Enantiomeric Separation by Capillary Electro chromatography with a Novel β-cyclodextrin-hyperbranched Carbosilane–silica Polymer Coating

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Summary: A new type of β-cyclodextrin-modified hyperbranched carbosilane (β-CD@HBC) stationary phase was synthesized by combining hyperbranched carbosilane with the -OH group of β-cyclodextrin, and then the β-CD@HBC was used as astationary phase to fix it on the inner wall of capillary column, application in chiral drug separation. The chromatographic behaviour of the stationary phase was studied. The preliminary test results showed that the β-CD@HBC coated OT column has the ability to separate a variety of enantiomers, such as clenbuterol, ibuprofen, enrofloxacin, hlorpheniramine, isoproterenol hydrochloride, epinephrine, Phenylalanine, and Ketoprofen. The β-CD@HBC coated OT column has good repeatability and stability. The relative standard deviations (RSD) between runs, between days, and between columns were less than 1.5%, 1.86%, and 4.83%, respectively. This paper shows that hyperbranched polymers have great potential and advantages in the application of capillary electrochromatography for chiral separation.

Keywords: β-cyclodextrin; Capillary electrochromatography; chiral stationary phase; Enantiomeric separation; Hyperbranched polymers

Introduction

Hyperbranched polymer is a three-dimensional structure polymer. Due to its special hyperbranched structure, a large number of terminal groups that can be modified and functionalized [1-11], low viscosity, no chain entanglement and good solubility [12], hyperbranched polymer is one of the research hotspots of polymer materials researchers in recent years. Hyperbranched polymers have been used in separation science, which are an essential branch of science and technology, such as capillary electrophoresis, [5, 6, 13] gas chromatography separation, [14] polymer solar cells,[15] biological imaging, [16] biomimetic biomedical materials [17, 18] and protein separation. [11] Most of the development of chiral separation methods [19-21] during the past few decades has been based on the fact that enantiomers have different qualitative or quantitative activities in their toxicological, biological, pharmacological and physiological behaviors on the human body, while capillary electrochromatography (CEC) has recently attracted more attention.[22] CEC is a technique used to separate enantiomers. The technology has the characteristics of high efficiency, low consumption of samples and solvents , and short analysis time.[1] It is suitable for solving practical problems in various industries, including chemistry, pharmacy, biomedicine, food and environmental.[1, 19] Currently, β-cyclodextrin (β-CD) and its derivatives are still the most common chiral selectors in capillary electrophoresis. The development of chiral analysis methods is a necessary and challenging task for the development and research of chiral materials.[23-28] Enantiomers are optical isomers, which show similar physical and chemical properties and are difficult to separate from each other. The chiral environment is key to chiral recognition and separation. The interaction between the chiral environment and enantiomers will enable the separation of isomers; this interaction is formed during or before the separation process, forming labile diastereomeric or stable diastereoisomer complexes, respectively.

Generally, enantiomeric resolution can be performed by the chromatography of a racemic mixture on a chiral stationary phase (CSP). These typically include several derivatives of CDs, crown ether, amylose and cellulose, cyclodextrin, and macrocyclic antibiotics. The β-CD is a commonly used chiral selector.[29-34] β-CDs and derived β-CDs are widely used in chromatography, such as capillary electro chromatography, [35-41] gas chromatography [3] and high-performance liquid chromatography.[23,42] the internal hydrophobicity and external hydrophilicity of cyclodextrins enable it to form clathrates with many organic and inorganic molecules. Its mechanism of action can depend on van der Waals force, static electricity, hydrogen bond, repulsive force and π-π bond or the dipole moment

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caused by uneven charge distribution, and the hydrophobic interactions between host and guest molecules.[40,43-48] Therefore, enantioseparation is based on the different electrophoretic mobilities of the isomer, and β-CD associates and their interactions.

Here, we studied dendritic macromolecules and the technology of chiral separation and analysis. A β-cyclodextrin-modified hyperbranched carbosilane (β-CD@HBC) was synthesized and fixed on the inner wall of a fused silica column, and then used as a stationary phase for a capillary open tubular column and the successfully separated several kinds of analogues, including clenbuterol, enrofloxacin, ibuprofen, hlorpheniramine, isoproterenol hydrochloride, and epinephrine. The hyperbranched macroporous structure presents more chiral sites after modification of the β-CD, which results in more advantages for chiral separation.[49] The significance of this topic lies in the following: chirality is closely related to life processes. Along with the development of materials, chemistry, molecular biology and other disciplines, finding a stable, high-efficiency, wide-ranging chiral stationary phase is important. Use of a hyperbranched polymer for the capillary column shows great potential for applications. The design and synthesis of a novel stationary phase material to realize the separation of chiral drugs for analysis are very important for strengthening research into the recognition mechanism for the chiral stationary phase and chiral separation technology. Herein, a novel carbosilane-hyperbranched macromolecule was synthesized. Based on this, β-CD was modified before chiral separation by CE. The β-CD@HBC polymer was used as the stationary phase for racemic separation that attempt to combine the capillary separation technology and hyperbranched polymers.

Experimental

Chemical Reagents

Methyldichlorosilane (purity ≥ 97%) was purchased from Adamas Reagent Co., Ltd. Allyl magnesium chloride (purity ≥ 98.0%) was purchased from Acros organics (NJ, USA). Karstedt's catalyst (Bis(1,3-divinyl-1,1,3,3-tetramethyldisiloxane)-platinumcomplex;) was purchased from Shanghai Aladdin Reagent Co., Ltd. Magnesium sulfate anhydrous (purity ≥ 99.0%), anhydrous calcium chloride, ammonium chloride (purity ≥ 99.5%), phosphoric acid (H_3PO_4) (purity ≥ 85%), N, N-dimethylformamide (DMF) ， disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium hydroxide (NaOH), and triethylamine (TEA) (purity ≥ 99.0%) were purchased from Guangzhou Chemical Regent Factory (Guangzhou, China). Tetrahydrofuran (purity ≥ 99.9%), hexane (purity ≥ 97.5%), platinum chloride and methylene chloride (purity ≥ 99.9%) were purchased from Beijing inokai Technology Co., Ltd (Beijing, China). The enantiomeric samples, which include clenbuterol hydrochloride, enrofloxacin, hlorpheniramine, isoproterenol hydrochloride, epinephrine, and ibuprofen, tyrosine, norfloxacin, propranolol hydrochloride, ephedrine hydrochloride, tryptophan, norepinephrine hydrochloride, were obtained from Shanghai Aladdin Reagent Co., Ltd. The purity of the enantiomers used in this experiment is all above 99%. The stock solutions for the chiral drugs were prepared in ultra-pure water at a concentration of 10 μg mL^{-1} each solution and diluted as needed before use.

