Synthesis of CD-C4AP-Based Chiral Stationary Phase

0.2g of TFTPn was added to a suspension of  $\beta$ -CD-functionalized silica gel (2g) in 24mL of DMF at 100°C. Then, 0.5mL EPI, C4A (0.1g) in DMF (8mL), and 0.95M NaOH (16mL) were added and the resulting mixture was heated at 100°C for 3h. Following a natural cooling process at room temperature, DMF, water, and EtOH were used to wash the product. The material was dried under vacuum at 60°C to get the CD-C4AP-based chiral stationary phase.

### 2.2.3 | Column Packing

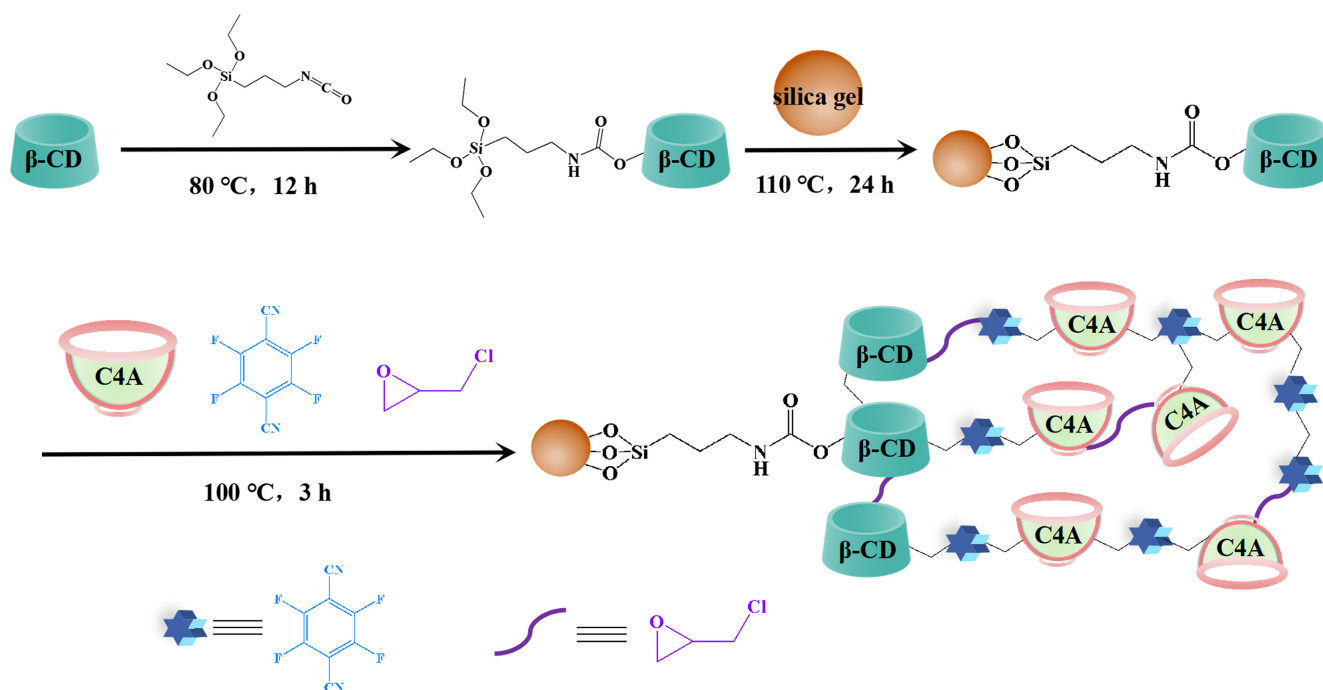
About 5g of the prepared stationary phases was dispersed in an appropriate volume of isopropanol and packed into a stainless-steel column (250×4.6mm i.d.) at a pressure of 40MPa. The newly chiral CD-C4AP column was connected to the HPLC system, which was equilibrated using methanol as the eluent. For comparison, the chiral  $\beta$ -CD column was also prepared and packed with  $\beta$ -CD-functionalized silica gel.

