Preparation and Characterization of Cellulose Membrane Modified with β-Cyclodextrin for Chiral Separation

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Abstract: In this study, it is first time to obtain a complete separation of using β-cyclodextrin (β-CD) modified cellulose microfiltration membrane. Commercially cellulose membrane with the pore diameter of 0.22μm was functionalized with β-cyclodextrin (β-CD) by aldolization at the solid–liquid interface. Filtration experiments were carried out using a dead-end filtration cell holding a flat sheet membrane with effective area of 36 mm^2. Aqueous solution of racemic tryptophan (0.05g/L, 150 mL) was forced to permeate through the cellulose membrane immobilized by β-CD at a flow rate of 0.1 ml/min through the membrane microdevice. Chiral ligand exchange chromatography was used to determine the concentration and ratio of D- and L-tryptophan in the filtrate. A complete separation of racemic tryptophan can be obtained by using this novel composite membrane-based separation system. In addition, a multi-stage filtration separation was applied in order to obtain higher permselectivity. The objective of this study is to obtain an easy prepared chiral membrane with good reproducibility and can be applied to a variety of chiral separations.

Keywords: Cellulose membrane, Enantiomeric separation, Glutaraldehyde, Tryptophan, Multi-stage filtration.

1. INTRODUCTION

Chirality is a widespread phenomenon that plays a crucial role in nature. The majority of drugs were racemic mixtures which have two enantiomers [1-5]. In general, one of them has the effective therapic function while another one may lead to some negative effect or toxicity [6-9]. Therefore, single enantiomer of chiral substances with each enantiomer having different biological activity is in demand [10, 11].

Membrane-mediated enantiomer separation process is a newly emerging and meanwhile promising technology, which has been receiving ever-increasing attentions recently. Membrane-based enantiomer separation is often found several advantages, such as low energy consumption, simplicity in set-up, and continuous operation mode [12-15]. Chiral separation membranes can be divided into liquid and solid membranes. As per the chiral resolution method developed earlier, liquid membranes possess good permeability and enantioselectivity, but they have poor durability and stability. Compared with liquid membranes, solid membranes provided good stability, favorable permeability and enantioselectivity. Hence, solid membranes were applied in industrial production. One of the main difficulties of chiral separation by solid membranes is the low separation factor compared to conventional methods, making the realization of a single-step membrane process difficult [16-20]. A multi-stage cascade in the membrane process has the advantage of using elements from existing analytical separation methods, and significantly enhancing the enantiomeric excess, providing an alternative for the current large-scale enantiomer separation processes [21].

Compared to other chiral selectors such as proteins and enzymes, cyclodextrins are still attractive candidates for chiral separation due to lower cost, wider applicability and higher tolerance in various environments [22]. One outstanding ability is to form inclusion complexes by virtue of a series of weak intermolecular forces. In spite of its potential as a chiral selector, there are only several reports concerning the immobilization of β-CD in solid polymer membranes by chemical binding and their use for the separation of organic isomer mixtures [22-28]. Although these studies have demonstrated the feasibility of performing chiral separation using β-cyclodextrin-immobilized membranes, cellulose dialysis membrane is expensive and β-cyclodextrin requires derivatization.

In the present study, we focused on a new method to directly immobilize underivatized β-CD on the surface of cellulose microfiltration membrane by aldolization. The commercialization microfiltration membrane is advantageous for achieving high flux and good reproducibility. The prepared membrane was tested for the separation of organic isomers, with tryptophan isomer as the model feeds, in a constant flow rate (0.1mL/min) which makes chiral substances and chiral resolving agent have the same interaction time. In the optimized method, D- and L-tryptophan were separated completely with satisfactory repeatability by a multi-stage filtration process. This is a

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report, for the first time, that the optical resolution of enantiomers is accomplished by filtering enantiomers for several times.

2. EXPERIMENTAL

2.1. Chemicals and Materials

The commercial cellulose membranes with pore diameter labeled 0.22μm were purchased from Shanghai Xinya Purification Company. β-cyclodextrin, D- tryptophan, L- tryptophan, DL-tryptophan, L-phenylalanine, sodium cyanoborohydride, periodate, glutaraldehyde 50% (v/v) aqueous solution were purchased from Aladdin Co. (Shanghai, China). Anhydrous sodium, sulfuric acid, phenol, dipotassium hydrogen phosphate were obtained from Nanjing Chemical Reagent Company. All reagents used in the experiments were of analytical grade. Deionized water was used throughout all experiments.

