Chiral Separation of D,L-Tyrosine Through Nitrocellulose Membrane

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ABSTRACT: Enantioselective membrane was prepared using nitrocellulose as membrane material. The flux and permselective properties of membrane using water solution of D,L-tyrosine as feed solution were studied. The top surface and cross-section morphology of the resulting membrane were examined by scanning electron microscopy. The optical resolution of over 85% enantiomeric excess was achieved when the enantioselective membrane was prepared with 25 wt % nitrocellulose and 15 wt % *N,N*-dimethylformamide in the casting solution of methanol, 10°C temperature of water bath for the gelation of the membrane, and the operating pressure and the feed concentration of the D,L-tyrosine were 6 kgf/cm^2 and 0.25 mg/mL, respectively. Since the nitrocellulose contains a large amount of chirality active carbons on the backbone structure and is possible to form helical structure, it is considered to be the reason for the enantioselectivity of the membrane. This is the first report that nitrocellulose can be used as a membrane material. This work indicates that the large-scale purification of chiral molecules from racemic mixtures will be realized by the enantioselective membrane technique in the near future and that the enantioselective nitrocellulose membrane could soon become very attractive for industrial uses. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 5187–5193, 2012

Key words: D,L-tyrosine; nitrocellulose; enantioselective membrane; chiral separation

INTRODUCTION

Chiral specificity is fundamental in pharmacology and chemical biology because stereochemistry plays a central role in controlling molecular recognition and interaction.[1] Many pharmaceutical and flavoring compounds are racemic mixtures with chiral isomers having nearly identical physical and chemical properties. Under many circumstances, only one enantiomer could meet specific needs while the other one possesses less or even negative effect. The increasing need for single enantiomers in pharmaceutical and chemical industry has stimulated a significant demand for efficient processes to resolve racemic mixtures. However, the separation of enantiomers is an arduous and challenging task because of extremely similar physiochemical properties in nature with respect to enantiomers.[2–4]

Currently, enantioseparations are typically performed by fractional crystallization, microbiological method, kinetic resolution technology, asymmetric catalysis, and chromatographic separation.[5–8] Due to incomparable preponderance over, such as low energy and time saving, set-up simplicity, large processing capacity, and the possibility to be used in continuous mode, membrane separation systems have potentials to be applied in large-scale industrious enantiomer separation processes.[9–13]

Various membrane configurations have already been proposed for separating a large number of species, including amino acids, drugs, and their derivatives.[14–20] In spite of the many kinds of membrane materials reported, unfortunately, only a few polymers can really be used as membranes for industrial-scale use. It is even more difficult to find an enantioselective membrane material which not only has good chiral resolution ability for some racemates, but also has good membrane properties. The main difficulty in developing an optical resolution membrane is that no enantioselective membrane material has been found for industrial-scale use. Up till now, only about two articles of enantioselective membrane have been published every year in various journals,[21–34] and their %e.e. and fluxes may reach to 98% and 40 mg/m^{2}h, respectively.

Historically, natural polymers, such as cellulose or starch components, were the first to be used as chromatographic chiral selectors due to their inherent chiral nature and ready availability. This led to further use of cellulose and other polysaccharides as the starting material in the preparation of selectors

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to be used in chiral stationary phases.[35] Cellulose derivatives have been widely used as preparation of polymer membrane for reverse-osmosis, nanofiltration, ultrafiltration, microfiltration, etc.[36] Nitrocellulose (CN), an excellent material to prepare various membrane,[37,38] possesses multichiral carbon atoms in its molecular structure unit and is possible to form helical structure. These characteristics could be the reason that nitrocellulose membrane could form certain chiral environment and make the membrane capable of optical resolution.[39] To the best of our knowledge, there is no example described of enantiomer separation by nitrocellulose membrane. This is a report, for the first time, that the nitrocellulose is used as an optical resolution membrane material.

EXPERIMENTAL

Materials

The nitrocellulose (esterify: 70% content, MW = 100,000) was purchased from Hengshui Eastern Chemical Industry, (China) and D,L-tyrosine was obtained from Acros (Belgium). All reagents were of analytical grade and were used without any further purification. Pure water was used as solvent of feed solution.

