Enantioselective Permeation of α-Amino Acid Optical Isomers through Crosslinked Sodium Alginate Membranes

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ABSTRACT: A pressure-driven separation process, using self-supporting crosslinked sodium alginate (SA) membranes with different swelling indices, enabled the optical resolution of α-amino acids, including the optical isomers of tryptophan and tyrosine. The SA membranes were prepared by the casting and drying of SA solutions on an acryl plate, followed by crosslinking with glutaraldehyde. They were characterized with analytical methods, such as Fourier transform infrared and swelling index measurements. During optical resolution with the SA membranes, the effects of experimental factors were studied, such as the concentration of the feed solutions, the operating pressure, and the degree of crosslinking of the membranes. When an SA membrane with a swelling index of 80% was used, a good optical resolution of tryptophan isomers was obtained: an enantiomeric excess of 54.0% and a flux of 24.8 mg/m^2 h. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 3046–3051, 2003

Key words: •••

INTRODUCTION

For optical resolution, many conventional methods, such as preferential crystallization, chemical modification by an optical resolution agent, and high-performance liquid chromatography (HPLC) with a chiral stationary phase, have been developed for a long time. However, each of these conventional methods has been known to have some limitations in its applications. For instance, the HPLC method is very good for resolution but difficult to apply for large-scale separation. Therefore, many people have sought a resolution method useful for the production of large amounts of single enantiomers. The membrane separation process, having several advantages over conventional methods, such as ease of handling, instrumental simplicity, and energy efficiency, has naturally been considered as an alternative method for optical resolution. The membrane process has been attracting considerable attention for the production of food, pharmaceuticals, and agricultural products.

There are several separation methods using membranes, and some have been commercialized. A typical example is a membrane reactor with an enzyme (multiphase) used for the production of diltiazem, which was developed by Lopez and Matson.[1] There have also been several reports on optical resolution through solid enantioselective membranes by Aoki et al. and other groups.[2–10] However, the membrane process for optical resolution is in the beginning stages of development and has to be improved much more if membranes are to be used for practical applications. In particularly, for the development of enantioselective membranes capable of separating optical isomers with high separation factors, considerable efforts are needed.

In this study, we selected sodium alginate (SA), one of the polysaccharide containing anionic groups, to prepare enantioselective membranes for the separation of α-amino acids, such as tryptophan and tyrosine, because of its excellent hydrophilicity and large content of chiral centers. These two qualities seemed to be necessary for the membranes to have proper membrane properties for the separation of water-soluble α-amino acid isomers such as tryptophan and tyrosine. Because of its excellent hydrophilicity, SA has often been used for the formation of hydrophilic membranes for the separation of water/alcohol and methyl tert-butyl ether (MTBE)/methanol mixtures, and it has shown good separation performances.[11–13] The large content of chiral centers is an important factor for the formation of a chiral environment in the membrane, which is critical for the separation of optical isomers. Without a chiral environment, it is almost impossible for a membrane to have enantioselectivity.

On this basis, SA membranes crosslinked with glutaraldehyde (GA) were prepared and used for the optical resolution of tryptophan and tyrosine; the de-

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gree of crosslinking of the membranes, the operating pressures, and the feed concentrations were controlled. Tryptophan and tyrosine, used in this study as optical isomers, are typical and common of the 19 types of racemic α-amino acids, and there was no specific reason for selecting them for this study. The results of this study are elaborated in this article.

EXPERIMENTAL

Materials

SA was purchased from Showa Chemical (Tokyo, Japan) and was used for the formation of the membranes. D,L-Tryptophan (Trp) and D,L-tyrosine (Tyr), purchased from Sigma–Aldrich (Milwaukee, WI), were used for the formation of the feed solution, without further purification. GA (25 wt % in water), hydrochloric acid (35.5 wt %), and acetone were purchased from Junsei Chemical (Tokyo, Japan). Ultrapure deionized water was used as a solvent of the feed solutions. All other chemicals were used without any further purification.