2.2. Membrane Modification Process

Cellulose membrane immobilized β-cyclodextrin was prepared by aldolization at solid-liquid interface (Figure **S1**). Firstly, the cellulose microfiltration membrane was soaked in purified water for 1h to remove residual storage chemical, sodium azide, which prevents the membrane from bacteria propagation. Then the membrane was immersed in periodate (H_5IO_6, 0.25mM, 5mL) and stirred at a constant temperature of 25°C (150r/min). After that, the membrane was rinsed with purified water three times. The activated membrane was immerged into 20mL of the mixed solution of sodium sulfate (2g) and sulfuric acid (0.10mL) containing β-cyclodextrin (0.1mM). The 10mL mixed solution of sodium sulfate (1g) and sulfuric acid (0.05mL) containing glutaraldehyde (0.1mM) was added dropwise with stirring. The membrane was agitated at 50°C for 2h, afterwards, followed by washing with purified water three times. Later, the crosslinked membrane was placed into the phosphate buffer solution (500mM, pH 6.0) containing sodium cyanoborohydride (0.25mM) and agitated at 20°C for 2h. Finally, the modified membrane was washed with purified water three times and let the membrane stand at 25°C for test.

2.3. Characterization of the Membranes

An investigation of the membrane morphology was performed by scanning electron microscopy (SEM) using a Hitachi 3400N scanning electron microscope (Hitachi, Tokyo, Japan) prior to which the membrane samples were first dried in a freeze drier, fractured in the liquid nitrogen and then coated with platinum.

2.4. Infrared Spectroscopy of β-Cyclodextrin Polymers Crosslinked by Glutaradehyde

The β-cyclodextrin (0.1mM) was dissolved in aqueous solution (pH=3.0). And glutaraldehyde (0.1mM) was added dropwise with stirring. Then the mixed solution was stirred at a constant temperature of 50°C for 2h. Having been cooled to room temperature, the mixed solution was adjusted to pH 7.0 and stirred for a further 0.5h at 50°C. After that, the reaction solution was dried to constant weight in a vacuum oven at 30°C. Then, the unreacted glutaradehyde was removed with anhydrous ethanol. Finally, the reaction product was dried to constant weight in a vacuum oven at 30°C.

Fourier transform infrared (FT-IR) were recorded on a Shimadzu 8400S spectrometer. For the infrared absorption spectra, the samples were formed into pellets with certain KBr and conducted in the wave-number region of 400-4000cm^{-1}. The different spectra were obtained by subtracting the background of the unloaded sample. The formation of β-cyclodextrin polymers is accompanied by changes in IR spectra of β-cyclodextrin polymers as compared with β-cyclodextrin and glutaradehyde.

2.5. The Validation of the Unbound β-Cyclodextrin in the Modified Membrane

Collect the last lotion after the reduction reaction of sodium cyanoborohydride. Pipette 2.0 mL of the last lotion into the standard colorimetric tube and add 1.0mL of phenol solution. Then add 5.0mL sulfuric acid and mix rapidly. With pure water as the blank control, phenol-sulfuric acid method detects whether the modified membrane contains adsorbed β-cyclodextrin by colorimetric method.

2.6. Rejection Experiments

Rejection experiments were characterized with 1.0g/L BSA aqueous solution through the membrane test device (Figure **S2**) at 0.1MPa. The concentrations of BSA in permeate and feed were determined by UV-spectrophotometer (UV1800, SHIMADZU) at 280nm. The BSA rejection was calculated according to formula (1) [29]:

$$R(\%)=100(1-C_p/C_f) \quad (1)$$

Where C_p and C_f are the concentration of BSA in permeate and feed solutions, respectively.

2.7. Membrane Filtration System

Filtration experiments were carried out using a dead-end filtration cell holding a flat sheet membrane with effective area of 36 mm^2. Aqueous solution of racemic tryptophan (0.05g/L, 150mL) was forced to repeatedly permeate the cellulose membrane immobilized β-CD at a constant flow rate of 0.1ml/min in the membrane microdevice. Each filtrate was collected for HPLC analysis.