## 3 | Results and Discussion

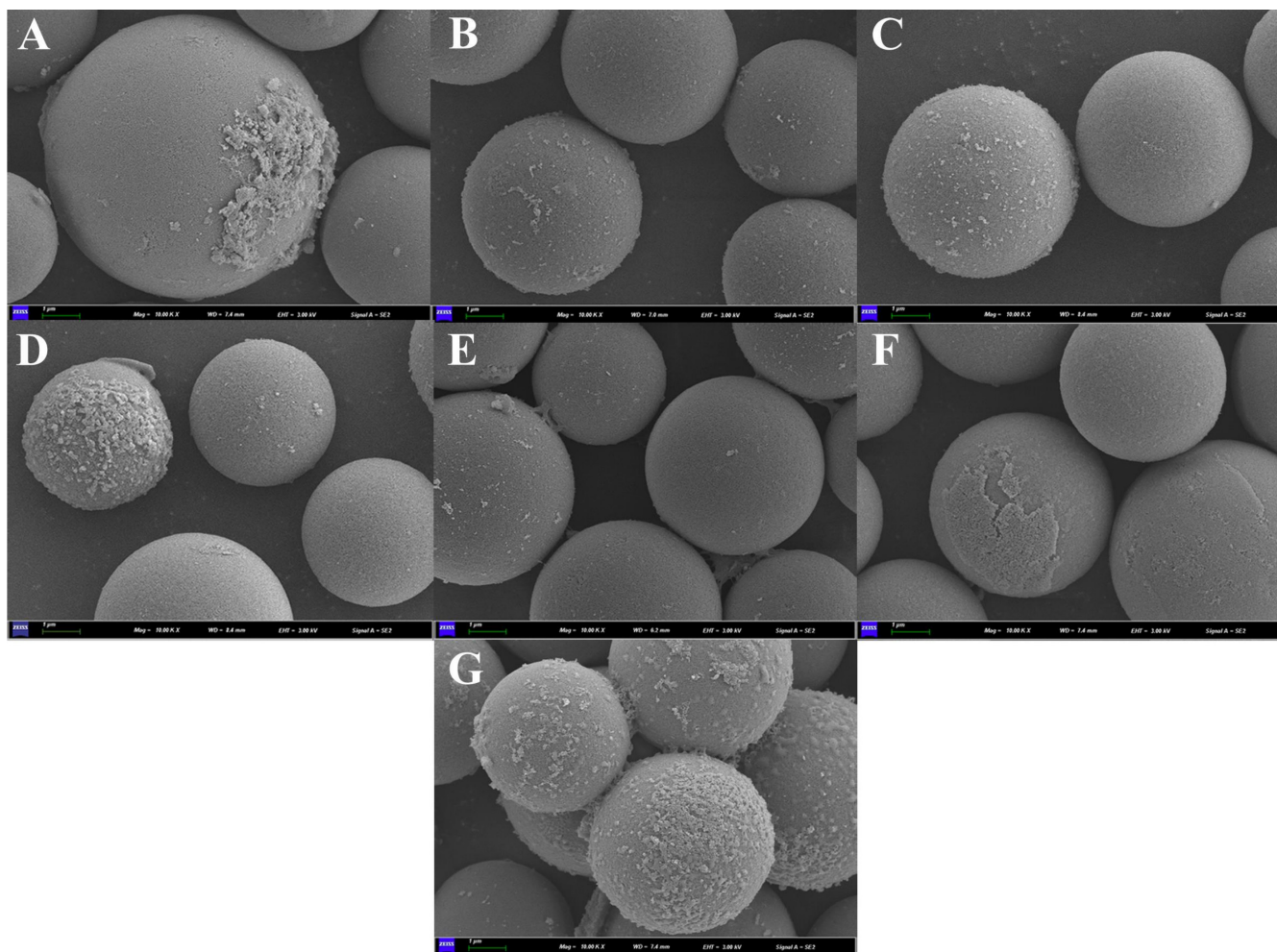
### 3.1 | Optimization of Synthetic Procedure of CD-C4AP-Based Chiral Stationary Phase

Many factors could affect the modification procedure and morphologies of stationary phases, such as the reaction time, reactant amount, temperature, etc. Therefore, the reaction time and temperature of C4AP modification, as well as the amount of TFTPn and C4A, were optimized. The SEM images and the enantioseparation performance of CD-C4AP-based chiral stationary phase under different prepared conditions were shown in Figure 1 and Figure S1, respectively.

As shown in Figure 1A–C, the surface morphology was inhomogeneous when the reaction time of C4AP was 1h. There was no obvious difference in the surface morphology between the 3- and 6-h reaction times of C4AP. In Figure S1A, it can be seen that the baseline separation was achieved when the reaction time of C4AP was 3 or 6h, and these two conditions showed a similar enantioresolution. For simplifying the synthesis process, 3h was chosen to prepare the chiral column.



**SCHEME 1** | Scheme for the fabrication of CD-C4AP-based chiral stationary phase.



**FIGURE 1** | The SEM images of CD-C4AP-based chiral stationary phase under different prepared conditions. (A) Reaction time of C4AP for 1 h, reaction temperature of C4AP with 100°C; the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (B) Reaction time of C4AP for 3 h, reaction temperature of C4AP with 100°C; the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (C) Reaction time of C4AP for 6 h, reaction temperature of C4AP with 100°C; the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (D) Reaction time of C4AP for 3 h, reaction temperature of C4AP with 80°C; the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (E) Reaction time of C4AP for 3 h, reaction temperature of C4AP with 120°C; the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (F) Reaction time of C4AP for 3 h, reaction temperature of C4AP with 100°C; the amount of TFTP and C4A were 0.1 and 0.05 g, respectively. (G) reaction time of C4AP for 3 h, reaction temperature of C4AP with 100°C; the amount of TFTP and C4A were 0.4 and 0.2 g, respectively.

The effects of reaction temperature of C4AP modification were tested at 80°C, 100°C, and 120°C (Figure 1D,B,E). After immobilizing C4AP on  $\beta$ -CD-functionalized silica gel surfaces at 80°C, the attachments on a few CD-C4AP-functionalized silica surfaces were clearly changed, while the majority of CD-C4AP-functionalized silica gel surfaces had rather smooth morphologies. When the reaction temperature was 100°C, the mass particles of C4AP were homogeneously distributed on  $\beta$ -CD-functionalized silica gel surfaces. Further improving the reaction temperature to 120°C, the spherical silica gel was beginning to link with each other, which could affect its dispersion. We speculated that the lower reaction temperature was insufficient for C4AP modification; the higher reaction temperature would induce the cross-linking between TFTP and the hydroxyl moieties on the  $\beta$ -CD-functionalized silica gel surface, resulting in the connections between the spherical silica gels. From Figure 1B, it can be observed that the enantioseparation of the prothioconazole was significantly enhanced when

the reaction temperature of C4AP was increased from 80°C to 100°C. Further increasing the reaction temperature to 120°C, the resolution decreased from 1.88 to 1.52. Thus, the reaction temperature of C4AP modification was chosen as 100°C.

