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Enantioselective Membranes Based on Chitosan for The Separation of D- And L-Tryptophan

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Abstract: Chitosan membranes crosslinked with glutaraldehayde that contained chiral environment were prepared. The chitosan membranes were characterized using FTIR and swelling index measurements. Their swelling index in water ranged from 100 to 70%, depending on the crosslinking time. The separation of D- and L-isomers of tryptophan was achieved through a pressure driven membrane separation process, using the self-supporting crosslinked chitosan membranes. The chiral separation performance of the membranes depended strongly on the swelling index of the membranes and the separation conditions such as concentration of feed solutions and different operating pressures. Especially when a chitosan membrane with a swelling index of 70% was used, almost complete optical resolution of D- and L-tryptophan was obtained ; enantiomeric excess (ee %) of 97.92% and flux of 2.26 $g/m^2 \cdot h$.

Keywords: *Chitosan membrane, Chiral separation, Optical isomer, Crosslinking, Pressure driven process*

1. Introduction

Chiral separation using enantioselective membranes has recently been attracting considerable attention, with increasing importance of the chiral compounds in the drug industries. Using single enantiomers makes almost half of the commercialized drug products worldwide. The several kinds of difficulties located in front of the conventional methods for the separation of optical isomers such as preferential crystallization, chemical modification by an optical resolution agent and high-performance liquid chromatography (HPLC) with a chiral stationary phase are another reason for strong consideration of the membrane process as an alternative process for the separation of chiral compounds.

There have been several reports on the optical resolutions by polymeric enantioselctive membranes by aoki. Et al. and other groups[1-7]. They used the enantioselective membranes made from modified L-glutamate containing chiral carbons in its main chain or the membranes prepared by blending conventional achiral polymers such as poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) with chiral polymers for the separation of optical isomers. Some of the membranes they used showed a good separation performance, but the formation of the membranes was not easy enough for the membranes to be commercialized.

Based on these backgrounds for the development of enantioselective membranes, chitosan membranes that can be prepared easily by using commercially available chitosan that contains five chiral carbons in its repeating unit, and that is possible to form chiral conditions in its membranes were prepared to use for the optical resolution. Since the optical isomers are only distinguishable under chiral condition, the membranes that will be used should have chiral spaces in the membrane, through which the optical isomers being penetrated can interact with the membrane. The regios-

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pecific space formed in the membranes surrounded by the chiral moieties of the membrane material can interact with permeants with different degree of interaction and results in the different diffusion rates of the different optical isomers. Such different diffusion rate between the optical isomers is one of the separation mechanisms of the optical isomers through the polymeric membranes.

In this paper, D- and L-tryptophan were used as optical isomers to be separated. They were separated under different operating conditions. The details of the membrane preparation and the separation of the optical isomers are elaborated in the following sections.

2. Experimental

2.1. Materials

Chitosan was purchased from Sigma Aldrich and used as a membrane material. D- and L-tryptophan (Trp) purchased from Sigma Aldrich were used as optical isomers to be separated without further purification. Glutaraldehyde (GA) (25 wt% content in water), hydrochloric acid (35.5 wt% content), acetic acid and acetone were purchased from Junsei Chemical (Tokyo, Japan) and used for the crosslinking of the membranes. Ultra pure deionized water was used as a solvent of feed solutions. All other chemicals were used without any further purification.

2.2. Membrane Preparation

Chitosan solution of 5 wt% in water was prepared by dissolving 5 g of chitosan into 1 kg of water containing 5 wt% acetic acid at 50°C. The chitosan solution was cast onto a polyester film attached to a glass plate, using a Gardner casting knife, and dried at room temperature in a fume hood for 4 days. After which, the chitosan films were peeled off the polyester film and immersed into an acetone solution containing GA of 5.0 wt% and HCl of 1.0 wt% for their crosslinking. The degree of crosslinking of the chitosan films was controlled adjusting the immersion time in the acetone solution from 6 to 48 hrs. The chitosan films crosslinked so were taken out of the solution and washed out several times with an excess amount of deionized water at 50°C for 24 h, and then dried under vaccum for 24 h. The chitosan films prepared were then used as membranes for the separation of the optical isomers. The thickness of the membranes was in the range of 50 to 60 μm.