Membrane preparation

The CN membranes were prepared through the phase inversion technique. About 10 g of the CN was dissolved in mixed solvent of methanol (24 g) and *N*,*N*-dimethylformamide (DMF, 6 g) to obtain a nitrocellulose solution. An ultrasonic bath was applied to help the free up of the air bubbles that were entrapped in it. Under the condition of 40% humidity and 10°C temperature, the resulting homogeneous solution was cast on the surface of a glass plate using an adjustable casting knife. After evaporating the CN membrane on the glass plate for 3 min, the nascent membrane was immersed into a water coagulation bath at 10°C at least 30 min. The membrane was washed in pure water at 10°C for 24 h to remove the DMF and acetone. Finally, the membrane was prepared and stored in pure water until use.

Characterization of membranes

Scanning electron microscopy (XL30ES-EM-TMP, Holland) was used to characterize the morphologies and the structures of CN membranes. The wet samples of CN membrane prepared according to the experimental method were first immersed in propanol and subsequently treated with heptane to retain their original structures, and then snapped in liquid nitrogen to give a generally clear break of the cross-section for the cross-section scan. Before scanning analysis, the surface and cross-section of resulting membrane were coated with gold.[40]

A FTIR spectroscopy (Bruker Tensor 27, Germany) was used to characterize the membranes.

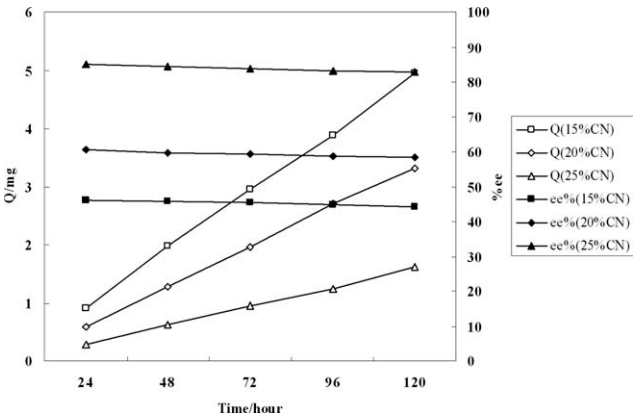

Figure 1 Quantity of the solute permeated (*Q*) and percentage enantiomeric excess (%e.e.) in enantioseparation of D,L-tyrosine through CN membranes fabricated with 15, 20, and 25 wt % CN and 15 wt % DMF. The operating pressure and the feed concentration of the D,L-tyrosine were 6 kgf/cm^2 and 0.25 mg/mL, respectively.

Preparation of the feed solution

Dissolved 25 mg of the D,L-tyrosine in 100 mL pure water, and stored at 10°C until use.

Permeation experiment

The permeation experiments could be conducted using a membrane cell whose effective membrane area is 7.0 cm^2 and volume is 100 mL.[23] The membrane was fixed tightly in the cell and 20 mL of 0.25 mg/mL of feed solution was added to the membrane cell. For the permeation test, a constant pressure could be applied to the membrane cell using nitrogen gas input through a knob located on the top of the cell. The rate of feed solution addition was controlled by adjusting the regulator attached to the gas container, confirmed by the pressure gauge of the cell. The optimal operating pressure was 6 kgf/cm^2. All the experiments were carried out at room temperature. Membranes were used only once.

HPLC analysis

The HPLC system was equipped with a Waters 514 liquid delivery pump, UV–vis detector (USA, Waters 7419). The chiral analysis was performed using a chiral column CHIRALPAK CR (4.6 mm i.d. × 250 mm, Daicel, Japan) and pure water was used as mobile phase at 30°C. The detection was examined at 254 nm, and the flow rate of the mobile phase was