2.8. Analytical Methods

2.8.1. Membrane Separation Performance Analysis

Each collected filtrate was analyzed using a chiral ligand exchange chromatography method [30] on a 1200 infinity series HPLC (Agilent Technologies, USA) with a 4.6mm I.D.×250mm C_{18} column (Baseline Ltd., China). The chromatographic system was equipped with a 20μL sample loop. Chiral analysis was performed at 35℃ using a mobile phase containing 0.375 mM L-phenylalanine and 0.075 mM copper sulfate as chiral ligand exchange reagent as well as methanol/water (20:80, v/v) at a flow rate of 1.0 ml/min. Detection wavelength of tryptophan was 278nm. Chiral separation membrane performance that was expressed on the enantiomeric excess (e.e.) could be expressed by Eq. (2).

$$e.e.=100(A_D-A_L)/(A_D+A_L) \quad (2)$$

Where, A_D and A_L denote D- and L-tryptophan peak area in the chromatogram of the filtrate, respectively.

2.8.2. Determination of Tryptophan Configuration in the Chromatogram

DL-tryptophan solution (0.05 g/L) and the mixed solution containing DL-tryptophan (0.05g/L) and L-tryptophan (0.05g/L) were analyzed respectively with the above chromatographic method. Observe the change of the above two solutions in the chromatographic peak area.

2.9. Stability of the Chiral Separation Performance

Racemic tryptophan filtrate was collected continuously in the seventh filter. The receiver was changed every 2 hours within 12 hours. The filtrate was analyzed respectively with the above chromatographic method to investigate the stability of the chiral separation effect within 12 hours.

3. RESULTS AND DISCUSSION

3.1. Characterization of Chiral Cellulose Membrane

The changes in membrane morphology have been demonstrated by SEM observation as shown in Figure **1**. By comparing the SEM pictures in Figure **1a** and **b**, it is clear that the membrane becomes rougher and nodules where β-cyclodextrin aggregates are observed after β-cyclodextrin functionalization.

3.2. Infrared Spectroscopy of β-Cyclodextrin Polymers Crosslinked by Glutaradehyde

The 4000-400 cm^{-1} FT-IR spectra of β-cyclodextrin, glutaradehyde and β-cyclodextrin polymers crosslinked by glutaradehyde are shown in Figure **2**. The major peak for β-cyclodextrin in Figure **2A** can be assigned as follows: the characteristic adsorption bands of O-H

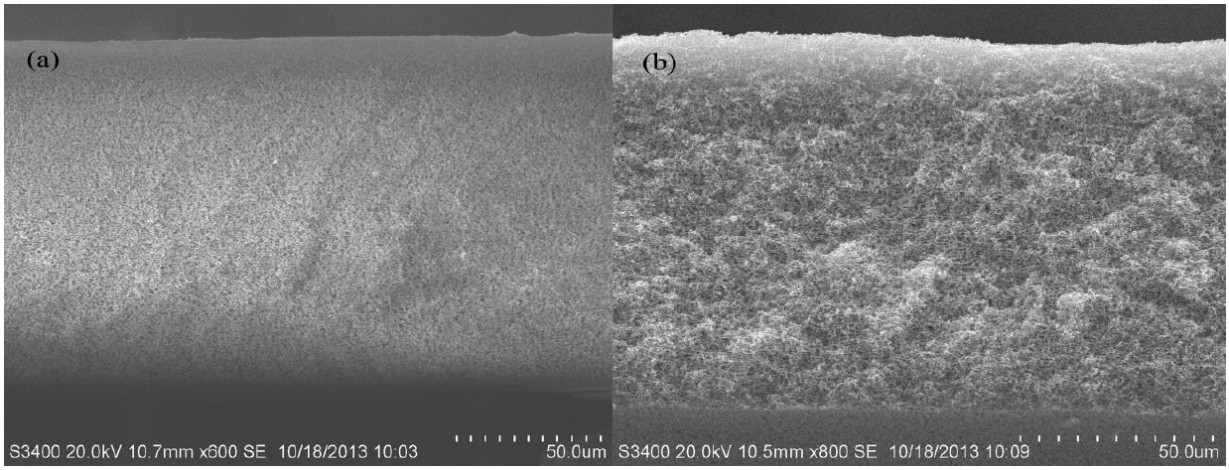

Figure 1: The membrane cross section morphology analysis by SEM (**a**) and (**b**) cellulose membrane before and after β-cyclodextrin functionalization.