Membrane preparation

A 5 wt % SA solution in water was prepared by the dissolution of 50 g of SA in 1 kg of water at 50°C. The SA solution was cast onto a polyester film attached to a glass plate, with a Gardner casting knife, and dried at room temperature in a fume hood for 4 days. Afterward, the dry SA films that formed were peeled off the polyester film and immersed in an acetone solution containing 5.0 wt % GA and 1.0 wt % HCl for the crosslinking of the SA films. For control of the degree of crosslinking of the SA films, the immersion time in the acetone solution was adjusted from 6 to 48 h. The crosslinked SA films were then taken out of the solution, washed several times with an excess amount of deionized water, and dried *in vacuo* for 24 h. The SA films so prepared were used as membranes. The thickness of the membranes was 50–60 μm.

Swelling index (SI) measurements

The SIs of the membranes were measured for the indirect comparison of the degrees of crosslinking of the membranes. The SA membranes, crosslinked for different reaction time, were fully swollen in deionized water at room temperature until there was no difference in the weights of the swollen membranes. After the water that remained on the surfaces of the membranes was removed, the membranes were weighed to determine the weight of the swollen membranes (W_s). Afterward, they were dried *in vacuo* to a constant weight to determine the weight of the dried membranes (W_d):

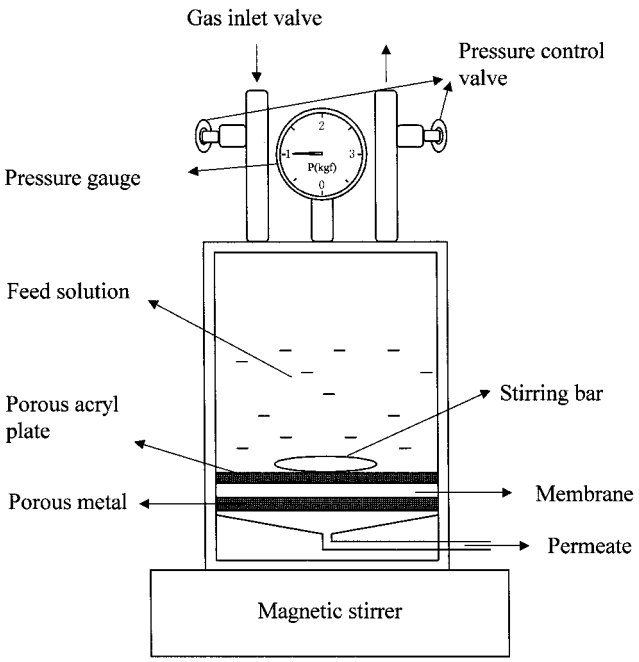

Figure 1 Schematic of the membrane cell.

$$SI = 100 \times (W_s - W_d)/W_d$$

Characterization of the membranes

The SA membranes were characterized with Fourier transform infrared (FTIR) spectrophotometry (model FTS-80, Digilab Division, Bio-Rad, Cambridge, MA) to determine their chemical structure. For the tests, SA films about 50 μm thick were used.

Permeation tests

For the permeation tests, a test cell, as shown in Figure 1, was designed and used. Constant pressure was applied through the gas inlet valve located on the top of the cell, with nitrogen gas used to apply the wanted pressure. The operating pressure was controlled by the adjustment of the regulator attached to the gas container and was confirmed by the pressure gauge located on the top of the cell.

The SA membranes were kept in distilled water for more than 24 h before being loaded into the cell. An excess amount of the feed solution (ca. 500 mL), compared with the volume of the permeates, was used to minimize the occurrence of a concentration difference in the feed solution throughout the permeation test. Also, to prevent a concentration polarization effect on the membrane surface during the operation, the feed solution was swirled by the magnetic stirrer located on the thin, porous plate above the membrane surface.

