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Chiral Separation by Capillary Electrophoresis based on Hyper-branched Materials

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In order to improve the chiral separation effect of capillary electrophoresis, a method based on hyper-branched materials was proposed. The mechanism of chiral separation in capillary electrophoresis was analyzed by using the capillary as a separation channel and high voltage direct current as the driving force. The experimental procedure was included preparation of the chiral separation by capillary electrophoresis, synthesis and preparation of hyper-branched materials and capillary, preparation of buffer solution and sample solution, and setting up chiral separation conditions of electrophoresis. Thus, combined with the chiral separation mechanism, the chiral separation by capillary electrophoresis was realized. Compared with the traditional separation method, the separation degree of the proposed separation technique was higher which indicates a significant improvement in the separation effect.

Keywords: Hyper-branched materials; Capillary; Electrophoresis; Chiral separation

1. INTRODUCTION

Capillary electrophoresis (CE) is a new type of liquid phase separation technology with capillary as a separation channel and high voltage DC electric field as the driving force. Capillary electrophoresis actually includes electrophoresis, chromatography and their cross contents [1]. It enables analytical chemistry from the microliter to the nanoliter scale, as well as single cell and single molecule analysis [2]. Electric double layer (EDL) is a kind of ion layer with different signs from the surface, which is composed of two parts of relatively fixed and free ions on the separation surface between two phases. In capillary electrophoresis, there are electric double layers on both the surface of charged particles and the surface of capillary tube wall [3, 4]. Capillary electrophoresis has a broad range of separation modes, allowing it to perform a wide range of tasks, and it is commonly used.

According to different separation modes, capillary electrophoresis can be classified into many different types, as shown in Table 1.

Table 1. Types of capillary electrophoresis

Type	Abbreviation	Explanation	Ref.
Single capillary	Capillary zone electrophoresis	CZE	[5]
Capillary isokinetic electrophoresis	CITP	Two different CZE buffers were used	[6]
Capillary isoelectric focusing	CIEF	The tube is filled with pH gradient medium, which is equivalent to pH gradient CZE	[7]
Micellar electrokinetic capillary chromatography	MECC	One or more micelles were added to CZE buffer	[8]
Microemulsionelectrokinetic chromatography	MEEKC	Polymer ion exchange in CZE buffer oil by adding oil in water emulsion	[9]
Capillary electrokinetic chromatography	PICEC	Polymer ions with micro phase separation were added into CZE buffer	[10]
Open tube capillary electrochromatography	OTCEC	The stationary phase coated capillary was used to separate the positive phase and the negative phase	[11]
Affinity capillary electrophoresis	ACE	Add affinity reagent into CZE buffer or tube	[12]
Non Gel Capillary Electrophoresis	NGCE	Polymer was added into CZE buffer to form a screening network	[13]
Polyacrylamide gel electrophoresis	PA-CGE	Polyacrylamide gel filled in tube	[14]
Agarose capillary electrophoresis	Agar-CGE	Agarose gel filled in tube	[15]
Packed capillary electrochromatography	PCCEC	The capillary is filled with chromatographic packing, which can be divided into positive phase and reverse phase for ion exchange	[16]
Capillary electrophoresis / nuclear magnetic resonance	CE/NMR	It is necessary to adopt the method of pause scanning sample peak	[17]

CE can usually separate and analyze materials that can be prepared into a solvent or suspension solution, ranging from inorganic ions to biological macromolecules and supramolecules, and even the entire cell [18, 19]. It's used in a variety of areas, including life science, medicinal science, clinical medicine, molecular biology, forensic and detection recognition, chemistry, climate, customs, agronomy, manufacturing process control, product quality testing, single cell and single molecule analysis, among others. Capillary electrophoresis detection technique is also widely used by drug analysts in the field of drug inspection. Drug analysis can be roughly divided into two parts: the first is the quantitative analysis of the original drug, the determination of impurities in the original drug, pharmaceutical analysis and the evaluation of their stability [20]. These methods require good selectivity, appropriate analytical sensitivity and reliable accuracy; the second is to study the absorption, distribution, metabolism and excretion of drugs or metabolites into the human body, that is, clinical drug analysis [21]. The determination of these two parts generally requires the combination of separation and detection methods.