stretching and bending at around 3400 cm^{-1} and 1640cm^{-1}, and at 2924 cm^{-1} confirms the H-C-H asymmetric stretching vibration. The peak at 939 cm^{-1} is due to the R-1, 4-bond skeleton vibration of β-cyclodextrin. The peaks at 1028 cm^{-1} and 1157 cm^{-1} correspond to the antisymmetric glycosidic v_a(C-O-C) vibrations and coupled v(C-C/C-O) stretch vibration [31]. The major peak for glutaradehyde in Figure **2C** can be assigned as follows: the peaks located at 1720 cm^{-1} and 2720 cm^{-1} correspond to C-O stretching vibrations and overtones of the –CHO groups. The FTIR spectrum shows the absorption peaks at about 3412 cm^{-1} for –OH group of glutaraldehyde condensates, and at 2945 cm^{-1} and 2870cm^{-1} for the asymmetric stretching vibration and symmetric stretching vibration of H-C-H. The Figure **2B** (β-cyclodextrin-glutaradehyde) illustrates the effect of glutaradehyde on the chemical structure of β-cyclodextrin. The peaks at about 3412 cm^{-1}, 1720 cm^{-1} and 2720 cm^{-1},which correspond to a broadened absorption peaks at –OH group and an absorption peaks at the unreacted aldehyde in β-cyclodextrin polymers was found. The spectral change was due to the reaction between the hydroxyl groups and glutaradehyde [32]. Thus, it can be concluded that β-cyclodextrin polymers crosslinked by glutaradehyde was prepared successfully.

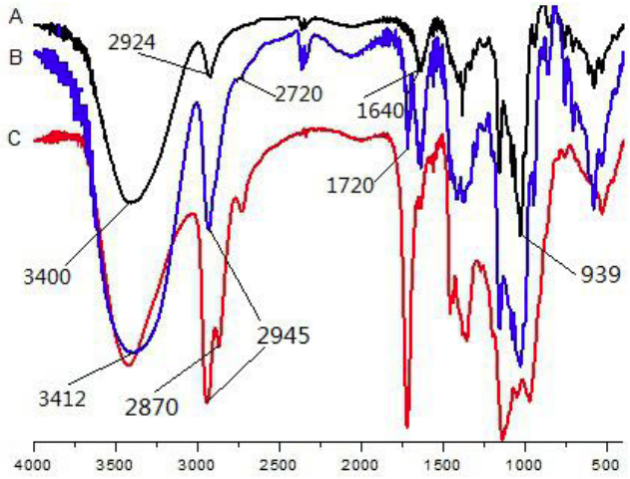

Figure 2: Infrared spectra of β-cyclodextrin (A), β-cyclodextrin polymers (B), glutaradehyde (C).

3.3. The Validation of the Unbound β-Cyclodextrin in the Modified Membrane

Phenol-sulfuric acid method is an easy and reliable method for measuring total content of the carbohydrates [33]. The chromogenic result indicated that the remaining unbound β-cyclodextrin was not found on the modified membrane.

3.4. Rejection Experiments

The linear regression equation of BSA concentration and absorbance was y=0.6447x+0.0027 (R^2=1). According to the formula (1), the retention of untreated membrane was 8.2% while the retention of the modified membrane was 28.5%. The results above can be explained by self cross-linking and bonding β-cyclodextrin which results in the smaller pore size.

3.5. Determination of Tryptophan Configuration in the Chromatogram

The chromatogram of DL-tryptophan solution (0.05 g/L) and the mixed solution containing DL-tryptophan (0.05g/L) and L-tryptophan (0.05g/L) was shown in Figure **3**. It is observed from the chromatogram that the peak area ratio of D-tryptophan and L-tryptophan is approximately 1 in Figure **3A** while 1/3 in Figure **3B**. The data suggests that the retention time of D-tryptophan is longer than L-tryptophan and the front peak is the peak of D-tryptophan.

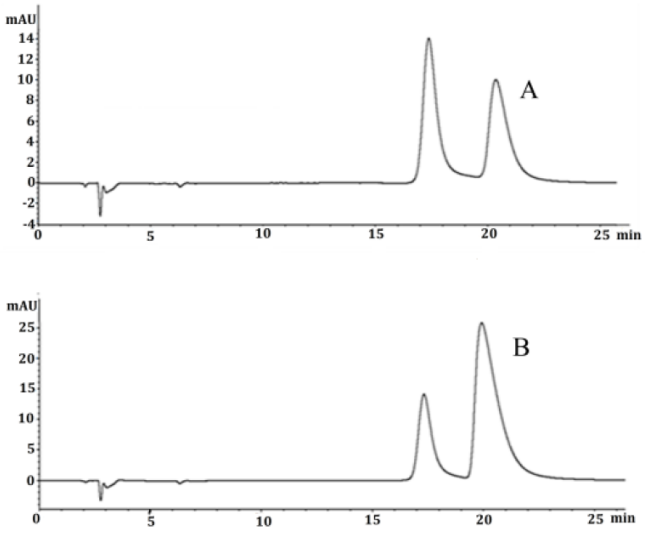

Figure 3: The chromatogram of DL-tryptophan solution (0.05 g/L, **A**) and the mixedsolution (**B**) containing DL-tryptophan (0.05g/L) and L-tryptophan (0.05g/L).