For the permeation tests, aqueous solutions of racemic tryptophan and tyrosine mixtures were used as feed solutions. Two concentrations of the feed solution

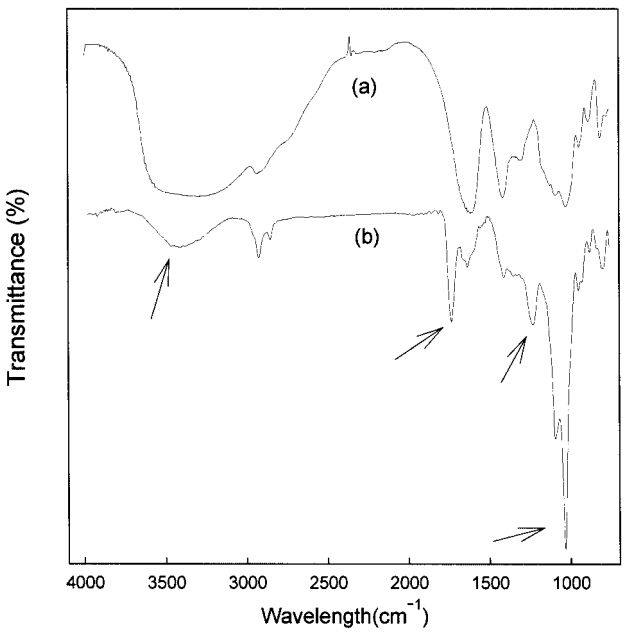

Figure 2 FTIR spectra of the SA membranes (a) before and (b) after crosslinking with GA for 48 h at room temperature.

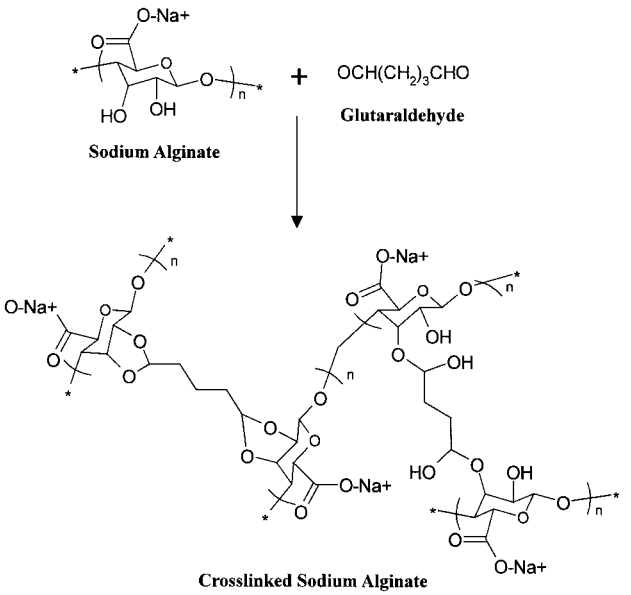

Figure 3 Schematic chemical structure of crosslinked SA.

were used (0.49 and 4.9 mmol/L). The operating pressures were 1.0 and 2.0 kgf/cm^2, and the operating temperature was 25°C. The compositions (the contents of D- and L-isomers) of the feed and permeates were measured with a liquid chromatograph equipped with a Chiralpak-WH column (Daicel Chemical Industries, Ltd., Tokyo, Japan) as an optical resolution column and a UV spectrophotometer (200 nm) as a detector. The enantiomeric excess (ee) of the permeates was determined from the peak areas of their two enantiomers, D-isomer (A_D) and L-isomer (A_L):

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

The flux was determined by the weighing of the amounts of the permeate that penetrated through the membrane per unit of time and per unit of membrane area.