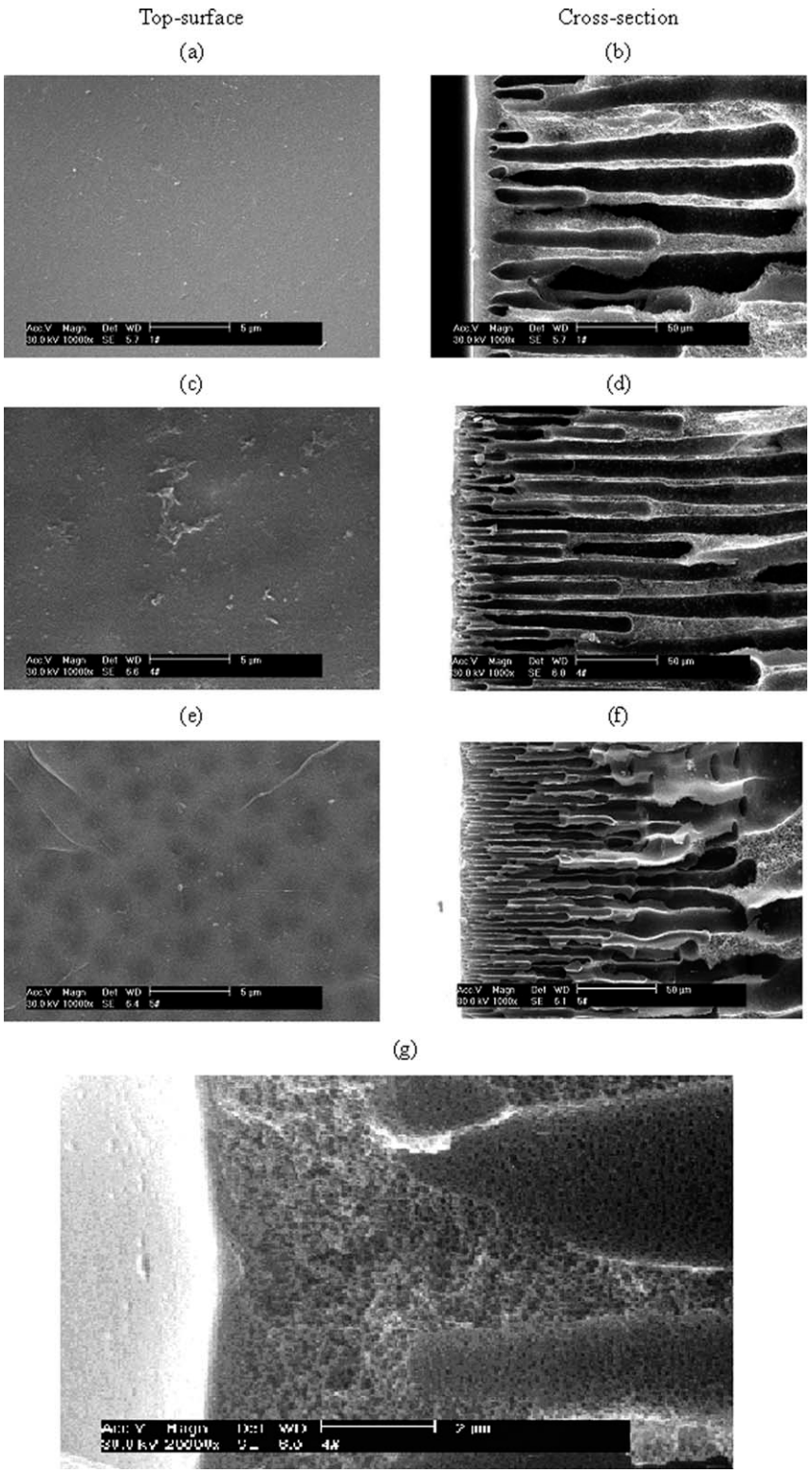

Figure 2 Scanning electron microscopy images of membrane prepared with 25 wt % (a, b), 20 wt % (c, d, g), 15 wt % (e, f) CN, and 15 wt % DMF, respectively.

0.4 mL/min. Each sample was introduced by injector with 20 μl. A personal computer equipped with a N2000 Workstation for the LC system was used to process the chromatographic data.

Membrane performance definitions

Membrane performance was evaluated by the flux and the percentage enantiomeric excess (%e.e.). Their equations are as follows:

$$\text{Flux } (\text{mg/m}^2 \cdot \text{h}) = \frac{Q}{At}$$

$$\text{e.e. } (\%) = \frac{A_D - A_L}{A_D + A_L} \times 100$$

where Q is the mass of the solute permeated for a given time, A the effective membrane area, and t the permeation time. A_D and A_L are the peak area of D- or L-isomer in the permeation, respectively.