Chirality exists widely in nature and represents asignificant symmetry characteristic in many disciplines [22, 23]. Chiral objects and their mirror images are called enantiomers, which are also called enantiomers in reference to molecular concepts. Chirality involves the origin of life and the survival and evolution of different animals and plants [24]. Almost all natural or semi synthetic drugs have chirality, of which more than 98% are optically active substances [25]. Hyper-branched materials are different from both inorganic porous materials and conventional organic complexes. Hyper-branched has both the flexibility of organic materials and the rigidity of inorganic materials. At

present, hyper-branched materials have also been reported in the chiral separation by capillary electrophoresis [26]. Chiral hyper-branched materials are prepared and coated in situ on the inner wall of capillary tubes with ZnO as a nucleating agent. A series of enantiomers of β-blockers are separated in CEC mode using carboxymethyl-β-CD as a chiral selector. Compared with the EKC mode without hyper-branched coating, the separation of chiral compounds can be significantly improved [27, 28]. Using poly (glycidyl methacrylate co ethyl dimethacrylate) monolithic column as the carrier, zeolite imidazole ester skeleton-8 hyper-branched is modified on the surface of the stationary phase byalayer by layer assembly mechanism. Furthermore, pepsin is covalently bonded to hyper-branched materials as chiral selector. Compared with the lack of hyper-branched materials and direct modification of pepsin on monolithic column matrix, the resolution of a series of chiral drugs such as chloroquine, nefopam and amlodipine could be greatly improved.

However, most of the chiral separation methods by capillary electrophoresis are used in conventional drugs, and there are few studies on the chiral separation of hyper-branched materials. Hyper-branched polymers, as homologues of dendrimers, have highly branched molecular structure, numerous end group functional groups and no entanglement between molecules, which makes them have some special properties compared with linear polymers. In this paper, hyper-branched materials are used as the research object to optimize the design and research of chiral separation method by capillary electrophoresis, in order to improve the separation efficiency of chiral separation method by capillary electrophoresis.

2. EXPERIMENTAL

2.1. Analysis of chiral separation mechanism by capillary electrophoresis

Capillary zone electrophoresis (CZE) is a separation method that uses an electric field to distinguish ions or charged particles based on their mobility in a free solution with a specific pH. Figure 1 shows schematic diagram of capillary electrophoresis instrument.

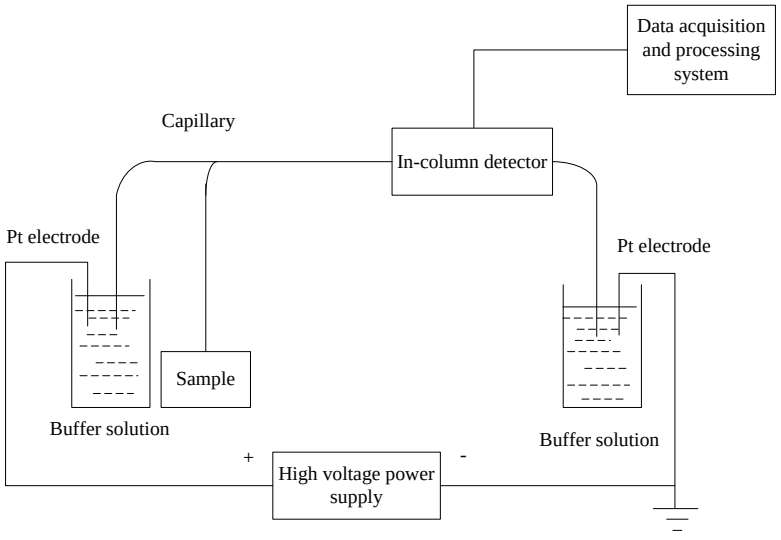

Figure 1. Schematic diagram of capillary electrophoresis instrument

2.1. Synthesis of hyper-branched materials

ABx monomers were used to make hyper-branched polymers. The polymerization of nucleated molecules and monomers produces the first generation of hyper-branched polymers, and the continuous proliferation of monomers produces dendrimers [29]. The synthesis mechanism is shown in Figure 3.