Next, the effects of the amounts of TFTP and C4A on the morphology of the chiral stationary phase were studied. By observing the images of SEM (Figure 1F,B,G), we can see that the surface of CD-C4AP-functionalized silica gel was rough but not uniform when the amounts of TFTP and C4A were 0.1 and 0.05 g, respectively. Along with the increase of the contents of TFTP and C4A (Figure 1B), the polymer coating became homogeneous. However, when the amounts of TFTP and C4A were 0.4 and 0.2 g, respectively, many rough particles were attached to the CD-C4AP-functionalized silica gel surface and there was adhesion between the silica gels. This illustrated that the higher amounts of TFTP and C4A could cause aggregation or conglomeration. The well morphology

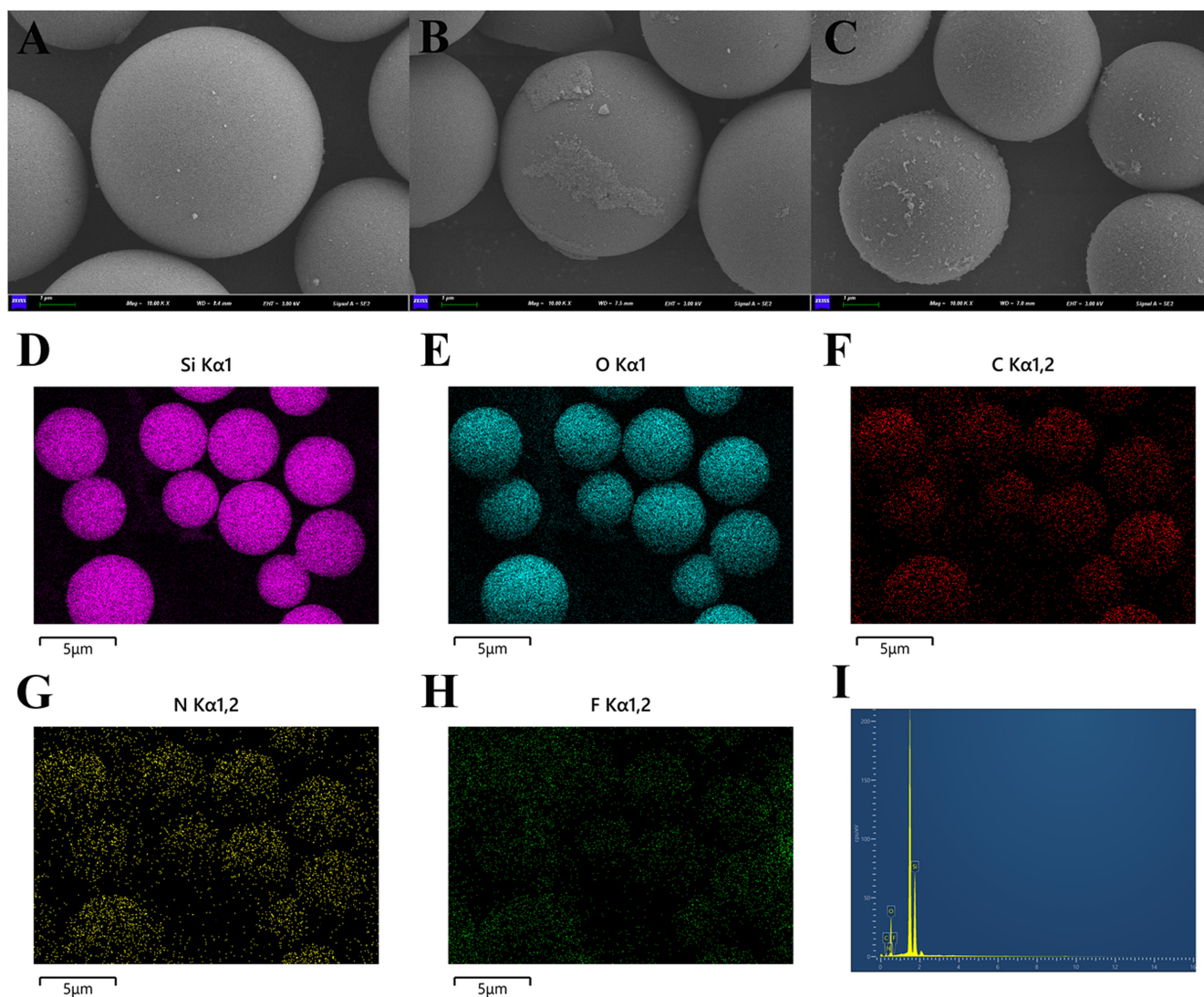
of CD-C4AP-functionalized silica gel was of great significance for the final chromatographic separation. It was clear that the CD-C4AP-based chiral stationary phase, which was synthesized with 0.2 g of TFTP and 0.1 g of C4A, has a stronger enantioseparation ability than the other two columns (Figure S1C). Therefore, the CD-C4AP-based chiral stationary phase was prepared with the amounts of TFTP and C4A being 0.2 and 0.1 g, respectively.

### 3.2 | Characterization of CD-C4AP-Based Chiral Stationary Phase

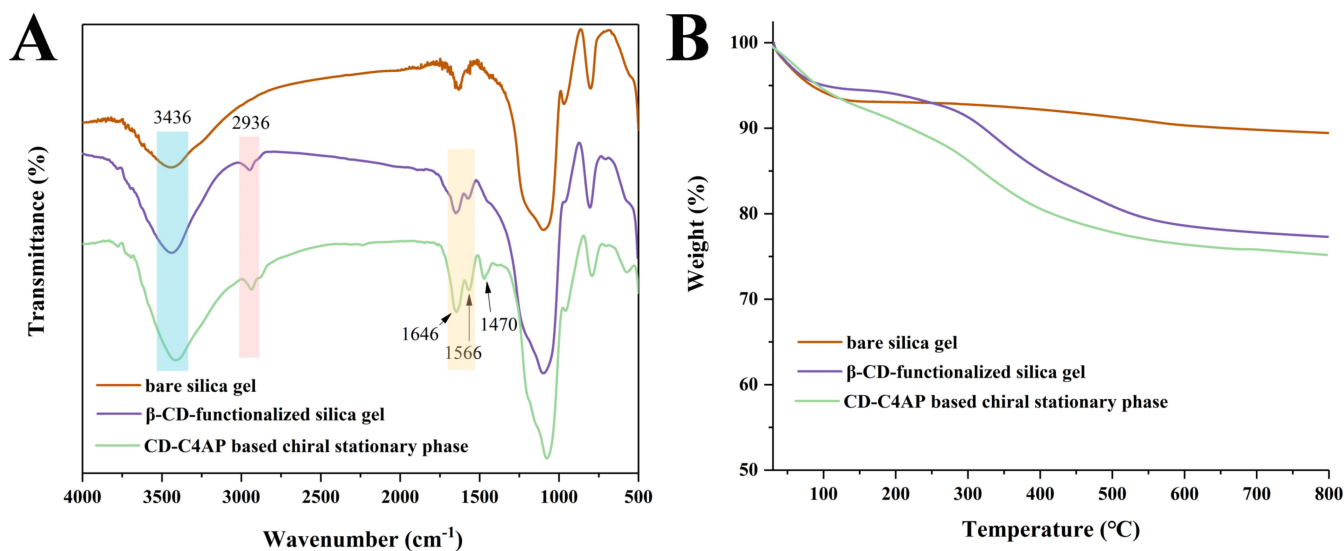
The CD-C4AP-based chiral stationary phase was characterized by several analytical techniques, including SEM, energy dispersive x-ray spectroscopy, FT-IR, and TGA; their characterizations were shown in Figures 2 and 3. The SEM images (Figure 2A–C) showed the differences in morphology among bare silica gel,  $\beta$ -CD-functionalized silica gel, and CD-C4AP-based chiral stationary phase. The bare silica gel exhibited a smooth and clean surface, and the  $\beta$ -CD-functionalized silica gel had a rougher