RESULTS AND DISCUSSION

Effect of CN concentration on the properties of membrane

It is known that the polymer concentration in the casting solution strongly influences the structure of the membrane. With 15, 20, and 25 wt % CN casting solution, respectively, three CN membranes were prepared by same preparation method. Figure 1 shows quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in enantioseparation of D,L-tyrosine through those CN membranes. When the CN concentration increased from 15 to 25 wt %, the flux through the membrane decreased. However, a high enantioselectivity was obtained for 25 wt % CN membrane. The reason is the tighter membrane structure for higher CN concentration.

Controlling the thickness of the membrane from 0.3 to 0.5 mm during the membrane preparation, the %e.e. did not change obviously, but the Q decreased from 0.3 to 0.05 mg, and the intensity of thin membrane decreased.

Figure 2(a–f) shows the SEM images of the top surface and cross-section morphological structure of CN membrane prepared with 15, 20, and 25 wt % CN and 15 wt % DMF, respectively. An additional high magnification (20,000×) of the cross-section of the top layer also shows the separation layer in more detail (Fig. 2g). When the CN concentration increased in the casting solution, the top surface morphologies of the membranes were smoother, and the amounts of macrovoids of the cross-section of membranes decreased. During the phase separation process, one-phase casting solution was converted into two-phase system consisting of a solid phase (CN-rich) that formed the membrane structure and a liquid phase (CN-poor) that formed the pores in the final membrane. Therefore, the higher the CN concentrations increased, the more compact the top surfaces of membrane changed, and the fewer the pores of cross-section possessed.

The IR spectra of CN membrane were measured and its characteristic peaks were as follows: 3444.60 cm^{-1} (O–H), 2918.12 cm^{-1} (C–H), 1651.15 cm^{-1} (O–N–O).

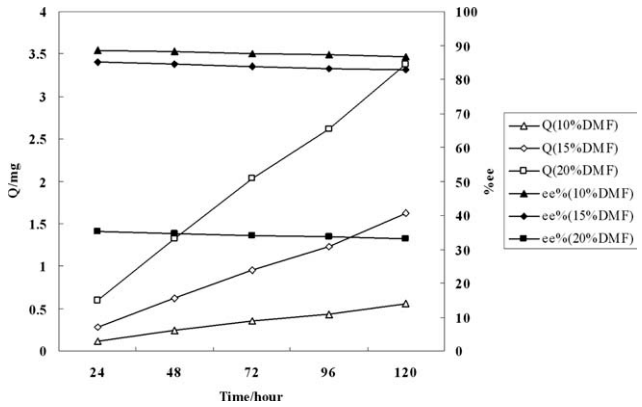

Figure 3 Quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in enantioseparation of D,L-tyrosine through CN membranes fabricated with 25 wt % CN and10, 15, and 20 wt % DMF. The operating pressure and the feed concentration of the D,L-tyrosine were 6 kgf/cm^2 and 0.25 mg/mL, respectively.

Effect of DMF content on the properties of membrane

To control the membrane structure, a low molecular weight component is frequently used as the additive in the membrane-forming system because it offers a convenient and effective way to develop a membrane with high performance. In this study, DMF was used as the non-solvent additive to modify membrane structures and properties. With an increase of DMF content in the casting solution, an increase in flux could be seen from Figure 3. The membrane of this case (15 wt % DMF) exhibited good enantioselective ability and moderate flux.

Figure 4 gives the membrane morphology of the top surface and the cross-section of CN membrane prepared with 10 and 20 wt % DMF, respectively. Together with Fig. 1(a,b), it could be seen that, there was coarse top surface and macrovoids of the cross-section of membrane for 10 wt % DMF. Increasing the content of DMF in the casting solution, the top active skin layers changed smooth and the macrovoids structure of CN membrane at the cross-section decreased. The possible reason might be that there was a bigger solubility of CN in DMF than that of in methanol.