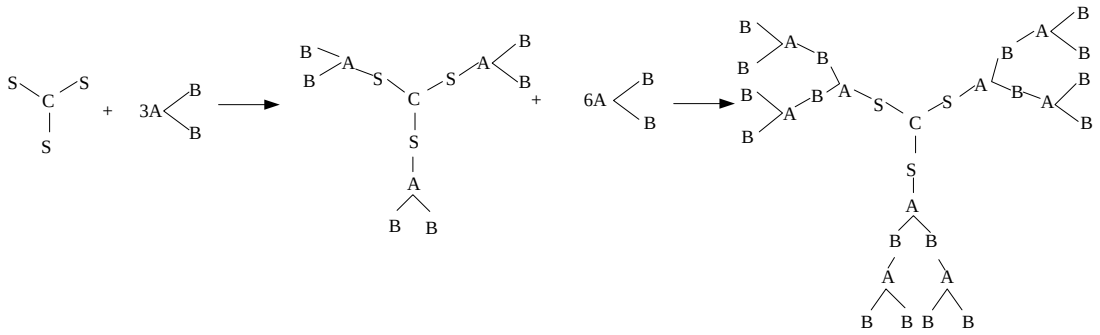

Figure 3. Synthesis mechanism of hyper-branched polymer

Controlling the volume of Karstedt catalyst, the hydrosilylation reaction, and adding monomer vinyltrisilane are all used to make hyper-branched polysiloxane macromolecules. A 50ml flask contains 6.0g monomer and 0.05g Karstedt catalyst, as well as a sufficient volume of p-benzoquinone to avoid double bond self-polymerization. The reaction is stirred by magnetic force for 4h at 40 ^{o}C. The reaction process is monitored by FTIR, and the reaction ended when the characteristic infrared absorption peak of the vinyl group at 1601cm^{-1} disappeared [30]. To dissolve the sample, 5 mL ether is added, followed by 50 mL acetonitrile to precipitate small molecules. Colorless transparent viscous oil, namely hyper-branched polysiloxane, is obtained after vacuum drying at 40 ºC for 12 hours. The first generation hyper-branched polysiloxane and the second generation hyper-branched polysiloxane can be prepared by changing the proportion of monomer and Karstedt catalyst. The branching degree of hyper-branched polymer is a factor to describe the structure of hyper-branched polymer expressed as follows:

$$DB = \frac{D+T}{D+T+L} \qquad (1)$$

where D is the number of fully branched units, T is the number of terminal units, and L is the number of linear units.

2.2. Synthesis of chiral ionic liquids

According to a previous paper [31], chiral ionic liquids were synthesized using a basic one-step procedure using readily available reagents. The following procedures were used to make chiral ionic liquids: First, 6mL water was used to dissolve 0.231g lithium carbonate and 0.388g boric acid. The mixture was then slowly added 1.83 g of (S)-mandelic acid. For 1 hour, the mixture was heated to around 55°C. The reaction was then cooled down to room temperature before adding 0.573g of

$BMIm^+Cl^-$. To extract the reaction substance, 40mL CH_2Cl_2 was applied after around 1 hour of stirring at room temperature (in the bottom layer). The organic layer of CH_2Cl_2 was washed five times with 3mL water. The viscous colorless liquid substance was obtained by drying the product under vacuum at 70°C overnight after CH_2Cl_2 evaporation.

2.3. Preparation of capillary tubes

Methanol is filtered by an oil membrane with a pore size of 0.45 μ m, then 1 mol / L HCl, 1 mol / L NaOH, 0.1 mol / L NaOH and purified water are filtered by a water membrane with a pore size of 0.45 μ m, and then degassed by ultrasonic for standby [32]. Then, the intercepted capillary was washed with methanol for 10 min to remove the residual organic matter in the new column. Then, the capillary column was activated by washing with purified water, 1 mol/ L HCl, purified water and 1 mol/ L NaOH for 30 min, and then washed with purified water, 0.1 mol/ L NaOH and purified water for 30 min. Finally, it was dried with nitrogen for standby [33]. In the coating process, dopamine (DA) was self-polymerized under oxygen and alkaline conditions to form polydopamine (PDA), and β-cyclodextrin (β-CD) was fixed on the inner wall of the capillary column through its adhesion. The preparation process of polydopamine/β-cyclodextrin coated capillary column is shown in Figure 2.