RESULTS AND DISCUSSION

Characterization of the membranes

FTIR

FTIR spectra of the SA membranes before and after crosslinking with GA are shown in Figure 2. Both spectra show the characteristic peaks of a polysaccharide structure,[14] around 1320 (C—O stretching), 1130 (C—C stretching), 1090 (C—O—C stretching), and 950 cm^{-1} (C—O stretching), and the characteristic peaks of SA, COO^- asymmetric and symmetric stretching peaks at 1620 and 1416 cm^{-1}.

Through the crosslinking with GA, the strength of the —OH groups of SA became much weaker because of the crosslinking reaction between the —OH groups of SA and the aldehyde groups of GA, as shown in Figure 3. With an increasing degree of crosslinking, the —OH groups transformed into —C—O—C— (acetal linkage) groups. Figure 2(b), obtained after the crosslinking of the membrane (SI = 80), clearly shows the results of this kind of crosslinking reaction. The strong, broad peaks, ranging from around 3000 to over 3500 cm^{-1}, of Figure 2(a) became much weaker and narrower, indicating the conversion of —OH groups of SA into acetal linkages. Also, the peak at 1320 cm^{-1} (C—O stretching) became stronger and sharper in Figure 2(b), confirming the formation of the acetal linkages. Additionally, in Figure 2(b), new peaks at 1742 and 1242 cm^{-1} can be observed, due to the symmetric and asymmetric stretching of —COOH groups formed by the reaction between $—COO^-Na^+$ and H^- of the crosslinking solution used as a catalyst.

SI measurements

Table I shows the SIs of SA membranes crosslinked with GA for different reaction times in crosslinking

TABLE I
SIs of the SA Membranes Used

Membrane	Crosslinking time (h)	SI (%)
SA-1	6	110
SA-2	12	100
SA-3	24	90
SA-4	48	80

solutions. With an increasing reaction time in acetone solutions containing 5.0 wt % GA and 1.0 wt % HCl as a catalyst, the SIs of the SA membranes decreased; this reflected the increase in the degree of crosslinking. As the reaction time reached 48 h, the SI became 80%.

Permeation tests

For optical resolution with a membrane, the membrane should have a chiral environment, such as the presence of chiral recognizing sites or things like that, which can then interact with optical isomers penetrating the membrane. The chiral environment formed in the membrane can interact specifically with the penetrating optical isomers, and this makes the membrane enantioselective. The interaction between the isomers and the chiral environment will be different, depending on the characteristics of the isomers or the chiral environment of the membrane, and will cause differences in the diffusion rates of the isomers. The different diffusion rates of the isomers will be a key factor for optical resolution through membranes because the optical isomers have the same chemical properties, such as the solubility parameter and polarity, except for their optical activity.

In this study, as explained in the introduction, for the development of such a chiral environment in a membrane, SA, containing a large amount of chiral carbons on the ring backbone structure and able to form helical structures, was used for the formation of enantioselective membranes.[15] Because SA has a backbone structure very similar to that of cellulose or its derivatives, which have often been used as chiral stationary phases of HPLC chiral columns, it is expected to have helical structures like cellulose and its derivatives. The helical structure will form chirally active small spaces in its main-chain backbone structure, and its assembly will be able to form certain larger chiral spaces in the membrane and make the membrane capable of optical resolution.

Figure 4 shows the amount of permeated tryptophan as a function of the operating time when an SA membrane with an SI of 80% was used for the optical resolution of a tryptophan racemate at 25°C. The permeation rates of the two optical isomers were different, the D-isomer being faster than the L-isomer. Therefore, with increasing operating time, the ee percentages of the permeates that accumulated for the entire operating time increased continuously. The difference between the areas of the respective HPLC peaks corresponding to the D-isomer and L-isomer increased with the operating time. When the operating time reached 100 h, it was about 54%, revealing the potential of this membrane as a good enantioselective membrane.