2.3. Characterization of the Membranes

The swelling index (SI) of the membranes in water was measured, using the following equation:

$$SI = 100 \times (Ws - Wd)/Wd$$

Where Ws and Wd are the weights of the films of swollen and dried, respectively.

The chemical structure change of the chitosan membrane by the crosslinking reaction was studied with FT-IR spectrophotometery (Bio-Rad, Digibal Division, model FTS-80, FT-IR). Sample thickness was about 50 μm.

2.4. Permeation Tests

A test cell used was a home made one. The gas pressure applied to the cell controlled the operating pressure. The operating pressure was 1 and 2 bar, the operating temperature was 25°C and the concentrations of the feed solutions were 0.49 to 4.9 mmol/L.

Before loading into the cell, the chitosan membranes were fully swollen in distilled water for more than 24 hours. Feed solution (about 500 mL) large enough not to make serious concentration difference during the separation process was used. Also rotating a magnetic stirrer located on the thin porous plate above the membrane surface minimized concentration polarization that was expected to happen during the separation process.

Flux was determined by calculating the weight of the optical isomers penetrated through the membrane:

$$Flux = Q/(At)$$

Where Q is the quantity of the solute permeated, t is the permeation time, and A is the surface area of the membrane. The composition (contents of D- and L-isomers) of the feed and permeates was measured by means of a liquid chromatography equipped with a Chiralpak-WH column (Diacel Chemical Industries, Ltd.,

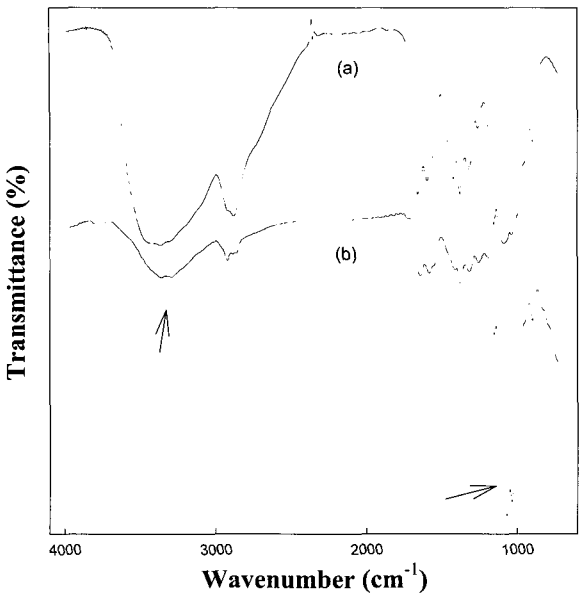

Fig. 1. FTIR spectra of the chitosan membranes (a) before and (b) after crosslinking with GA for 48 hrs at room temperature.

Japan) as an optical resolution column, and a UV spectrophotometer (254nm) as a detector. The enantiomeric excess (ee) of permeates was determined from the peak areas of their two enantiomers, D- (A_D) and L-isomers (A_L).

$$\% \text{ ee} = 100 \times (A_D - A_L)/(A_D + A_L)$$

3. Results and Discussion

3.1. Preparation of Membranes

Enantioselective chitosan membranes were prepared by crosslinking the chitosan films with glutaraldehyde (GA) as explained in the Experimental. Two aldehyde groups of a GA that was used as a crosslinking agent reacted with the hydroxyl groups of the chitosan molecules to form acetal linkages, resulting in the formation of the crosslinked chitosan networks in the film. The chemical structure change of the chitosan by the crosslinking reaction was studied with FTIR spectroscopy. Figure 1 shows the FTIR spectra of the chitosan membrane before and after the crosslinking reaction.