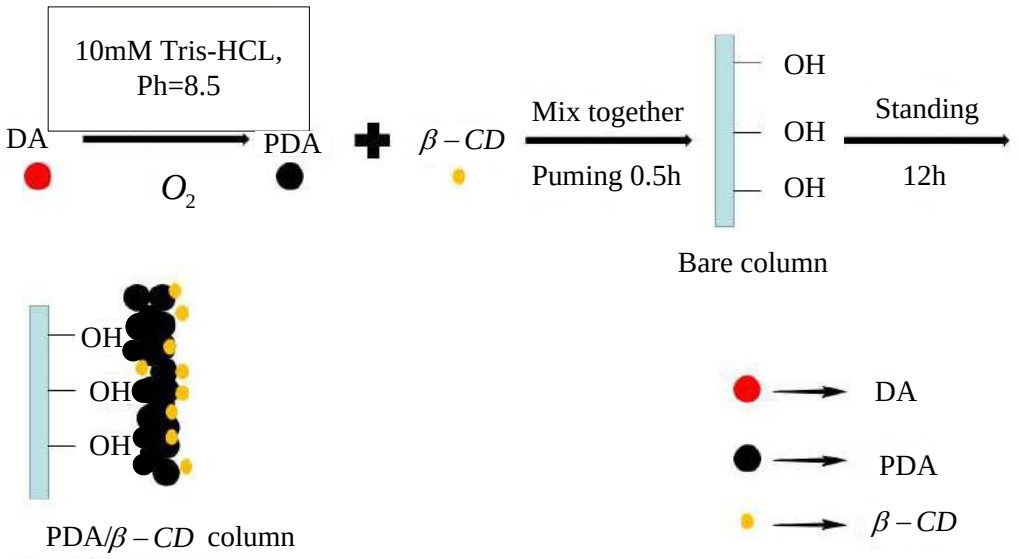

Figure 2. Preparation process of coated capillary column

2.4. Preparation of buffer solution and sample solution

The following steps were used for the preparation of a sample stock solution: i) accurately weigh 10 mg of ofloxacin reference substance, ii) dissolve it with 0.1 mol/ L sodium hydroxide, iii)

transfer it to a 10 ml volumetric flask, iv) fix the volume with water, and v) shake it up to obtain 1 mg/ml ofloxacin stock solution, and store it at 4ºC.

The following steps were used to prepare of sodium dihydrogen phosphate solution: i) precisely weigh a certain amount of sodium dihydrogen phosphate, ii) dissolve it with water, iii) transfer it to a volumetric flask, iv) dilute it with water, v) fix the volume and shake it well to obtain the required concentration of sodium dihydrogen phosphate solution for standby.

Preparation of the buffer solution includes following steps: i) precisely weigh a certain amount of copper acetate and L-amino acid, ii) dissolve them in the prepared sodium dihydrogen phosphate solution, iii) then weigh a certain amount of cyclodextrin, iv) dissolve them in the solution, v) transfer them to a 100ml volumetric flask, vi) dilute them with the prepared sodium dihydrogen phosphate solution, vii) fix the volume and shake them well. The buffer solution containing copper acetate, L-amino acid and cyclodextrin was obtained by measuring 50 ml of the above buffer solution and adjusting the pH to the required pH with phosphoric acid.

The steps for making a 0.1mol/l sodium hydroxide solution are as follows: i) weigh about 0.4g sodium hydroxide particles in a beaker, ii) dissolve them with water, iii) transfer them to a 100ml volumetric flask, iv) fix the volume and shake them well.

In addition, the preparation of test solution in chiral separation by capillary electrophoresis will be modulated according to the separated drug object. Taking Ofloxacin separation object as an example, 1ml Ofloxacin stock solution is accurately measured in a 10ml volumetric flask, diluted with water to a constant volume and shaken well to obtain a test solution with Ofloxacin mass concentration of 100 μg/ml, and stored in a refrigerator at 4 ºC. In the electrophoretic chiral separation environment, the separation method in Figure 3 was used to realize the chiral separation of hyper-branched material samples.

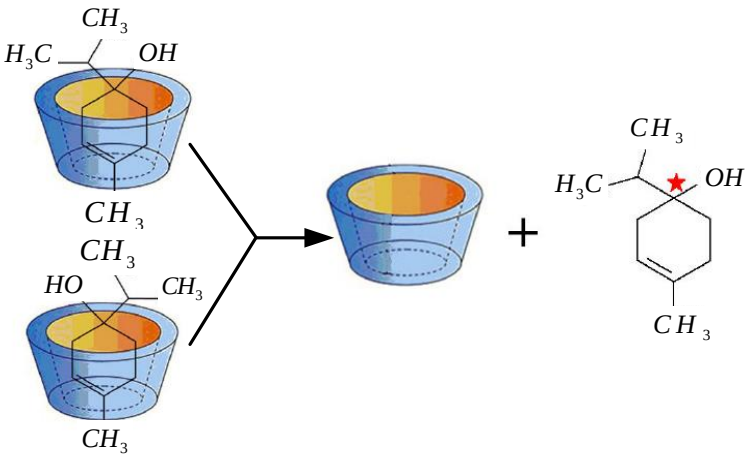

Figure 3. Schematic diagram of separating subject and object of hyperbranched enantiomers by capillary electrophoresis