Instruments

The CEC device manufactured in the laboratory consists of a UV-Vis detector and a TriSep-2100 high-voltage power supply (Unimicro Technologies, USA). HW-2000 workstation (Shanghai Qianpu Software Co., Ltd., China) was used for data acquisition and analysis. In the experiment, a PHD 2000 syringe pump (Harvard Instruments, Holliston, Massachusetts, USA) was used to inject reagents and sample solutions into the capillary column.The pH value for the phosphate buffer solution was measured using an AG PB-10 pH meter (Sartorius Mechatronics T&H GmbH Co., Ltd.). A fused silica capillary column (50 microns in inner diameter) was purchased from the Hebei Yongnian Ruipu Chromatogram Equipment Company (Handan, China). Scanning electron microscopy (SEM) photos of the capillaries were obtained using a ZEISS EVO 18 Research emission scanning electron microscope (Carl Zeiss, Germany). The oven used to modify the capillary was a component of a 7890A series gas chromatograph (Agilent Technologies, CA, USA).In addition, the polymer synthesis equipment built in the laboratory includes: DF-101D heat-collecting constant temperature heating magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd.), JX50 24 single-phase capacitor running motor (Shanghai Shenshun Biological Technology Co., Ltd.), SIGMA 2-16K centrifuge (Bolixing Instrument Co., Ltd.), DK-8AX electric heating constant temperature water tank，and DHG-9035A Heating and Drying Oven.

Preparation of the hydrophobic β-CD@HBCs

The necessary synthesis changes of the β-CD@HBC polymer were determined by referencing the work of Flory,[3] and Y. Lu [51] The first step in

the synthesis of methyl diallyl silane is as follows: Methyl diallyl silane is synthesized with a Grignard reagent (allyl magnesium chloride) and dichloromethylsilane as the principal raw materials using ice. First, 22.6 mL of Grignard reagent (allyl magnesium chloride) was cooled in an ice bath in a 250 mL three-necked flask, and then 50 mL of a dichloromethylsilane THF solution was added dropwise into a three-necked flask at a rate of 10 μL min^{-1} with stirring overnight. 50 mL of saturated aqueous NH_4Cl was sufficient to terminate the reaction by gradually adding with stirring and cooling. After stratification, the oil layer was collected and the solvent evaporated. After drying treatment, methyl diallylsilane (MeHSi (CH_2-CH=CH_2)$_2$) was obtained. The chemical changes can be expressed by the equation in Fig. 1.

The second step for synthesizing allyl carbosilane-hyperbranched macromolecules was achieved by the following procedures: Methyl diallylsilane (MeHSi (CH_2-CH=CH_2)$_2$) can be constructed by a self-assembly method based on a hydrosilylation reaction. This reaction was catalyzed by a Karstedt catalyst and reacted under ultra-dry THF as solvent, with the temperature held at 80°C for 10 hours under a nitrogen atmosphere. The solvent and redundant reactants were evaporated with the nitrogen atmosphere. Allyl-terminated hyperbranched carbosilane (HBC-allyl) was then obtained. The chemical changes can be expressed by the equation in Fig. 2.

The typical structure of the allyl-terminated hyperbranched carbosilane is shown in Fig 3.

The third step is to modify the hyperbranched macromolecules using cyclodextrin. The allyl-terminated hyperbranched carbosilane macromolecules reacted with $MeHSiCl_2$ to form hyperbranched polymers with active chloride groups. The reaction was catalyzed with platinum chloride acid and reacted under ultra-dry n-hexane as solvent, with the temperature held at 80–90°C for 6 hours under nitrogen atmosphere. The excess reactant and solvents were evaporated in a nitrogen atmosphere. HBC_{-Cl} was obtained. The chemical change can be expressed by the equation in Fig. 4.

The molecular structure of chloride-terminated hyperbranched carbosilane (HBC_{-Cl}), a chlorinated terminal silane hyperbranched macromolecule, as shown in Fig 5.

During the combination of β-CD and chloride-terminated silane-hyperbranched macromolecules HBC_{-Cl}, trimethylamine acts as a catalyst and absorbs acid agent in this reaction, and the hydrogen atom transposition on the cyclodextrin ring bonded with dechlorination of hyperbranched carbosilane (HBC). Of these terminating groups, those with allylic chlorine might be expected to show the highest activity.[52] The chlorination of the terminating groups is key to successfully binding β-CD to hyperbranched macromolecules

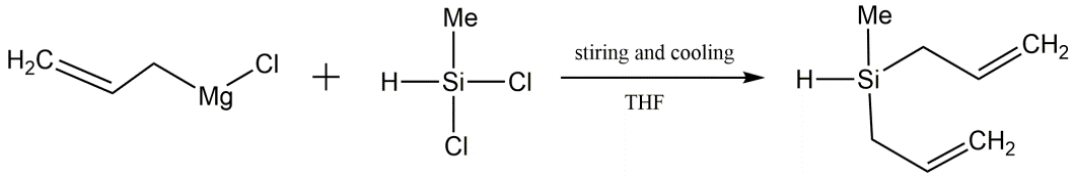

Fig. 1: The reaction formula for this chemical reaction is the synthesis of Methyl diallylsilane (MeHSi (CH_2-CH=CH_2)$_2$).

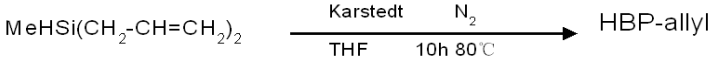

Fig. 2: The reaction formula for this chemical reaction is the synthesis of allyl-terminated hyperbranched carbosilane (HBC-allyl).