surface. After the modification of C4AP, the CD-C4AP-based chiral stationary phase showed a coating layer assembled with small particles; this indicated that CD-C4AP was modified successfully on the  $\beta$ -CD-functionalized silica gel. The element mapping and EDS results of the CD-C4AP-based chiral stationary phase were shown in Figure 2D–I. The element mapping images and EDS results demonstrated that the elements (C, Si, N, O, and F) were distributed on the surface of the CD-C4AP-based chiral stationary phase. The above results demonstrated that the CD-C4AP-based chiral stationary phase was successfully fabricated.

The FT-IR spectra of bare silica gel,  $\beta$ -CD-functionalized silica gel and CD-C4AP-based chiral stationary phase were analyzed and presented in Figure 3A. The broad peaks at around  $3436\text{ cm}^{-1}$  were attributed to the stretching vibration generated by  $-\text{OH}$  and  $\text{N}-\text{H}$  groups. The adsorption peaks at  $2936\text{ cm}^{-1}$  were the stretching vibration of  $\text{C}-\text{H}$ . In the  $\beta$ -CD-functionalized silica gel spectrum, the peaks at  $1646$  and  $1566\text{ cm}^{-1}$  were the stretching vibrations of  $\text{C}=\text{O}$  groups and the in-plane bending vibration of  $\text{N}-\text{H}$  groups, respectively, demonstrating the



**FIGURE 2** | SEM images of bare silica gel (A),  $\beta$ -CD-functionalized silica gel (B), CD-C4AP-based chiral stationary phase (C); Si, O, C, N, and F element mapping for CD-C4AP-based chiral stationary phase (D–H); EDS result of CD-C4AP-based chiral stationary phase (I).



**FIGURE 3** | FT-IR spectra (A) and thermogravimetric curve (B) of bare silica gel,  $\beta$ -CD-functionalized silica gel, and CD-C4AP-based chiral stationary phase.

formation of covalent bonds of  $\text{-NHCOO-}$  through the reaction of the  $\text{-OH}$  moieties of  $\beta$ -CD with isocyanate. Additionally, the intensities of the absorption bands around  $1646$  and  $1566\text{ cm}^{-1}$  in the CD-C4AP-based chiral stationary phase spectrum were enhanced compared to those in the spectrum of  $\beta$ -CD-functionalized silica gel, indicating the presence of benzene rings in the structure of the CD-C4AP-based chiral stationary phase. The signal at  $1470\text{ cm}^{-1}$  was also identified as the skeleton vibration of benzene rings.

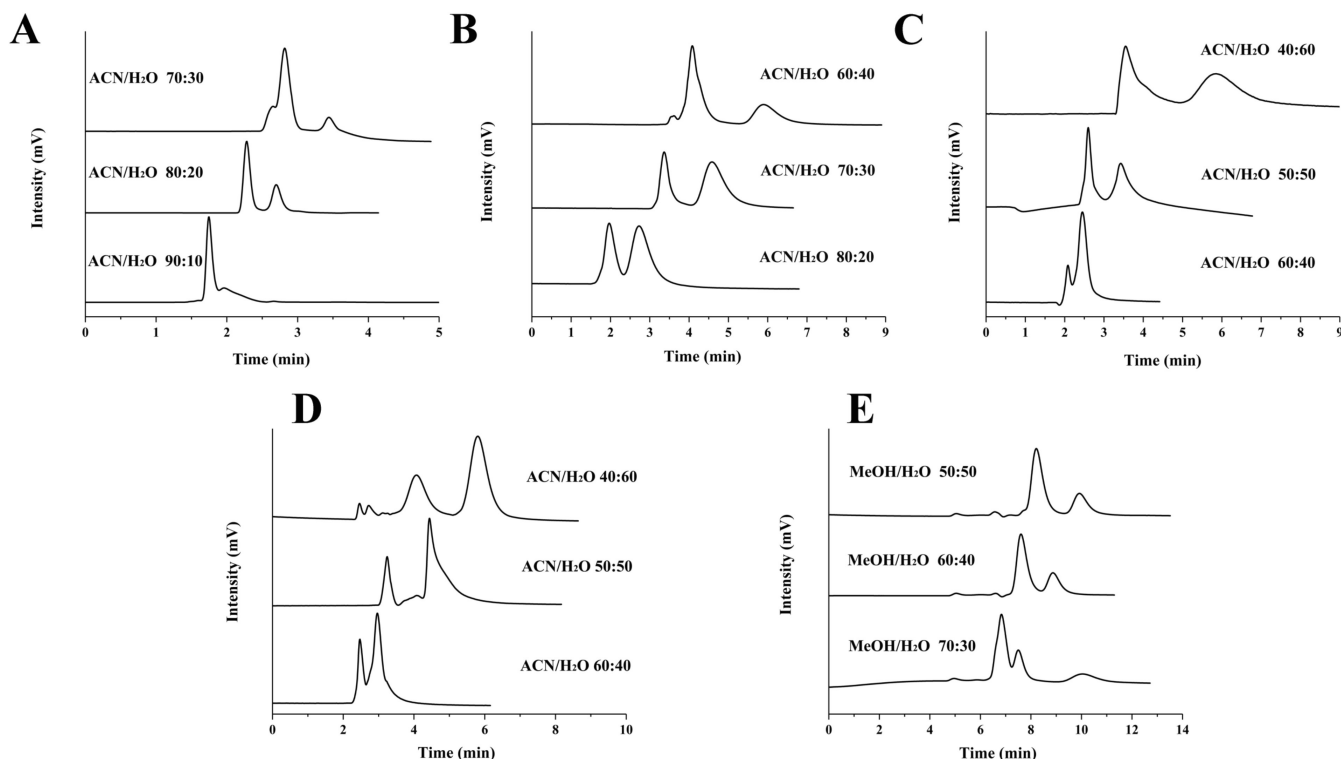
The TGA experiments with bare silica gel,  $\beta$ -CD-functionalized silica gel, and CD-C4AP-based chiral stationary phase were conducted between  $30^\circ\text{C}$  and  $800^\circ\text{C}$  at a heating rate of  $10^\circ\text{C min}^{-1}$  under a nitrogen atmosphere. As shown in Figure 3B, the weight loss at temperatures below  $120^\circ\text{C}$  can be explained by the loss of absorbed water from the material. In the temperature range of  $120^\circ\text{C}$ – $800^\circ\text{C}$ , the CD-C4AP-based chiral stationary phase showed higher weight loss compared with  $\beta$ -CD-functionalized silica gel, indicating that C4AP was successfully bonded to the surface of  $\beta$ -CD-functionalized silica gel. Furthermore, the decomposition temperature of the CD-C4AP-based chiral stationary phase was approximately  $250^\circ\text{C}$ ; therefore, the CD-C4AP-based chiral stationary phase was suitable for use in chromatography.