3. RESULTS and DISCUSSION

The electrophoretic separation parameters are calculated by the following formula.

$$\begin{cases} \eta = \dfrac{t_2}{t_1} \\ R_s = 2\dfrac{(t_2 - t_1)}{(\omega_1 + \omega_2)} \end{cases} \quad (2)$$

where η and R_s denote the separation factor and separation, respectively, the parameter t denotes the electrophoretic migration time, ω represents the peak width, and subscripts 1 and 2 denote the first and second enantiomeric separation peaks. Taking Ofloxacin as an example, under the optimal conditions of 5mmol/L sodium dihydrogen phosphate, 8mg/ml carboxymethyl-β-cyclodextrin, 8mmol/L Cu(II), Cu(II) and L-histidine molar ratio of 1:1.3, pH5.0, separation voltage of 15kV, the enantiomer of Ofloxacin can be separated in 10min, and the separation is 2.24. The electrophoretogram of the separation is shown in Figure 4.

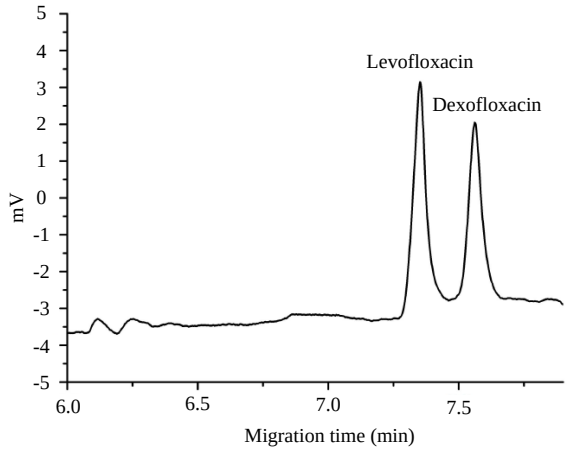

Figure 4. Electropherograms of Chiral compounds separated by capillary electrophoresis with Ofloxacin for hyper-branched enantiomers

In order to test the design of the chiral separation method by capillary electrophoresis based on hyper-branched materials, comparative experiments were used to show the advantages of the design method. In addition to the designed chiral separation method, the chiral separation method by capillary electrophoresis based on ionic liquid was also set up. In order to avoid the limitation of a single experiment, different chiral selectors were set in the experiment, and the experiment is divided into several groups, and the final separation results are obtained under the operation of two different separation methods [36].

The pH of 30mmol/L NaH_2PO_4 buffer was adjusted to 2.2 with phosphoric acid, and 10mmol/L β-cyclodextrin was taken as chiral selector. The electropherograms of Chiral compounds separated by capillary electrophoresis with β-cyclodextrin as chiral selector based on hyper-branched materials and ionic liquids are shown in Figure 5. Through the application of three chiral separation methods, the separation was 2.42 and 2.19 for Chiral separation in capillary electrophoresis based on hyper-branched materials and ionic liquids, respectively.