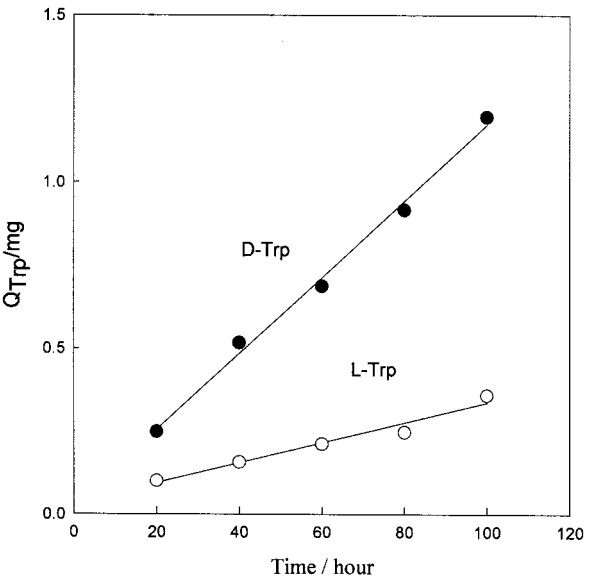

Figure 4 Characteristics of the optical resolution of tryptophan racemates with an SA membrane with an SI of 80%. The concentration of the feed solution and the operating pressure were 0.49 mmol/L and 1 kgf/cm^2, respectively.

Effect of the degree of crosslinking

The SA membranes also have excellent hydrophilicity because of the sodium carboxylate groups located on every repeating unit of SA molecules; this is considered good for the high flux of the α-amino acids from the good affinity of the membrane toward the water-soluble α-amino acid isomers. However, SA membranes need to be crosslinked for the optical resolution of the α-amino acids dissolved in water solutions, or they will be dissolved. The crosslinking reaction will also secure the chiral environment in the membrane.

Therefore, the SA membranes should be crosslinked and the degree of crosslinking of the membrane should be controlled for good enantioselectivity. The degree of swelling of the membranes will affect the characteristics of the chiral environment of the membranes. The swelling reaction will increase the intermolecular distances of the molecules and make large chiral spaces (a kind of free volume) in the membranes. The large chiral spaces of the membranes will then allow the isomers to pass through the membranes with less interaction with the chiral environment, and this will result in a low separation capability of the membranes.

Figure 5 shows the effect of the degree of crosslinking of the membranes on the optical resolution of a 0.49 mmol/L tryptophan racemate solution. With increasing SI (less crosslinking), the flux improved, but the ee percentage decreased gradually. From this result, it became evident that larger membrane swelling was unfavorable for high optical resolution. The 54% ee obtained from a membrane with an SI of 80%

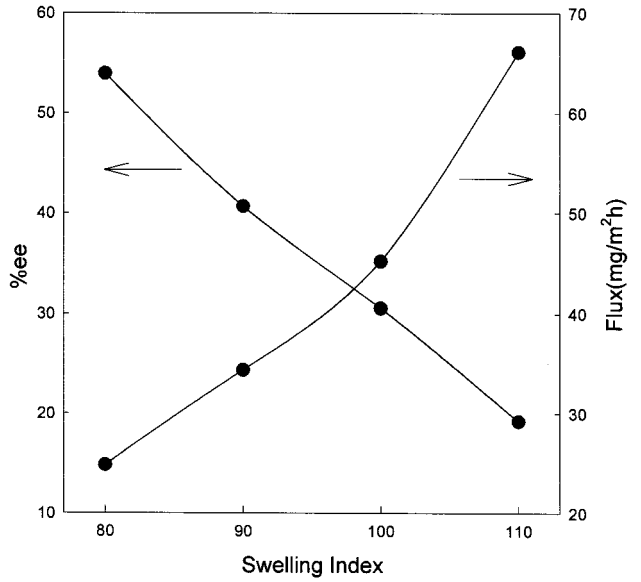

Figure 5 Characteristics of the optical resolution of tryptophan racemates as a function of the SIs of the SA membranes. The concentration of the feed solution and the operating pressure were 0.49 mmol/L and 1 kgf/cm^2, respectively.