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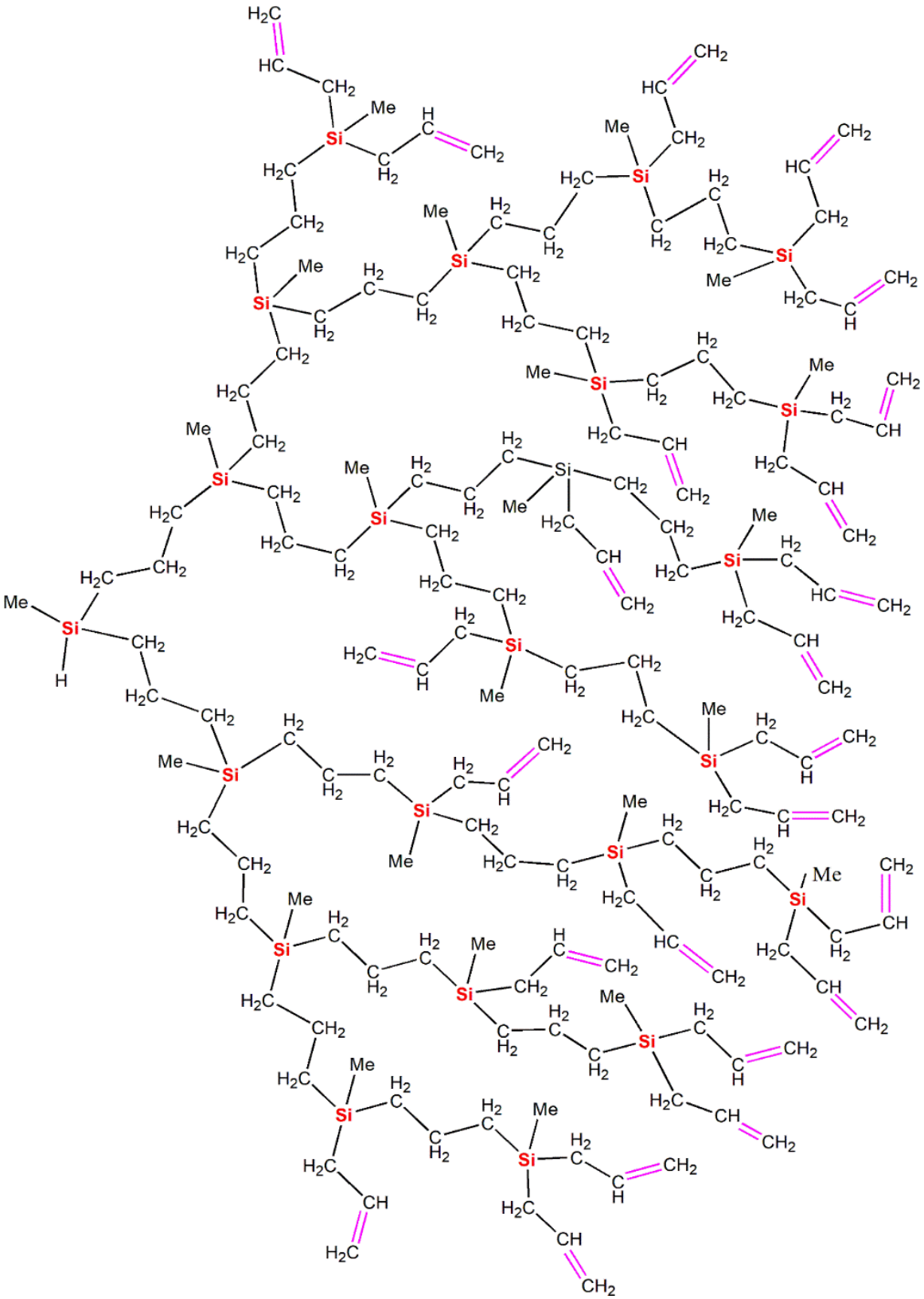

Fig. 3: Structure diagram of the allyl-terminated hyperbranched carbosilane.

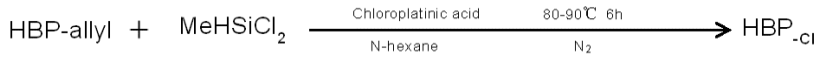

Fig. 4: The reaction formula for this chemical reaction is the synthesis of Chloride -ended hyperbranched carbosilane (HBC_{-Cl}).