Effect of evaporation time on the properties of membrane

Figure 5 demonstrates that the evaporation time of the liquid membrane on the surface of a glass plate could change the properties of the membranes prepared by phase inversion technology. Increasing the evaporation time from 2 to 4 min, caused the flux to increase also, but the best %e.e. in the optical resolution of D,L-tyrosine was obtained at 3 min. This behavior could be explained by the vaporization of

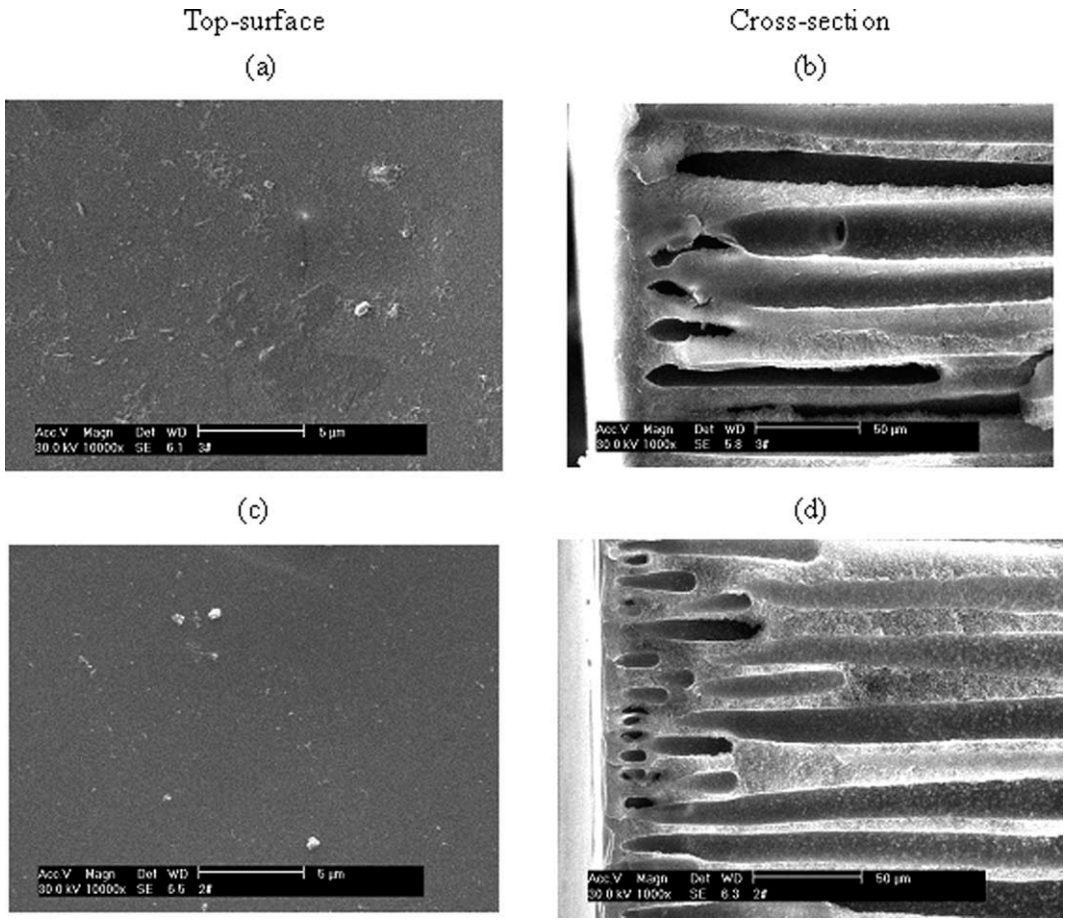

Figure 4 Scanning electron microscopy images of membrane prepared with 10 wt % (a, b), 20 wt % (c, d) DMF and 25 wt % CN, respectively.

the solvent of the liquid membrane, resulting in a "skin" layer at the surface. In general, the increase in evaporation time leads to the increase in thickness of a skin layer, which results in the decrease of transport rate. However, if the evaporation time is too long, the volatilization of solvent inside the nascent membrane may continues to pass through original surface pores so that these pores are expanded to give the opposite results.