Both spectra show the characteristic peaks of the chitosan structure, peaks around 1651 (Amide I), 1587 (Amide II), 1380 (-CH_2 bending), and 1160 cm^{-1} (anti-symmetric stretching of the C-O-C bridge), 1075 and 1040 (skeletal vibration involving the C-O stretching). The spectrum (b) of the chitosan membrane having a swelling index of 70% exhibits the clear difference from that (a) of the uncrosslinked chitosan membrane. By the reaction with GA, the strength of the OH groups of the chitosan ranged from about 3,000 to over 3,500 cm^{-1} became much weaker, with the conversion of OH groups into acetal linkages. More reaction with GA, more OH groups transformed into C-O-C- (acetal linkage) groups, increasing the degree of crosslinking of the chitosan membranes. The peak at 1320 cm^{-1} (C-O stretching) became even stronger and sharper by the crosslinking with GA, confirming the formation of the acetal linkage.

3.2. Swelling Index Measurements

The free volume of the membrane that is fully swollen by the feed solution during the separation process is thought to be important for the separation of optical isomers. When considering the difference between the diffusion rates of D- and L-isomers of tryptophan through the membrane as the major mechanism for their optical resolution, the interaction occurring between the permeate being penetrated through the free volume of the membrane and the functional groups forming the chiral environment in the membrane is one of the most important factors contributing to the separation of optical isomers by a membrane. The degree of interaction between permeate and membrane should be very much dependent on the swelling index of the membrane used.

Table 1 shows the swelling indices of chitosan membranes crosslinked with GA. With increasing reaction time in the crosslinking solution, the swelling index of the chitosan membranes decreased. When the reaction time was 48 hours, the swelling index of the membrane obtained became 70%.

Table 1. Swelling indices of the chitosan membranes used

	Crosslinking time (hr.)	Swelling Index
Chitosan-1	6	100
Chitosan-2	12	90
Chitosan-3	24	80
Chitosan-4	48	70

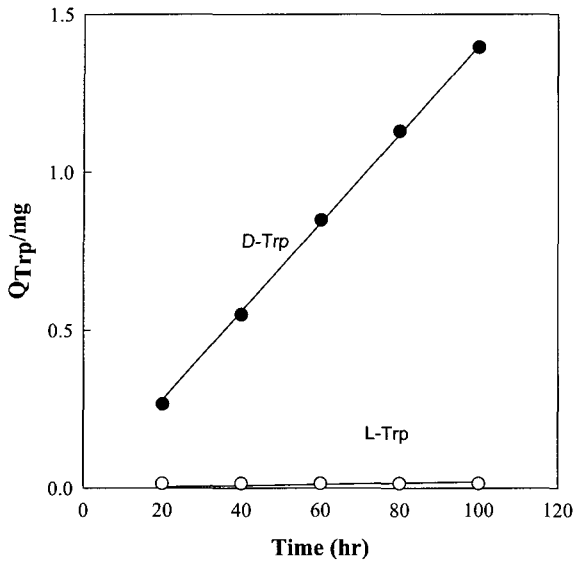

Fig. 2. The accumulated amount of the D- and L-tryptophan penetrated through the chitosan membrane with a swelling index of 70% as a function of operating time. The concentration of the feed solution and operating pressure were 0.49 mmol/L and 1 kgf/cm^2, respectively.

3.3. Optical Resolution

The chitosan membrane separated the D- and L-isomers of tryptophan with pressure difference as a driving force under different separation conditions. Figure 2 shows the performance of the chitosan membrane with swelling index of 70% for the separation of the D- and L-tryptophan. As one can see, the permeation rate of the D-tryptophan was much faster than that of the L-isomer. Up to 100 hrs of operation, the behavior of the optical resolution has not been changed, the slope of the flux of the D- and L-tryptophan as a function of operating time was linear. The % enantiomeric excess of the permeate (% ee) was about 97%. This result strongly suggests that the chitosan membrane is possible to separate the optical isomers of the a-amino acids including tryptophan, tyrosine and others.