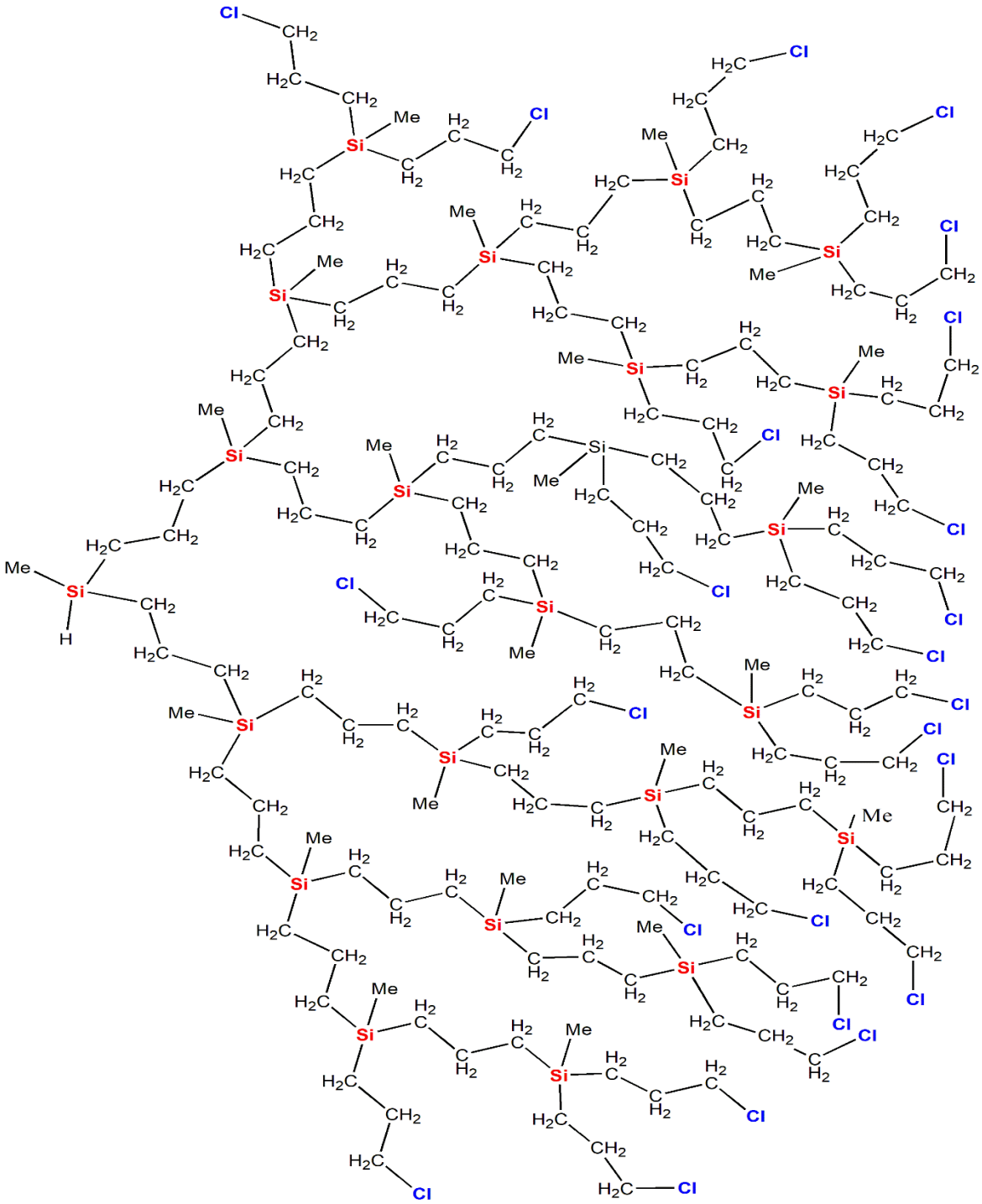

Fig. 5: Structural diagram of the chlorine-ended hyperbranched carbosilane.

A mixture of 0.3 g of sufficiently dried β-CD, 2 mL of triethylamine, and 15 mL of THF was stirred overnight under nitrogen atmosphere. Approximately 5 mL of THF was added to the reaction system, once its temperature dropped to ambient level, and then stirred for 20 minutes. Then, 20 mL of deionized water was added to the reaction system and the solution was stirred for approximately 10 minutes. The addition of deionized water causes the temperature of the reaction system to rise. After the system was cooled to room temperature; the solution was filtered to remove floating cross-linked impurities. The supernatant liquid in the filtrate was separated and dried with anhydrous magnesium sulfate for 4–5 hours. After filtration and evaporation of the Tetrahydrofuran solvent, β-CD@HBC was obtained. Figs 6 and 7 showed the network structure formed by the multi-site combination of hyperbranched macromolecules on the inner wall of the capillary. From the perspective of the unique physical structure of hyperbranched macromolecules, at the end of the silane macromolecule, the strong reaction activity of the chlorine ion was used to deacidify the hydroxyl of the β-CD molecule, and the end of the silicon carbide macromolecule was bound and modified with β-CD.

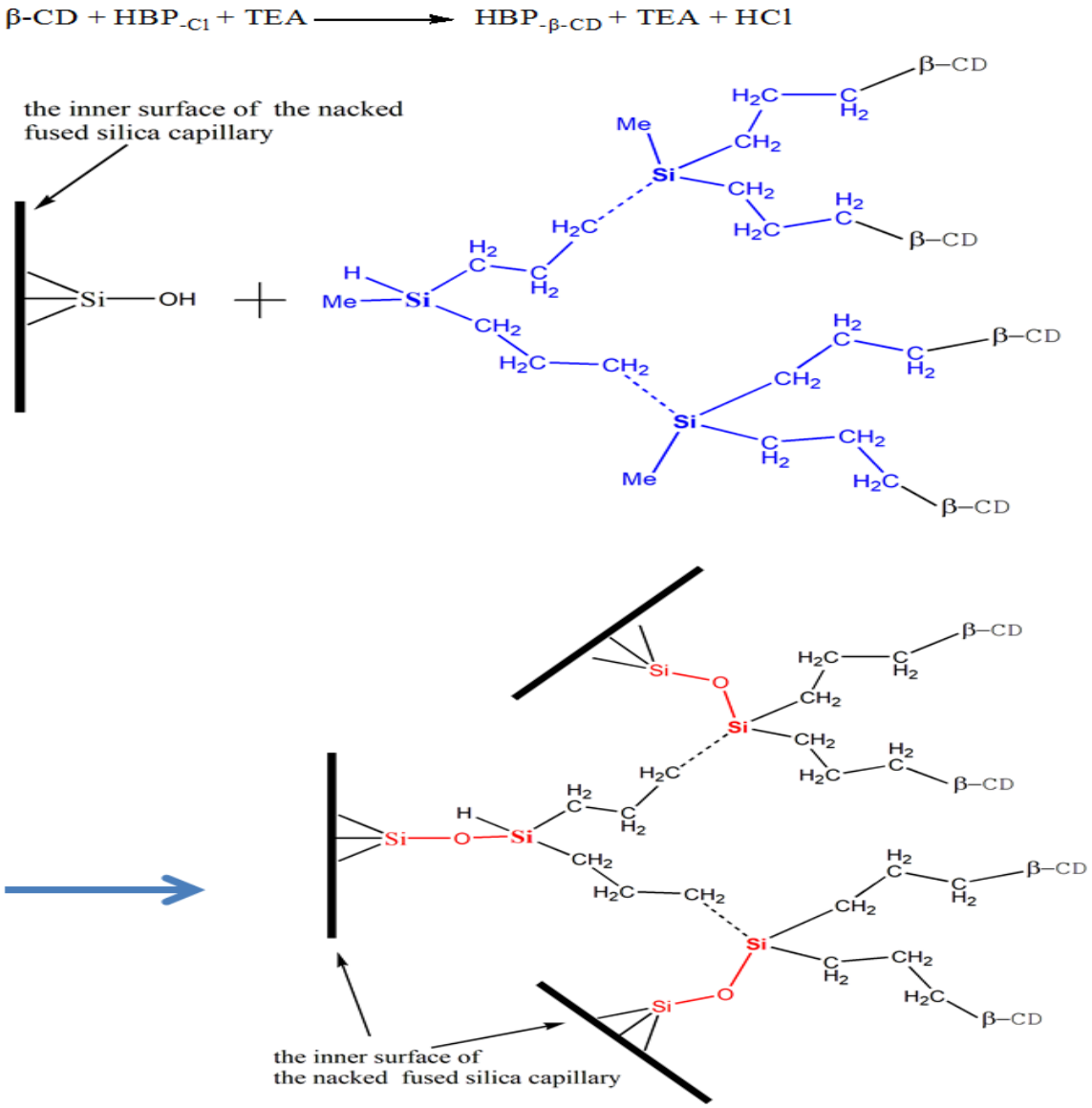

Fig. 6: Schematic diagram of the synthesis process of β-CD terminal group HBC and its immobilization on silica.