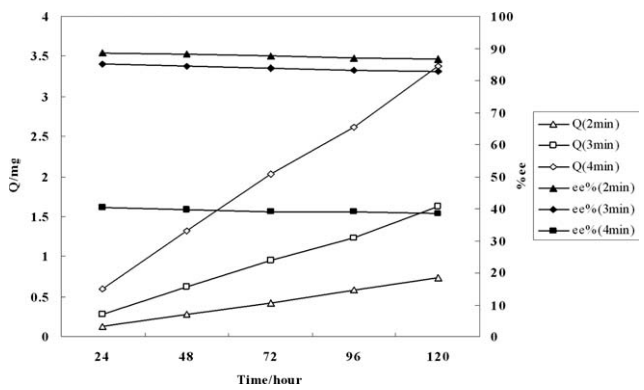

Figure 5 Quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in enantioseparation of D,L-tyrosine through CN membrane prepared with 25 wt % CN, 15 wt % DMF and different evaporation times. The operating pressure and the feed concentration of the L-Tyrosine were 6 kgf/cm^2 and 0.25 mg/mL, respectively.

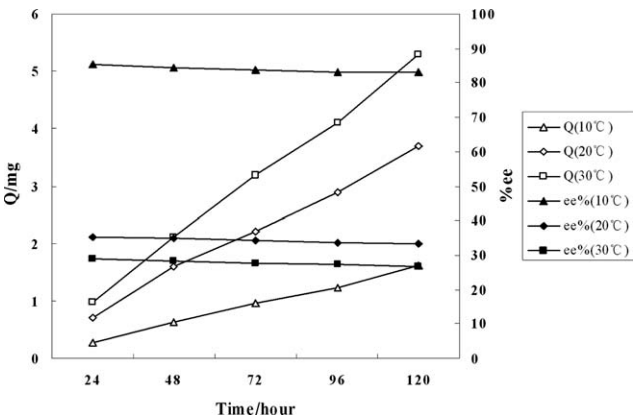

Figure 6 Quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in the enantioseparation of D,L-tyrosine through CN membrane prepared using 25 wt % CN, 15 wt % DMF and 10, 20 and 30°C water coagulation baths. The operating pressure and the feed concentration of the D,L-tyrosine were 6 kgf/cm^2 and 0.25 mg/mL, respectively.

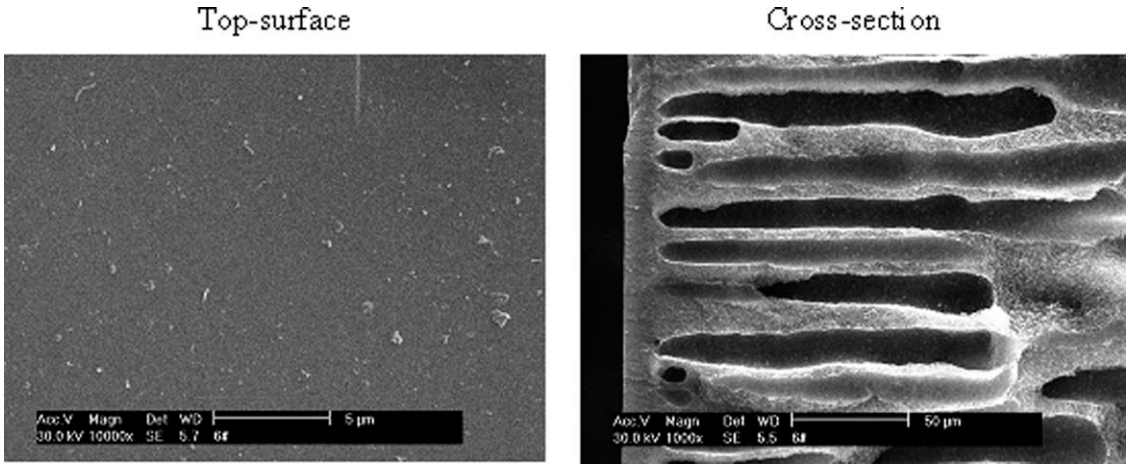

Figure 7 Scanning electron microscopy images of membrane prepared at 20°C with 25 wt % CN and 15 wt % DMF.

Effect of the coagulation bath temperature on the properties of membrane

Since the membranes were prepared by the phase inversion method, the temperature of the water coagulation bath influenced the properties of the membranes. Increasing the temperature of the water coagulation bath from 10 to 30°C, it resulted in an increase of the flux, but a decrease of the percentage enantiomeric excess (Fig. 6), because the solvent of membrane casting solution was faster diffused into the water with higher temperature of water coagulation bath. Thus, the membrane prepared under this condition was porous, which resulted in high flux and low selectivity.