### 3.3 | Enantioseparation Performance of CD-C4AP-Based Chiral Stationary Phase and $\beta$ -CD-Based Chiral Stationary Phase

The enantiomeric separation performance of the CD-C4AP-based chiral stationary phase was evaluated in reversed phase mode with 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol as model analytes. In the system of ACN and water, the CD-C4AP-based chiral column successfully separated four chiral compounds, including prothioconazole, promethazine, propranolol, and bisoprolol, while 1-phenylethanol was successfully separated in the MeOH/water system. In order to obtain the optimal chiral separation

conditions, the proportion of the mobile phase was adjusted and optimized. As shown in Figure 4, a higher water content in the mobile phase resulted in a longer retention time and improved enantiomeric resolution. However, there were some peak broadening and tailing phenomena in the separation of all chiral compounds as the water content increased, which could be attributed to the improved adsorption interactions between the analyte and the stationary phase. The separation data of all chiral analytes were summarized in Table 1. The results showed that 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol were baseline separated with resolutions of 1.62, 1.88, 1.74, 1.71, and 1.80, respectively. Additionally, the impacts of flow rate, temperature, and injection volume were also studied with prothioconazole as an example (Figures S2–4). These findings indicated that these three separation factors had a minor impact on the retention behavior and selectivity of prothioconazole on the CD-C4AP-based chiral column.

Furthermore, the enantioseparation of these chiral analytes was performed on  $\beta$ -CD-based chiral stationary phase for comparison. As shown in Table S1 and Figures S5–7, the stereoselectivity of these chiral analytes improved when the C4AP was introduced onto  $\beta$ -CD-based chiral stationary phase, probably due to the more chiral recognition interactions between enantiomers and stationary phase. As can be seen from Table S1, the  $\beta$ -CD-based chiral column was unable to enantioseparate propranolol and bisoprolol, performed poorly in terms of enantioseparation of prothioconazole and promethazine with the best resolutions below 0.9. The  $\beta$ -CD-based chiral column only separated one of the chiral analytes (1-phenylethanol) within 20 min with a resolution of 1.51. Thus, it can be seen that the fabricated CD-C4AP-based chiral column exhibited higher enantioselectivity and improved separation performance for the studied chiral analytes when compared to the  $\beta$ -CD-based chiral column. However, the chromatographic efficiencies of the CD-C4AP-based chiral column were not great especially in comparison to some earlier literature [20, 22–26]. The chiral discrimination ability of the CD-C4AP-based chiral column was influenced by the synergetic effects of  $\beta$ -CD and modified C4AP.



**FIGURE 4** | HPLC chromatograms of prothioconazole (A), promethazine (B), propranolol (C), bisoprolol (D), and 1-phenylethanol (E) on the CD-C4AP-based chiral column at different mobile phase compositions.

**TABLE 1** | The optimal separation results of chiral analytes on CD-C4AP-based chiral stationary phase.

| Chiral analytes              | $t_1$ | $t_2$ | $\alpha$ | $R_s$ | Mobile phase                      |
|------------------------------|-------|-------|----------|-------|-----------------------------------|
| 1-Phenylethanol <sup>a</sup> | 7.61  | 8.87  | 1.17     | 1.62  | MeOH/H <sub>2</sub> O (v/v=60:40) |
| Prothioconazole <sup>b</sup> | 2.28  | 2.70  | 1.18     | 1.88  | ACN/H <sub>2</sub> O (v/v=80:20)  |
| Promethazine <sup>b</sup>    | 3.30  | 4.57  | 1.38     | 1.74  | ACN/H <sub>2</sub> O (v/v=70:30)  |
| Propranolol <sup>b</sup>     | 2.60  | 3.50  | 1.36     | 1.71  | ACN/H <sub>2</sub> O (v/v=50:50)  |
| Bisoprolol <sup>b</sup>      | 3.20  | 4.42  | 1.38     | 1.80  | ACN/H <sub>2</sub> O (v/v=50:50)  |

<sup>a</sup>Flow rate of 0.4 mL min<sup>-1</sup> at 30°C.