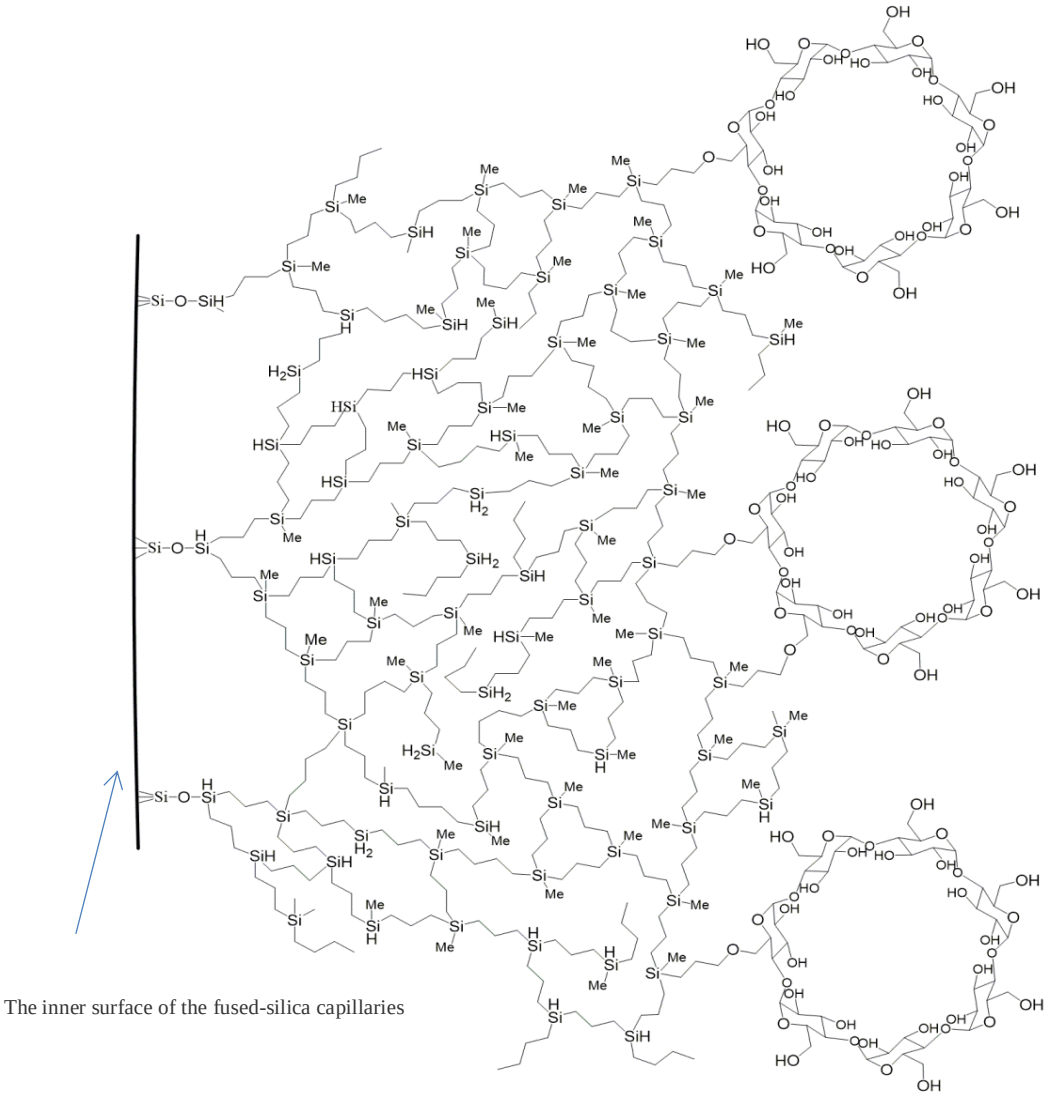

Fig. 7: Schematic diagram of the bonding mode of hyperbranched carbosilane and β-CD.

Preparation of Background Sample Solutions and Electrolytes (BGEs)

Phosphate buffered saline (PBS) with different pH values were prepared by adjusting the mixing concentration of 40 mmol/L sodium hydrogen phosphate (Na_2HPO_4), 40 mmol/L sodium hydrogen phosphate (NaH_2PO_4), phosphoric acid (H_3PO_4), and NaOH solutions until the desired pH values were obtained. Before use, the BGEs were degassed for 30 min in an ultrasonic bath.

Immobilization of the β-CD@HBC on the Inner Wall of the Fused Silica Column

In order to clean and activate the inner surface of the bare fused-silica capillary and expose more hydroxyl groups, the capillary should be subjected to some pretreatments before the experiment. The capillary was first rinsed with 1 mol/L HCl (flushing time: 1 hour), then rinsed with deionized water (flushing time: 0.5 hour), 1 mol/L NaOH (flushing time: 1 hour), and deionized water (flushing time: 0.5 hour). All rinses were carried out at a flow rate of 0.1 mL min^{-1}. The fused-silica capillary column was dried with nitrogen at 50°C for 1 hour in the subsequent operation. The β-CD@HBC solution was injected into the capillary column and stored at room temperature for 20 minutes to promote the adhesion of the β-CD@HBC coating on the inner surface; the excess β-CD@HBC solution was removed by pressure with nitrogen. The column was then aged and dried for 1 hour under nitrogen atmosphere at 180°C.

CEC Tests

The CEC separation was performed in a prepared β-CD@HBC open-tubular column （OT column）. Before use, the β-CD@HBC OT column was flushed with background solution for 20 minutes, and then electrophoresis was run at 15 kV. The CEC test experiment of the enantiomers was not started until a stable baseline was equilibrated. The β-CD@HBC OT columns were rinsed with BGE for 10 minutes between each run. Racemic isomers were detected at 214 nm.

At the end of each day, first rinse the β-CD@HBC coated OT column with background solution and dichloromethane, then inject nitrogen to blow out the liquid in the column, and dry theβ-CD@HBC coated OT column at 180°C for 3 hours under nitrogen atmosphere and then store it at 40°C under nitrogen atmosphere in a drying oven overnight. Sample stock solutions of clenbuterol, enrofloxacin, hlorpheniramine, isoproterenol hydrochloride, epinephrine, and ibuprofen (each at a concentration of 10 μg mL^{-1}) were diluted as required before use.