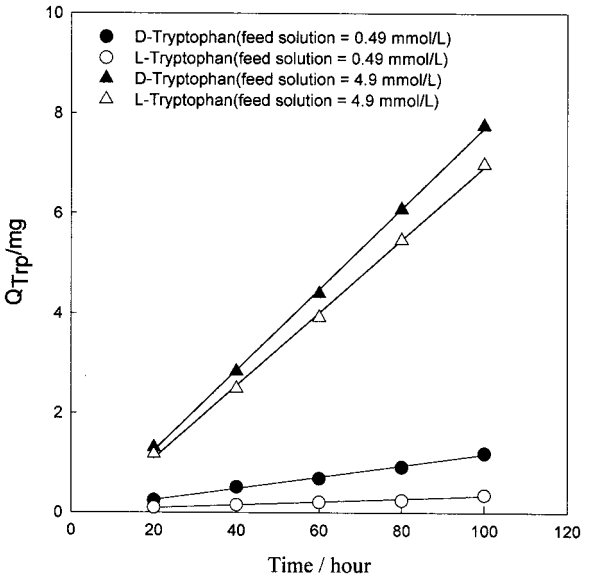

Figure 6 Effect of the concentration of the feed solutions on the characteristics of the optical resolution of tryptophan racemates with an SA membrane with an SI of 80%. The operating pressure was 1 kgf/cm^2.

became less than 20% as the SI of the membrane reached 110%. As expected, the larger chiral space formed by the high swelling in the membrane probably allowed the isomers to pass easily through the membrane with less interaction, and there was not enough difference in the diffusion rates between the optical isomers to show good separation.

Effect of the feed concentration

The feed concentration is one of the most important operating factors of a membrane separation process. Particularly for a pressure-driven process using a nonporous membrane, such as the reverse osmosis process, the feed concentration affects the solute flux according to the solution diffusion model, which uses the following equation for the flux of solutes:

$$J_A = -D_{Am}K_A\Delta c_A/\delta$$

where J_A is the solute flux, D_{Am} is the diffusion coefficient of the solute in the membrane, K_A is the distribution constant, Δc_A is the solute difference between the two sides of the membrane, and δ is the membrane thickness.

Figure 6 shows the effect of the feed concentration on the separation properties of a tryptophan racemate through an SA membrane with an SI of 80%. With a 10-fold increase in the concentration of the feed solution, the amount of tryptophan that penetrated through the membrane increased drastically, and this reduced the enantioselectivity of the membrane. From this result, it can be suggested that with the increase in the concentration of the feed solution, too much of both isomers was absorbed into the membrane, and then the absorbed L-isomers interfered with the diffusion of the D-isomers, whereas they were being pushed by the D-isomers. As a result, the amounts of both the D-isomers and L-isomers that penetrated through the membrane per unit of time increased without much of a difference between them, and this resulted in the low enantioselectivity.

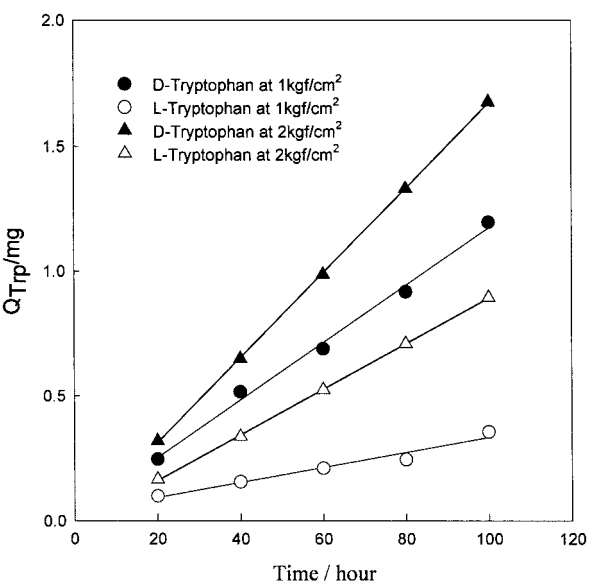

Figure 7 Effect of the operating pressure on the characteristics of the optical resolution of tryptophan racemates with an SA membrane with an SI of 80%. The concentration of the feed solution was 0.49 mmol/L.