<sup>b</sup>Flow rate of 0.8 mL min<sup>-1</sup> at 30°C.

The C4A groups of C4AP have several special interaction sites, such as benzene rings ( $\pi$ - $\pi$  interaction and hydrophobic interaction), hydroxyl groups (hydrogen bonding interaction), and macrocycle cavities (inclusion interaction). The co-existence of  $\beta$ -CD and C4A might serve to improve the separation ability of CD-C4AP-based chiral column. The studied chiral analytes contained aromatic rings, which enhanced the  $\pi$ - $\pi$  interaction with the stationary phase. These compounds also attached the hydroxyl group or amino group to their chiral centers, which can form hydrogen bonds with hydroxyl groups on the stationary phase. The appropriate molecular structure of analytes

facilitated the formation of the inclusion complexation with CD and C4A groups on the stationary phase. Therefore, the chiral recognition mechanism between the CD-C4AP-based chiral column and chiral analytes was complicated and probably involved multiple interactions, such as hydrogen bonding,  $\pi$ - $\pi$  interaction, and inclusion effect. These results indicated that the CD-C4AP-based chiral column had a good potential application prospect as a novel type of chiral stationary phase in HPLC enantioseparations.

### 3.4 | Chromatographic Separation Performance of CD-C4AP-Based Chiral Stationary Phase and $\beta$ -CD-Based Chiral Stationary Phase Toward Polycyclic Aromatic Hydrocarbons

The applicability of CD-C4AP-based chiral column was further explored by separating two groups of polycyclic aromatic hydrocarbons (PAHs). As can be seen in Figure S8, two groups of analytes, naphthalene (logP 3.3) and anthracene (logP 4.45), anthracene and pyrene (logP 3.79) can be separated using MeOH/H<sub>2</sub>O as the mobile phase. When the ratio of MeOH to H<sub>2</sub>O was 90/10 (v/v) and 70/30 (v/v), respectively, the two groups of PAHs were baseline separated. The retention time of PAHs was decreased with the increment of MeOH content, which demonstrated the hydrophobic interaction was involved in the separation process.

The elution sequence of these PAHs was naphthalene < anthracene < pyrene; it was accorded with the increasing number of aromatic rings; however, it was inconsistent with their logP orders. We speculated that the increased number of benzene rings in

pyrene resulted in more  $\pi$ - $\pi$  interaction sites and stronger interactions with the CD-C4AP-based chiral column; subsequently, pyrene was the last one to be eluted out. The  $\pi$ - $\pi$  interaction was crucial in the resolution process of PAHs.

Naphthalene and anthracene achieved baseline separation within 20 min using MeOH/H<sub>2</sub>O (60:40, v/v) as the mobile phase on the  $\beta$ -CD-based chiral column. Meanwhile, the CD-C4AP-based chiral column had an absolute advantage in separating these two analytes (Figure S9). Compared with the separation results of anthracene and pyrene on the CD-C4AP-based chiral column, the  $\beta$ -CD-based chiral column showed a shorter retention time and a higher resolution for these two analytes (Figure S10). There might be two reasons to explain this result: one reason was that the four-membered aromatic ring of pyrene can enhance the  $\pi$ - $\pi$  interaction with the CD-C4AP-based chiral column, which induced strong retention and tailed peaks; another reason was that the C4A polymer had a certain space structure that could influence the interaction between analytes and the stationary phase.

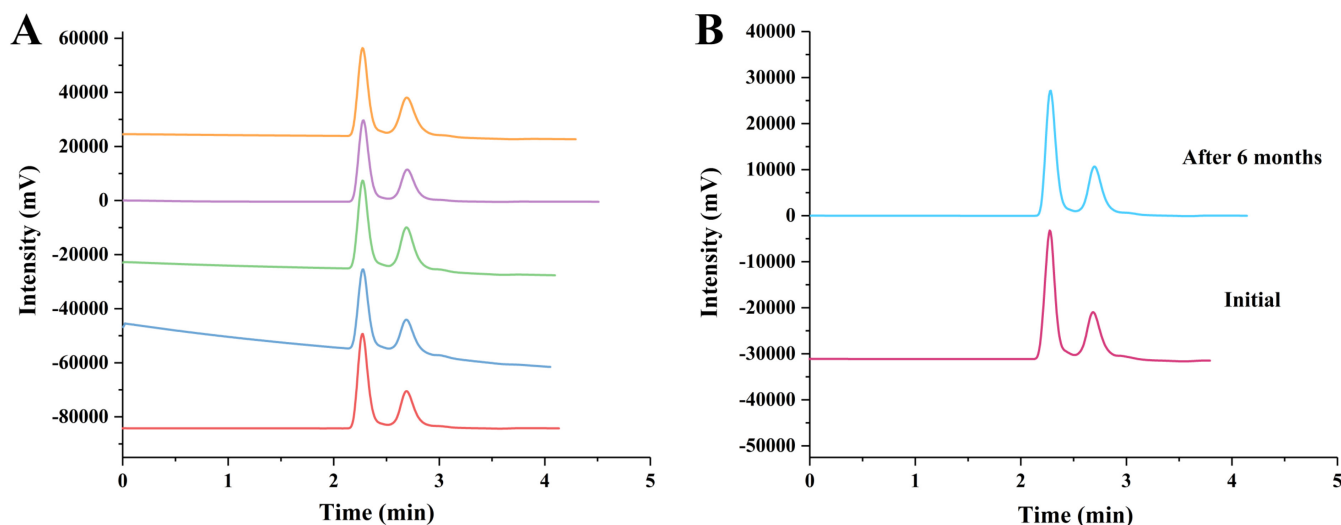
### 3.5 | CD-C4AP-Based Chiral Column Compared With Other Commercial Chiral Columns