EOF Measurement

Electroosmotic flow is an important technology in chemical separation, especially in Capillary electrophoresis. When an electric field acts on a charged surface (such as the inner wall of a capillary with pH > 3) and the surface is in the electrolyte solution, the solution will flow at a fixed speed, which is electroosmotic flow (EOF). EOF can reflect various effects in electrophoresis experiments, such as buffer composition, acid-base conditions, capillary surface chemical modification, buffer temperature, applied radial voltage and so on. In this experiment, the effect of chemical modification of the inner surface of capillary on EOF was investigated to determine whether the inner surface of capillary was successfully modified. Thus, the EOF of the bare capillary and the β-CD@HBC-coated columns at different pH conditions are measured and compared. The EOF mobility, μ_{EOF}, was calculated by Eq. (1):

$$u_{EOF} = \frac{L_t L_e}{VT}, (1)$$

where L_t and L_e are the total length (65 cm) and the effective length (40 cm) of the capillary, respectively, T is the migration time of the EOF marker, and V is the separation radial voltage.

Results and Discussion

Characterization of the β-CD@HBC coated OT Column Preparation

DMF is used as an EOF marker because it is electric neutral and does not adsorb on the β-CD@HBC coated OT column. The EOF behaviour of β-CD@HBC coated OT columns with different pH (from 3.0 to 10) of phosphate buffer(40 mM) was studied and compared with a bare capillary, as shown in Fig. 8. As shown in Fig. 8b, the inflection point was observed between pH 4 and pH 5. The pH 4.8 is an inflection point, and the direction of electroosmotic flow changes. At pH 3.0, the direction of the EOF for the β-CD@HBC coated OT column was toward the anode, which is different from the bare capillary. For the bare capillary, when the pH of the solution increases, the ionization of Si-OH ionization on the inner surface increases, the charge density increases, and the EOF increases, that is, the electroosmotic flow increases with the increase of pH value. The inner surface of the bare capillary is negatively charged, and the direction of EOF is toward the cathode.For the β-CD@HBC coated OT column (as shown in Fig 8b), when the pH value is lower than 4.8, The EOF direction of the β-CD@HBC-coated OT column flows to the anode due to the protonation of hydroxyl in acidic environment. When the pH value is higher than 4.8 (pH=4.8-10.0), The EOF direction is to the cathode due to the ionization of β-cyclodextrin hydroxyl group. When the pH value is higher than 8.0 (pH = 8.0-10.0), the magnitude of EOF remains stable because the ionization of cyclodextrin hydroxyl groups and phosphate ions in phosphate buffer are adsorbed on the β-CD@HBC membrane as a bidentate surface substances through Lewis acid base interaction [53,54], resulting in the reduction of negative charge on the β-CD@HBC-coated OT column inner surface. The experimental results showed that β-CD@HBC was successfully fixed on the inner wall of the capillary.

Characterization of the β-CD@HBC coated OT Column

The β-CD@HBC coated OT column was characterized by scanning electron microscope (SEM). The morphology of the inner surface of the OT column is shown in Fig 9. From Fig 9a shows that the inner surface of a bare capillary is pretty smooth. Fig 9b shows that the inner surface of the β-CD@HBC coated OT column became somewhat rough after being modified with β-CD@HBC and presents a layered state. These SEM results indicate that β-CD@HBC was successfully immobilized on the inner wall of capillary.

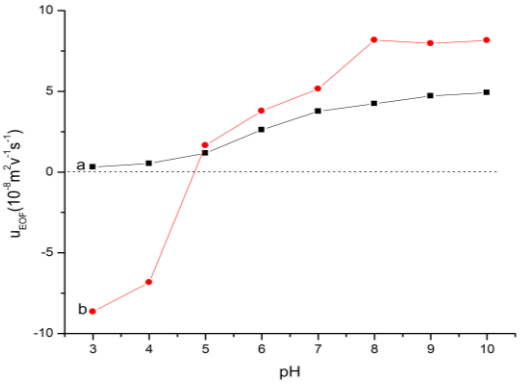

Fig. 8: Comparison Fig of the influence of BGE pH value on EOF mobility in different chromatographic columns. (a) Bare capillary; (b) β-CD@HBC coated OT column. BGE (pH 3.0-10.0); separation voltage, 20 kV. Experimental conditions: capillary, inner diameter 50mm, length 62.9cm (effective length 36.7cm);; temperature, 25°C; detection wavelength, 214 nm.

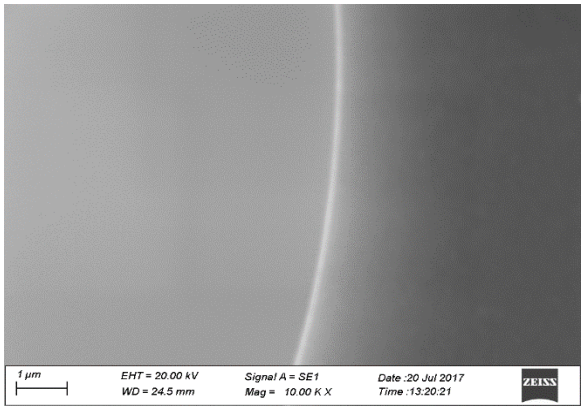

Fig. 9: Typical SEM images of bare capillary (a), β-CD@HBC coated OT columns (b).

Separation of Racemic Drugs with a β-CD@HBC coated OT Column

The separation ability of the β-CD@HBC coated OT column was evaluated for racemic drugs in capillary electro chromatography (CEC). Eight racemic drugs, including clenbuterol, enrofloxacin, ibuprofen, chlorphenamine maleate, Isoprinosine hydrochloride, adrenaline, phenylalanine, and ketoprofen, were used as analytes. The chromatographic performance was tested by separating several kinds of analogs, with the results shown in Fig. 10. A variety of chiral isomer separation studies were performed in the experiment, and some of them have a certain separation effect, but the separation efficiency was not fully optimized. The chromatographic performance test results are shown in Fig. 11.

Generally, the migration time and peak width of two adjacent peaks are read out on the electrophoretic map, and Resolution (Rs) is calculated according to the following formula.

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \quad (2)$$

Subscripts $(t_R)_A$ and $(t_R)_B$ represent the detection time of two adjacent substances a and B respectively, $(t_R)B > (t_R)A$, W_A and W_B are the baseline peak widths corresponding to samples A and B expressed in time. The denominator indicates the effect of component broadening on separation at this time interval. The resolution(Rs) of each tested enantiomer is shown in Table-1. when the resolution is higher than 1.0, the tested object can be completely separated.

Table-1: The resolution (R_S) of the tested enantiomers in the β-CD@HBC coated OT column.