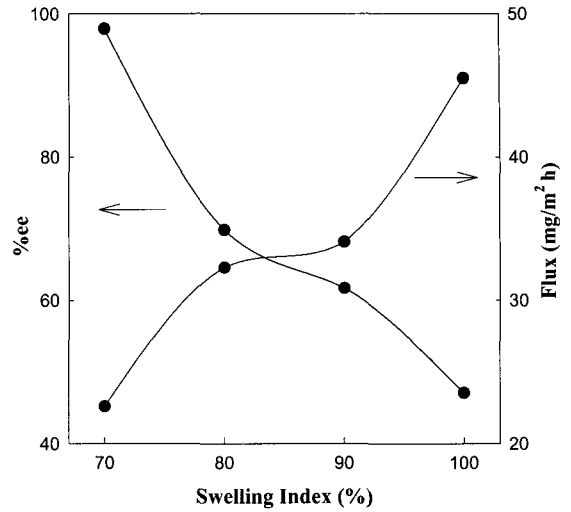

Fig. 3. Behavior of enantiomeric excess (%) of permeates and flux for the separation of D- and L-tryptophan as a function of the swelling indices of the chitosan membranes. Concentration of the feed solution and operating pressure were 0.49 mmol/L and 1 kgf/cm^2.

From this result, it is possible to speculate that the interaction between the L-tryptophan and the membrane is much higher than that of the D-tryptophan with the membrane. In other words, D-tryptophan that does not well fit to the regeospecific chiral spaces formed in the membrane by the chiral carbon of the chitosan molecules penetrates more easily, while the L-tryptophane is hindered in its proceed, interacting more, by the well fitting chiral spaces of the membrane.

Effect of degree of crosslinking : Figure 3 shows the enantiomeric excess (%ee) of the permeate and the flux for the optical resolution of D- and L-tryptophan as a function of the swelling index of the membranes. For this study, four chitosan membranes with different swelling indices such as 70, 80, 90 and 100% were used. With increasing swelling index of the membrane, in other words decreasing degree of crosslinking and increasing free volume of the membrane, the % ee of the permeate decreased and flux increased. The % ee of the permeate obtained by using the membrane with swelling index of 70% was almost 97%, while that was only about 46% when the membrane with swelling index of 100% was used. Of course, the flux

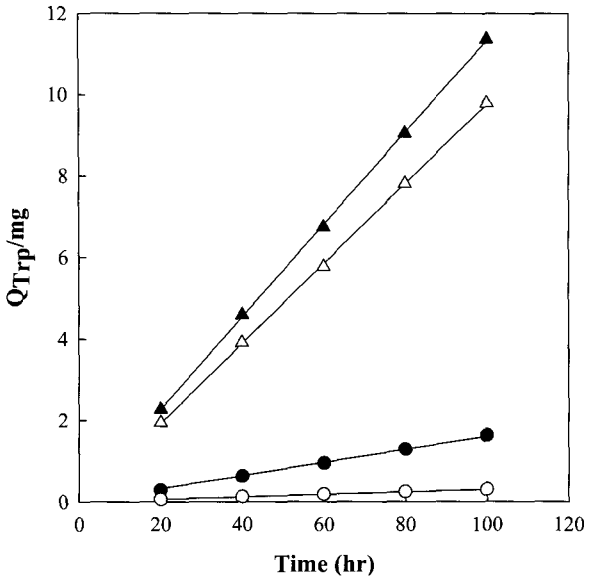

Fig. 4. Effect of the concentration of the feed solutions on the characteristics of the optical resolution of tryptophan racemates, using a chitosan membrane with 80% of swelling index: (●,○) Concentration of feed solution = 0.49 mmol/L, (△,▲) Concentration of feed solution = 4.9 mmol/L; (●,▲) D-tryptophan, (○,△) L-tryptophan.