The enantioseparation performance of the CD-C4AP-based chiral column toward 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol was compared with other commercialized chiral columns, namely, Astec CYCLOBOND I 2000 DMP chiral column, Lux Amylose-2 chiral column, Chiralpak OD-RH chiral column, and Lux Cellulose-1 chiral column. As displayed in Table S2, the Astec CYCLOBOND I 2000 DMP chiral column showed a better enantioseparation performance toward 1-phenylethanol, which provided shorter retention time and higher resolution. Prothioconazole and promethazine had longer retention times on the Astec CYCLOBOND I 2000 DMP chiral column than those of the CD-C4AP-based chiral column. Furthermore, propranolol and bisoprolol obtained good enantioseparation on

the CD-C4AP-based chiral column, while these two analytes cannot be chiral separated on the Astec CYCLOBOND I 2000 DMP chiral column. In reversed-phase mode, propranolol and bisoprolol were separated on the Lux Amylose-2 chiral column with the basic mobile phase [42]. Prothioconazole and propranolol can be separated on the Chiralpak OD-RH chiral column with the acidic mobile phase, while promethazine and bisoprolol were not. When using the basic mobile phase, the resolution of propranolol was increased to 8.96; promethazine and bisoprolol still cannot be separated [42, 43]. As for the Lux Cellulose-1 chiral column, prothioconazole, propranolol, and bisoprolol showed superior resolution ( $R_s > 5.00$ ) [44, 45]. From the above results, we can see that some commercial chiral columns possessed higher enantioselectivity and broader chiral discrimination ability than the CD-C4AP-based chiral column. However, the resolutions of 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol on the CD-C4AP-based chiral column were above the midrange when compared to other commercial chiral columns. On the other hand, the CD-C4AP-based chiral column exhibited the advantage of rapid analysis of the aforementioned chiral analytes without any acid or basic additives in the mobile phase.

### 3.6 | The Repeatability of CD-C4AP-Based Chiral Column

The repeatability of the CD-C4AP-based chiral column was assessed using the chiral analyte prothioconazole, and the results were displayed in Figure 5. We could see that there was no significant change in the chiral separation performance toward prothioconazole after five repeated injections (Figure 5A). The relative standard deviation (RSD%) value of the retention time was below 0.16% ( $n = 5$ ). In addition, after 6 months of use, the separation performance of the CD-C4AP-based chiral column for prothioconazole enantiomers did not significantly decrease (Figure 5B). These results indicated that the CD-C4AP-based chiral column had good repeatability and stability.



**FIGURE 5** | (A) The HPLC chromatograms on the CD-C4AP-based chiral column for five replicate separations of prothioconazole at 30°C using ACN/H<sub>2</sub>O (v/v = 80:20) as the mobile phase under a flow rate of 0.8 mL min<sup>-1</sup>. (B) The HPLC chromatograms for enantioseparation of prothioconazole on the CD-C4AP-based chiral column at different times.

**TABLE 2** | Comparison with the enantioseparation performance of CD-C4AP-based chiral column and other  $\beta$ -CD or C4A-based columns.

| <b>Analytes</b>                                                                       | <b>Chiral selector</b>                                                                                       | <b>Preparation time (h)</b> | <b>Separation mode</b>                 | <b><math>t_{R1}</math> (<math>k_1</math>)*</b> | <b><math>R_s</math></b> | <b>Ref.</b> |
|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|-----------------------------|----------------------------------------|------------------------------------------------|-------------------------|-------------|
| Isoxazolines, dansyl amino acids and flavonoids                                       | Mono-6 <sup>A</sup> -deoxy-6-allylimidazolium- $\beta$ -CD and allylimidazolium bridged bis( $\beta$ -CD)    | 79 and 67                   | Reversed phase                         | (2.37–11.1)                                    | 1.14–7.20               | [46]        |
| Flavanones, blockers, amino acids and common drugs                                    | Ethylenediamine dicarboxyethyl diamido-bridged bis( $\beta$ -CD)                                             | 194                         | Reversed phase and polar organic phase | (0.53–10.72)                                   | 0.55–4.35               | [16]        |
| Flavonoids, ticagrelor impurities, chiral quinoline alkaloids, and lansoprazole       | Star $\beta$ -CD polymer                                                                                     | 111                         | Reversed phase                         | —                                              | 0.315–2.11              | [47]        |
| DNB-amino acids, omeprazole, diclofenac methyl, (RS)-pregabalin, and DL-mandelic acid | Deoxycholic-calix[4]arene                                                                                    | >173                        | Normal phase                           | (0.85–6.10)                                    | 0.89–3.93               | [40]        |
| 19 Chiral drug compounds                                                              | Calix[4]arene-capped [3-(2-O- $\beta$ -CD)-2-hydroxypropoxy]propylsilyl-appended silica particles            | >66                         | Reversed phase                         | 3.02–20.18 (0.85–5.26)                         | 0.32–5.14               | [37]        |
| 17 Chiral drug compounds                                                              | 4-isopropylcalix[4]arene-capped (3-(2-O- $\beta$ -CD)-2-hydroxypropoxy)propylsilyl appended silica particles | >66                         | Reversed phase                         | (0.45–1.61)                                    | 0.87–6.54               | [38]        |
| 1-Phenylethanol, prothionazole, promethazine, propranolol, and bisoprolol             | $\beta$ -CD-calix[4]arene polymer                                                                            | 39                          | Reversed phase                         | 2.28–7.61                                      | 1.62–1.88               | This work   |

\* $t_{R1}$  is the retention time for the enantiomer eluted out first;  $k_1$  is the retention factor for the enantiomer eluted out first.