3.6. Membrane Separation Performance Analysis

Figure **4** and Table **1** shows the chromatogram of each collected filtrate and the enantiomeric excess with the gradually increasing number of filter. It can be seen that the concentration of L-tryptophan decreased gradually while the concentration of D-tryptophan basically unchanged with the increasing number of the filtration. D, L-tryptophan achieved a complete separation after 14 times filtration. This phenomenon indicates that β-cyclodextrin and L-tryptophan can

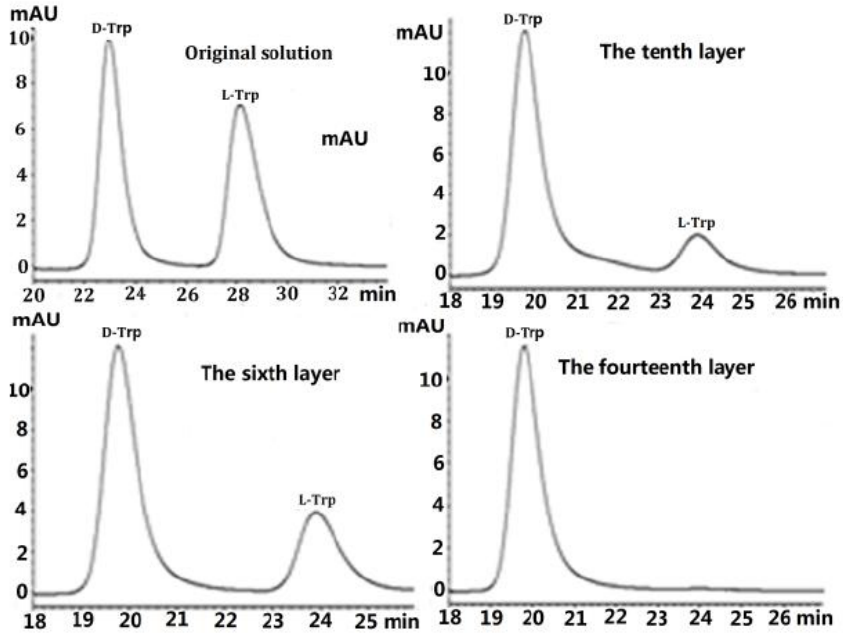

Figure 4: The chromatogram of each collected filtrate.

Table 1: The Enantiomeric Excess with the Gradually Increasing Number of the Filter Conditions[a]

Filter layer	0	6	10	14
e.e (%)	5.44	45.6	72.3	99.1

[a]Experimental conditions: aqueous solution of racemic tryptophan: 0.025g/L; flow rate: 0.1mL/min; membrane with effective area: 36mm^2; the data of e.e (%) is average of three tests.

more easily form inclusion complexation than D-tryptophan, which showed good consistency with the previous reports [34].

3.7. Stability of the Chiral Separation Performance

The RSD of the chiral separation performance within 12h was 3.4% which exhibited good stability.

4. CONCLUSION

Experimental results show that cellulose membrane can selectively adsorbs L-enantiomer of D,L-tryptophan, and a complete separation can be obtained using the multi-stage filtration system. This may be caused by the fact that the binding constant of L-tryptophan with β-CD is higher than that of D-tryptophan. Through our results, it can be found that the proposed multi-stage filtration separation can greatly increase the resolution effect of enantiomers, providing a viable alternative for the current large-scale enantiomer separation processes. As this method is suitable for wide range of chiral selectors including cyclodextrin and its derivatives and the system is relatively simple and straightforward using available modules, it has great application prospects for many chiral substances separation. Besides, it seems that complete resolution of a racemic mixture through one step operation with a membrane will be almost impossible. Multi-stage membrane permeation process will be the prototype for future industrial application of enantioselective membranes.

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SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

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