Figure 7 gives the scanning electron microscopy images of membrane prepared at 20°C with 25 wt % CN and 15 wt % DMF. The membrane possessed more coarse surface and similar cross-section comparing with Fig. 1(a,b). Therefore, 10°C temperature of water coagulation bath resulted in a high enantioselectivity.

Effect of operating pressure on properties of membrane

Under different operation pressures, the Q and %e.e. through CN membrane were measured (Fig. 8). It could be seen that there was an increase in flux and a decrease in percentage enantiomeric excess while operating pressure increase from 5 to 7 kgf/cm^2. This behavior came from the interaction between membrane and solutes. With an increase of operating pressure, the movement of solution accelerated, leading to decreasing diffusion selectivity and sorption selectivity. The high enantioselectivity and moderate flux were able to be obtained for the 6 kgf/cm^2 of operating pressure in this study.

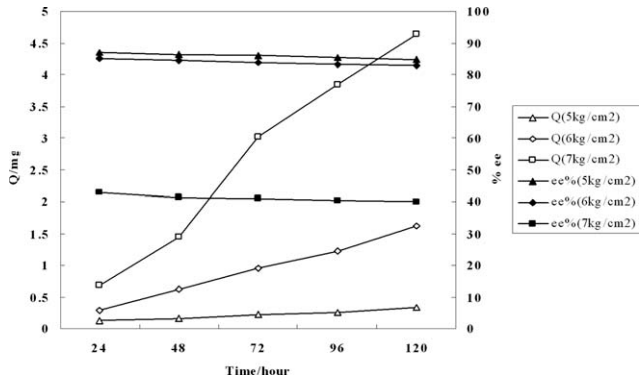

Figure 8 Quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in the chiral separation of D,L-tyrosine through CN membrane at 5, 6 and 7 kgf/cm^2 of operating pressure. The membrane was prepared with 25 wt % CN and 15 wt % DMF. The feed concentration of D,L-tyrosine was 0.25 mg/mL.

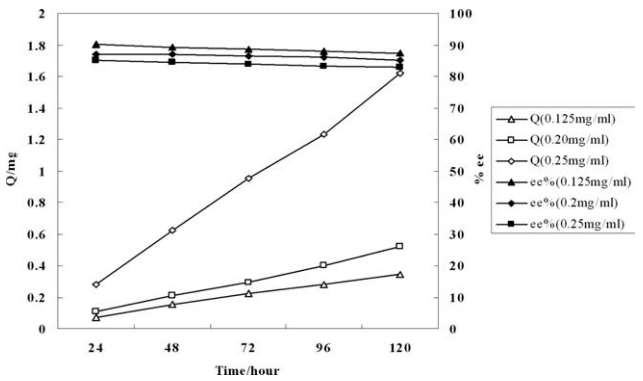

Figure 9 Quantity of the solute permeated (Q) and percentage enantiomeric excess (%e.e.) in the chiral separation of D,L-tyrosine through CN membrane prepared with 25 wt % CN and 15 wt % DMF. The concentrations of feed solution were 0.125, 0.20, and 0.25 mg/mL. The operating pressure was 6 kgf/cm^2.

Effect of feed concentration of racemate on properties of membrane

Increasing the feed concentration, too much of both the kinds of isomers (D and L) were absorbed into the membrane, the enantioselectivity of membrane would be decreased when the active sites of membranes were limited. Figure 9 shows the effect of feed concentration on the flux and enantiomeric excess. When the feed concentration increased from 0.125 to 0.25 mg/mL, the amount of the D,L-tyrosine penetrated through CN membrane increased dramatically, while decreasing the enantioselectivity of the membranes. For the 0.25 mg/mL of feed concentration, the high enantioselectivity and moderate flux were obtained.

CONCLUSIONS

Chiral separation of D,L-tyrosine is possible through CN membrane by a pressure driven process. The properties of the membrane can be influenced by changing the CN and DMF concentration in the casting solution, evaporation time of the liquid membrane, coagulation bath temperature, operating pressure, and feed concentration, respectively. The chiral recognition is the results of steric fit, dispersion, dipole–dipole, and hydrogen–bond interactions between the enantiomers and the gyroidal glucopyranose units of the membrane.

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