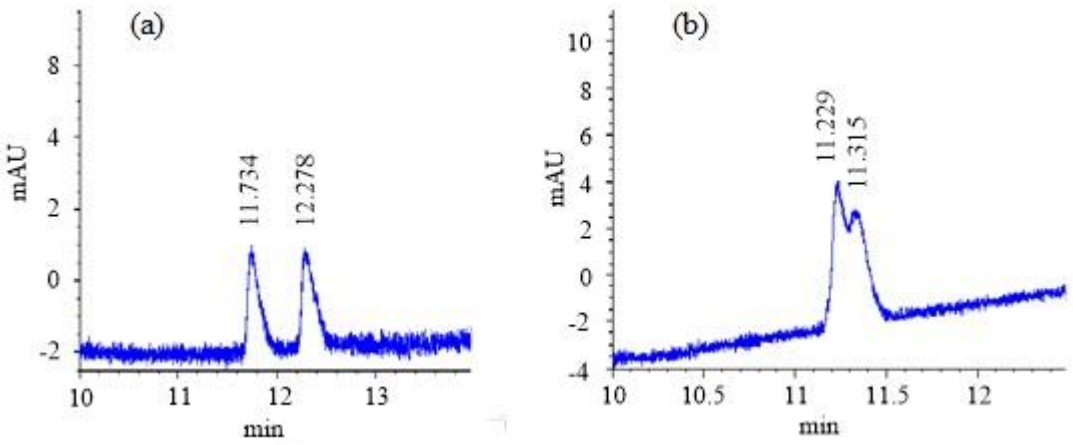

Figure 5. Electropherograms of Chiral compounds separated by capillary electrophoresis with β-cyclodextrin as chiral selector (a) based on ionic liquids (b) based on hyper-branched materials

Table 3 compares and contrasts the analytical performances of the reported enantiomeric resolutions values of chiral separation in this work with previous research. Capillary electrophoresis based on hyper-branched materials suggests a significant development in capillary electrophoresis.

Table 3. Comparison between the enantiomeric resolutions value of chiral separation in capillary electrophoresis based on hyper-branched materials in this work with previous researches

Technique	Drugs	Enantiomeric resolutions	Ref.
Chiral capillary electrophoresis	Econazole	6.5	[33]
Chiral separation of capillary electrophoresis	Tropicamide	1.26	[34]
Chiral ionic liquidsby Capillary Electrophoresis	Terbutaline	2.30	[32]
Chiral Separation by Capillary Electrophoresis	Nefopam hydrochloride	2.01	[37]
Chiral Separation by Capillary Electrophoresis	Ofloxacin	2.42	This work

In the same way, the separation results of other chiral selectors can be obtained. In order to achieve the quantitative comparison of the separation effect, two indexes were set, i.e. enantiomeric excess value and electrophoretic separation. The enantiomeric excess of the reaction product was determined by the established separation method, and the results are compared with those of the traditional separation technique based on ionic liquids. The results are revealed in Table 4.

Table 4. Determination of enantiomeric excess value of chiral separation (EE) for both separation techniques

Sample number	Chiral separation by electrophoresis based on ionic liquids	Chiral separation in capillary electrophoresis based on hyper-branched materials
1	95.0	94.5
2	94.0	93.7
3	96.0	95.4
4	97.0	96.3
5	95.0	94.2
6	97.0	96.7
7	77.0	76.4
8	79.0	78.9

The results show that the enantiomeric excess values determined by the chiral separation in capillary electrophoresis based on hyper-branched materials are in good agreement with those determined by that one based on ionic liquids. It has proved that the separation method based on hyper-branched is accurate and reliable, and can be used for the separation and analysis of chiral aromatic secondary alcohols and the determination of enantiomeric excess value. From the point of view of enantiomeric excess, the excess value of the propose method is less, that is, the separation accuracy is higher.

Furthermore, the quantitative comparison results of separation degree for both separation techniques can be obtained, as shown in Table 3.

Table 3. Quantitative comparison results of separation degree for both separation techniques

Sample number	Chiral separation by electrophoresis based on ionic liquids	Chiral separation in capillary electrophoresis based on hyper-branched materials
1	0.83	0.89
2	0.88	0.93
3	0.84	0.92
4	0.82	0.87
5	0.83	0.92
6	0.81	0.85
7	0.86	0.94
8	0.87	0.91

According to the quantitative comparison results in Table 2 and Table 3, it can be seen that the designed chiral separation method by capillary electrophoresis based on hyper-branched materials has better separation effect in practical work.

4. CONCLUSIONS

With the wide application of capillary electrophoresis in medicine, it will also bring more challenges. The future development trend of capillary electrophoresis in drug analysis is mainly in the complex biological sample systems including cells and tissues. Biological sample analysis will inevitably require the further development of complex sample pretreatment and enrichment technology, more sensitive detection technology and a series of problems with mass spectrometry, such as interface technology, sample utilization, concentration sensitivity, detection volatility, etc. In this work, the mechanism of chiral separation in capillary electrophoresis was analyzed by using the capillary as a separation channel and high voltage direct current as the driving force. The experimental procedure included synthesis and preparation of hyper-branched materials and capillary, preparation of buffer solution and sample solution, and setting up chiral separation conditions of electrophoresis. On this basis, combined with the chiral separation mechanism, the chiral separation by capillary electrophoresis was realized. Compared with the traditional separation method, the separation degree of the proposed separation method is higher which indicates a significant improvement in the separation effect.

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