### 3.7 | Comparison With the Enantioseparation Performance of CD-C4AP-Based Chiral Column and Other $\beta$ -CD- or C4A-Based Columns

In this work, we compared the enantioseparation performance of the CD-C4AP-based chiral column with other reported  $\beta$ -CD or C4A-based columns [16, 37, 38, 40, 46, 47]. As shown in Table 2, these reported  $\beta$ -CD- or C4A-based columns were constructed with  $\beta$ -CD or C4A derivatives, which needed a long time to synthesize, while the CD-C4AP-based chiral column was prepared directly with native  $\beta$ -CD and C4A. The preparation time of the CD-C4AP-based chiral column was greatly shortened. The studied analytes could be separated in a short time without any buffer. In addition, irritating chemicals were not used in the fabrication of the CD-C4AP-based chiral column, thus reducing the possible risk to operators in the experiment. However, it still has some limitations: (i) the chromatographic efficiencies were not great especially in comparison to the earlier more classical papers; the resolutions of studied analytes were not particularly good and the separable enantiomer species were limited. For example, Armstrong's group separated propranolol ( $R_s = 2.13$ ) and five other beta blockers ( $R_s$ : 1.50–3.16) on a commercial Cyclobond I column [48]. The reported C4A-capped  $\beta$ -CD CSP and 4-isopropyl-C4A-capped  $\beta$ -CD CSP showed a wider range of separable enantiomer species than our CSP [37, 38]; (ii) C4A was difficult to accurately adjust its spatial connection sites, which might influence the chromatographic separation performances. In summary, this work provided a new and convenient method for designing the chiral column. Ongoing work is aimed at developing more kinds of CDs and C4A derivatives for several kinds of CSPs, and further studying the chiral separation ability and chiral recognition mechanism of CSPs.

## 4 | Conclusions

In this work, a novel chiral CSP with  $\beta$ -CD and C4AP functionalization was prepared using a simple method. Benefiting from the synergistic inclusion effect, hydrogen bonding interaction, and rich  $\pi$ -binding sites of the CD-C4AP-based chiral column, it showed good chiral recognition ability and fast separation performance toward 1-phenylethanol, prothioconazole, promethazine, propranolol, and bisoprolol. The preparation time and analysis time of the CD-C4AP-based chiral column were superior to most related reported  $\beta$ -CD and C4A-based columns, holding great potential as a new type of CSP. Our work provided a new approach to combine the use of  $\beta$ -CD and C4A as CSP for chiral separation, and expanded the application of C4A in the field of enantiomeric separation.

### Author Contributions

Shuling Dong and Jiayi Sun contributed equally to this work.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The chromatograms of prothioconazole on CD-C4AP-based chiral stationary phase under different prepared conditions. (A) The influence of reaction time of C4AP on enantioseparation performance: a: 1 h, b: 3 h, c: 6 h, other conditions: reaction temperature of C4AP with 100°C, the amount of TFTP and C4A were 0.2 and 0.1 g, respectively. (B) The influence of reaction temperature of C4AP on enantioseparation performance: d: 80°C, b: 100°C, e: 120°C, other conditions: reaction time of C4AP for 3 h, the amount of TFTP and C4A were 0.2 and 0.1 g, respectively; (C) the influence of the amount of TFTP and C4A on enantioseparation performance: f: 0.1 and 0.05 g; b: 0.2 and 0.1 g; g: 0.4 and 0.2 g, other conditions: reaction time of C4AP for 3 h, reaction temperature of C4AP with 100°C. Separation conditions: ACN/H<sub>2</sub>O (v/v=80:20) was used as the mobile phase, column temperature 30°C, flow rate was 0.8 mL min<sup>-1</sup>. **Figure S2:** Effect of the flow rate on the enantioseparation performance of CD-C4AP-based chiral column toward prothioconazole. **Figure S3:** Effect of the column temperature on the enantioseparation performance of CD-C4AP-based chiral column toward prothioconazole. **Figure S4:** Effect of the injection volume on the enantioseparation performance of CD-C4AP-based chiral column toward prothioconazole. **Figure S5:** The comparison of enantioseparation performance of CD-C4AP-based chiral column with β-CD-based chiral column toward prothioconazole. Separation conditions: ACN/H<sub>2</sub>O (30:70) was used as the mobile phase for β-CD-based chiral column; ACN/H<sub>2</sub>O (v/v=80:20) was used as the mobile phase for CD-C4AP-based chiral column. All the separations were performed at a flow rate of 0.8 mL min<sup>-1</sup> at temperature of 30°C. **Figure S6:** The comparison of enantioseparation performance of CD-C4AP-based chiral column with β-CD-based chiral column toward promethazine. Separation conditions: ACN/H<sub>2</sub>O (60:40) was used as the mobile phase for β-CD-based chiral column; ACN/H<sub>2</sub>O (v/v=70:30) was used as the mobile phase for CD-C4AP-based chiral column. All the separations were performed at a flow rate of 0.8 mL min<sup>-1</sup> at temperature of 30°C. **Figure S7:** The comparison of enantioseparation performance of CD-C4AP-based chiral column with β-CD-based chiral column toward 1-phenylethanol. Separation conditions: MeOH/H<sub>2</sub>O (50:50) was used as the mobile phase for β-CD-based chiral column; MeOH/H<sub>2</sub>O (v/v=60:40) was used as the mobile phase for CD-C4AP-based chiral column. All the separations were performed at a flow rate of 0.4 mL min<sup>-1</sup> at temperature of 30°C. **Figure S8:** The separation performances of CD-C4AP-based chiral column toward two groups of PAHs. (A) Naphthalene and anthracene. (B) Anthracene and pyrene. **Figure S9:** The comparison of separation performance of CD-C4AP-based chiral column with β-CD-based chiral column toward naphthalene and anthracene. Separation conditions: MeOH/H<sub>2</sub>O (60:40) was used as the mobile phase for β-CD-based chiral column; MeOH/H<sub>2</sub>O (v/v=90:10) was used as the mobile phase for CD-C4AP-based chiral column. All the separations were performed at a flow rate of 0.8 mL min<sup>-1</sup> at temperature of 30°C. **Figure S10:** The comparison of separation performance of CD-C4AP-based chiral column with β-CD-based chiral column toward anthracene and pyrene. Separation

conditions: MeOH/H<sub>2</sub>O (70:30) was used as the mobile phase with a flow rate of 0.8 mL min<sup>-1</sup> at 30°C. **Table S1:** The optimal separation results of chiral analytes on β-CD-based chiral stationary phase. **Table S2:** Separations of chiral analytes on CD-C4AP-based chiral column and other commercial chiral columns.