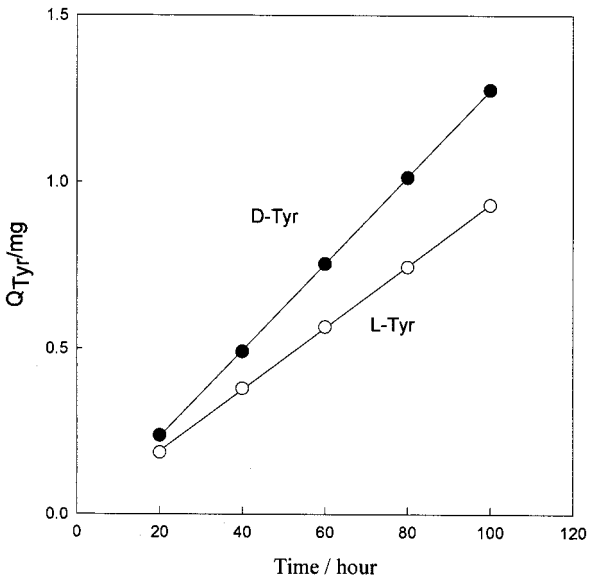

Figure 8 Characteristics of the optical resolution of tyrosine racemates with an SA membrane with an SI of 80%. The concentration of the feed solution and the operating pressure were 0.49 mmol/L and 1 kgf/cm^2, respectively.

Effect of the operating pressures

Figure 7 shows the effect of the operating pressure on the optical resolution of the tryptophan racemates in the same way used for the effect of the concentration, except for the pressure. With increasing pressure, the amount of the penetrated tryptophan increased. In particular, that of the L-isomers increased more, and this reduced 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 usually determines the selectivity of a dense membrane by both sorption selectivity and diffusion selectivity. The diffusion selectivity usually decreases with an increasing driving force for the transfer of solutes. This kind of effect also occurred in this study, and with increasing pressure (driving force), the enantioselectivity decreased with the increasing diffusivity of the L-isomers rather than that of the D-isomers.

Effect of the different feed solutions

To determine if the SA membrane could be used for the optical resolution of other α-amino acids, we used a tyrosine racemate as a feed solution. The separation conditions were the same as those of the tryptophan, with an SA membrane with an SI of 80%: 0.49 mmol/L feed solution and 1 kgf/cm^2 operating pressure.

Figure 8 shows the results of the optical resolution of the tyrosine racemate. Compared with that of tryptophan, the optical resolution of tyrosine by the SA membrane was possible, but the performance was less. It is not clear yet, but one of the possible major reasons for this kind of result could be the smaller size of tyrosine, that is, less interaction between the chiral environment of the membrane and the solutes penetrating the membrane. Therefore, it appears that the L-isomer of tyrosine penetrated more easily through the membrane, reducing the enantioselectivity of the membrane.

CONCLUSIONS

SA membranes crosslinked with GA are possible for the optical resolution of α-amino acids, especially tryptophan and tyrosine, by a pressure-driven process. The presence of five chiral carbons located on the ring structure of SA seems to produce helical molecular structures and chiral environments in the membrane like cellulose and its derivatives, making the membrane enantioselective. With an increasing degree of crosslinking, the membrane has better enantioselectivity through increased interactions between the functional groups of the chiral environment of the membrane and the penetrating solutes. The other factors that can reduce the intermolecular interaction between the membrane and solutes, such as an increase in the operating pressure, an increase in the concentration of the feed solution, and smaller solutes, act against the enantioselectivity.

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