Analytes	R_S of migration time (%)
Clenbuterol	0.83
Phenylalanine	0.9
Adrenaline	2.26
Chlorphenamine Maleate	1.07
Enrofloxacin	1.97
Isoprinosine hydrochloride	0.52
Adrenaline	2.26
Ibuprofen	1.23
Tyrosine	1.01
Norfloxacin	0.56
Propranolol hydrochloride	0.51
Ephedrine hydrochloride	2.83
Tryptophan	9.3
Norepinephrine	2.03

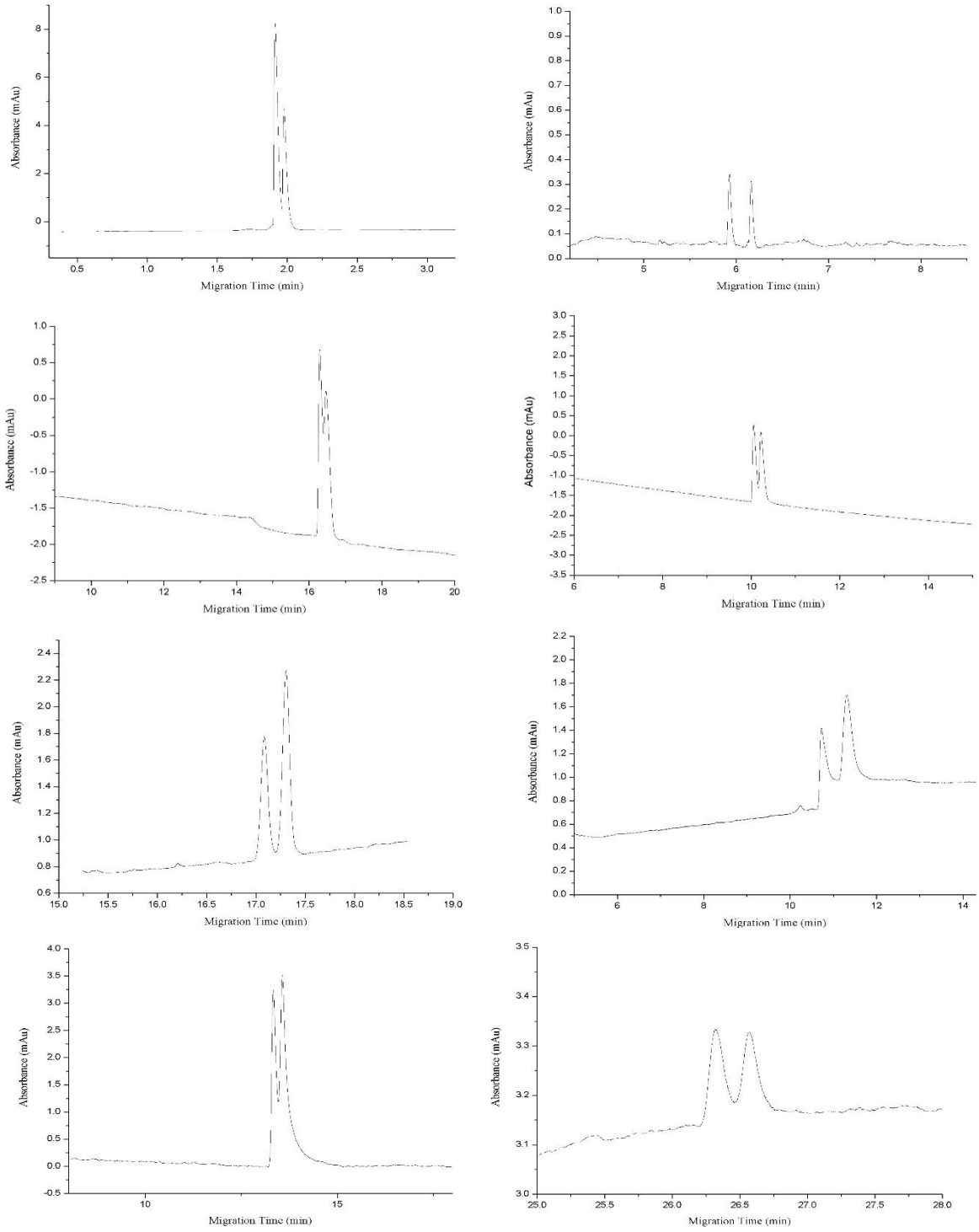

Fig. 10: CEC electropherogram on the β-CD@HBC coated capillary column used to separate isomers: a= clenbuterol. b= Enrofloxacin. c= Ibuprofen. d= Chlorphenamine Maleate. e= Isoprinosine hydrochloride. f= Adrenaline. g= Phenylalanine. h= Ketoprofen. Separation conditions: mobile phase, 40 mM phosphate buffer (a: pH 6.0, b: pH 7.0, c: pH 5.5, d: pH 4.0, e: pH 4.0, f: pH 4.0, g: pH 4.0, h: pH 5.5,); capillary, 70 cm total length. 50 mm i.d. (effective length 41 cm); detection wavelength, 214 nm; applied voltage, 16 kV; injection, 10 cm height for 10 s.