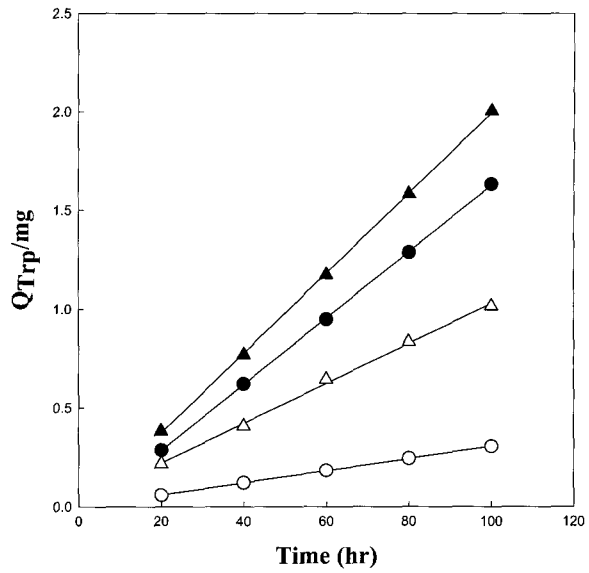

Figure 5. Effect of operating pressure on the characteristics of the optical resolution of tryptophan racemates, using a chitosan membrane with 80% of swelling index: (●,○) Pressure = 1 kgf, (△,▲) Pressure = 2 kgf ; (●, ▲) D-tryptophan, (○,△) L-tryptophan.

behaved exactly the opposite way. With increasing swelling index from 70 to 100%, it increased almost two times from 23 to 46 mg/m^{2}h.

This result confirms that the extent of interaction between permeates and membrane is the key factor for the efficiency for the separation of optical isomers by a membrane. As mentioned above, the interaction between the D- and L-tryptophan molecules being penetrated through the chiral spaces (free volume) located in the membrane and the functional groups of the membrane material, chitosan, such as hydroxyl and amine groups is an acting factors for the optical resolution.

Effect of the concentration of feed solutions : Figure 4 shows the effect of the concentration of tryptophan racemate on the separation of its optical isomers. For this study, the chitosan membrane with a swelling index of 80% was used at room temperature. As one can see, with 10 times increase in the concentration of the feed solution, the amount of the tryptophan penetrated through the membranes increased drastically, decreasing the enantioselectivity of the membrane.

From this result, it can be suggested that by the increase in the concentration of the feed solution, too much of both the isomers were absorbed into the membrane. The L-isomers absorbed fast, interacting tightly with the membrane interfered the diffusion of the D-isomers, while they were being pushed by the D-isomers. In consequence, with increasing concentration of the D- and L-isomers in the membrane, the difference between the degrees of interaction between D-and L-isomers with membrane became less in the membrane, resulting in the less difference in their diffusion rates. Therefore, the amount of both D- and L-isomers penetrated through the membrane per unit time increased, but the enantioselectivity became low.

Effect of operating pressures : Figure 5 shows the effect of operating pressure on the optical resolution of tryptophan racemates by the same way used for the effect of the concentration. With increasing pressure, the amount of the penetrated tryptophan increased. Especially, the L-isomer increased more in its amount, decreasing the enantioselectivity of the membrane.

This kind of result is one of the phenomena most commonly encountered in the membrane separation process. The solution diffusion model has usually determined the selectivity of a dense membrane by both sorption and diffusion selectivity. The diffusion selectivity usually decreases with increasing driving force for the movement of solutes, eventually decreasing over all permselectivity. This kind of effect occurred in this study too, and with increasing pressure (driving force), the over all enantioselectivity decreased with large improvement in the permeation of L-isomer.

4. Conclusions

Chitosan membranes crosslinked with GA are possible for the optical resolution of D- and L-tryptophan by a pressure driven process. The presence of five chiral carbons in the ring structured main chain of chitosan seems to make helical molecular structure and chiral environments in its membrane as cellulose and its derivatives do, making the membrane enantioselective. With increasing degree of crosslinking, the enantioselectivity of the membrane increases by increasing the interaction between permeates and membrane. On the other hands, the conditions that create less interaction between permeates and membrane such as increases in the concentration of feed and operating pressure are unfavorable for the high enantioselectivitiy.

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