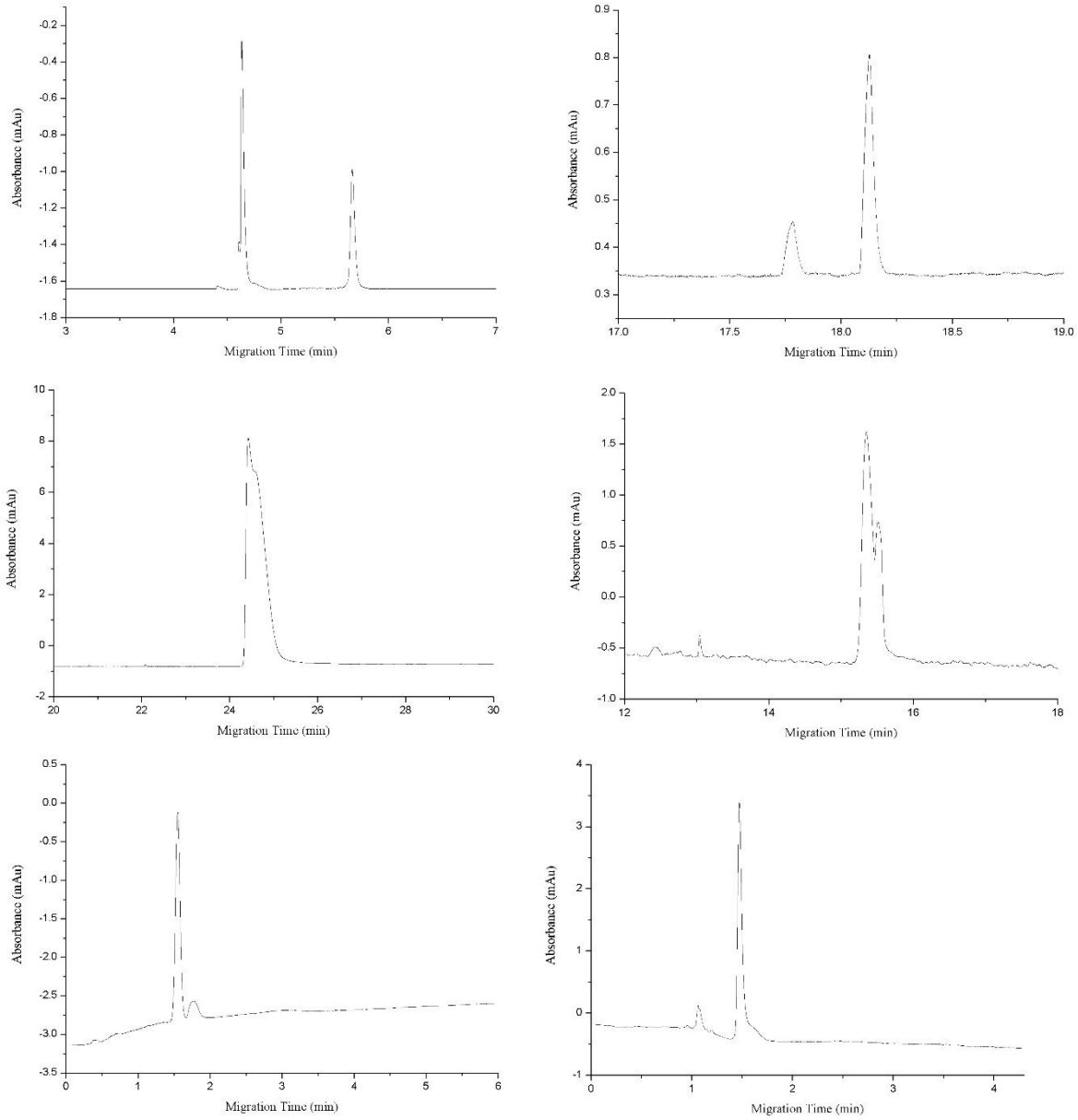

Fig. 11: CEC electropherogram on the β-CD@HBC coated capillary column used to separate isomers: a= Tyrosine. b= Norfloxacin. c= Propranolol hydrochloride. d= Ephedrine hydrochloride. e= Tryptophan. f= Norepinephrine hydrochloride. Separation conditions: mobile phase, 40 mM phosphate buffer (a: pH 4.0, b: pH 7.5, c: pH 4.0, d: pH 5.5, e: pH 6.0, f: pH 6.0); capillary, 70 cm total length. 50 mm i.d. (effective length 41 cm); detection wavelength, 214 nm; applied voltage, 16 kV; injection, 10 cm height for 10 s.

Fig 10 and 11 showed that the β-CD @ HBC column has a certain chiral separation capacity for various isomers. After proper optimization, separation performance could be obtained for these isomers. The hyperbranched structure with a large amount of hydroxyl groups greatly enhanced the chiral separation ability of the stationary phase. On one hand, the tridimensional structure of the hyperbranched carbonsilane is conducive to molecular "entrapment"; the abundance of hydroxyl groups in the functional layer of the capillary column are very important for the separation performance, while the β-cyclodextrin combined with hyperbranched structure of the β-CD@HBC provides more abundant hydroxyl groups. On the other hand, the Si–C–C dendritic branches of the β-CD@HBC also enhance the molecular

interactions with the coating layer. The increased and enhanced contact functional points between the hyperbranched structure and the analytehelps to improve column efficiency.[50] The β-CD@HBC synthesized in this project shows good performance for chiral analysis. The β-CD@HBC can increase intermolecular π–π interactions by narrowing the distance between the immobilized complexes and the target analyte by virtue of the synergism between the hydrophobicity of the derivative functional group and β-CD. The chiral separation ability of the polymers in both the positive and reverse phase is good.

Stability and Repeatability of the β-CD@HBC coated OT Column

The stability and repeatability of the β-CD@HBC coated OT column were evaluated by measuring the relative standard deviations (RSDs) of the migration times of the six enantiomers. The between runs, between days, and between columns repeat abilities were investigated, as shown in Table 2. The RSDs of the between runs, between days, and between columns were less than 1.5%, 1.86%, and 4.83%, respectively, which demonstrates that the β-CD@HBC coated OT column had good stability and repeatability.

Table-2: Repeatability of migration times of the tested enantiomers in the β-CD@HBC coated OT column.

Analytes	RSD of migration time (%)		
	between runs	between days	between columns
Clenbuterol	**0.68**	**1.2**	**4.83**
Chlorphenamine Maleate	**0.43**	**0.26**	**4.58**
Enrofloxacin	**1.3**	**1.1**	**3.3**
Isoprinosine hydrochloride	**0.45**	**0.54**	**3.9**
Adrenaline	**0.45**	**0.64**	**3.7**
Ibuprofen	**1.5**	**1.86**	**3.7**

Experimental condition: see Fig 10.

Conclusions

In this study, the Grignard reagent method was used to synthesize methylallyl silane, and then hydrosilylation was used to synthesize allyl carbosilane-hyperbranched macromolecules through self-assembly. Then, the end groups of the macromolecules were chlorinated, and the β-CD@HBC was synthesized by modifying cyclodextrin with a deacidification reaction of chloride ions and hydroxyl groups. The novel β-CD@HBC stationary phase was synthesized, and the β-CD@HBC column was characterized by CE chromatography and SEM. The findings indicated that that the β-CD@HBC column has a high chiral separation ability and good stability and repeatability. The future of hyperbranched polymers has infinite possibilities, but one can predict that hyperbranched structures will find more applications in chemical analysis and related subject fields. The combination of Chiral selectors β-CD with hyperbranched polymers and the synthesis of polymers, which have innovative applications in Capillary electrophoresis and chiral separation, which will help deepen people's understanding of the structure and properties of hyperbranched polymers and have academic theoretical and practical application values.

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