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– *Notes* –

Up-Hill Transport of Tryptophan through Polymer Inclusion Membrane Containing Aliquat 336

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Tryptophan, which is an essential amino acid, is used as a nutrient enhancer, a preservative, and a feed additive. Development of a cost-effective and straightforward separation technique is still required to extract and purify tryptophan in the bioproduction processes. We conducted permeation of tryptophan through poly(vinyl chloride)-based membrane containing Aliquat 336 as a carrier. Tryptophan successfully permeated through the membranes against its concentration gradient when the feed and receiving solutions were prepared by 0.1 mol/dm^3 sodium hydroxide solution and hydrochloric acid, respectively. The up-hill transport of tryptophan was achieved and more than 85% of tryptophan in the feed solution was recovered.

1. Introduction

Tryptophan, which is an essential amino acid, is used as a nutrient enhancer, a preservative, and a feed additive. Tryptophan is produced commercially by extraction from hydrolysate of proteins, chemical synthesis, fermentation, and enzymatic method [1]. In most cases, tryptophan was purified by various methods and recovered finally by isoelectric point crystallization under low temperature. Development of a cost-effective and straightforward separation technique is still required to extract and purify tryptophan in the bioproduction processes because of low product concentration with complex composition. A number of alternative processes for amino acid recovery have been proposed, including solvent extraction [2], adsorption [3], supported liquid membrane [4], electrodialysis [5] and aqueous two-phase extraction [6].

In our previous paper, we applied polymer inclusion membranes (PIMs) containing bi-functional ionic liquid consisting of Aliquat 336 and bis(2-ethylhexyl)phosphoric acid [A336][D2EHPA] or Aliquat 336 to the separation of phenylalanine [7]. Phenylalanine successfully permeated against its concentration gradient and the permeation rates through PIM containing [A336][D2EHPA] were higher than those using Aliquat 336. However, permeation rates were not significantly improved by using [A336][D2EHPA] compared to Aliquat 336. Moreover, synthesis of the bi-functional ionic liquids takes time and effort. Therefore, we used Aliquat 336 as a carrier of tryptophan in this study and the objective of this study was to achieve the up-hill transport of tryptophan with a high recovery. Although there have been some studies on the liquid membrane transport system of tryptophan using Aliquat 336 as a carrier [8-11], only one paper [10] has clearly demonstrated up-hill transport of tryptophan through the supported liquid membrane to the best of our knowledge. However, the maximum efficiency of tryptophan recovery into the receiving phase was 65 %

and not so high.

2. Experimental

2.1 Chemicals and membrane preparation

Aliquat 336 (Alfa Aesar), and other chemicals of analytical grade were used as received in this study. The PIM was prepared by the solution casting method [12]. A polymer solution was prepared by dissolving the PVC (n=1100, 0.6 g) and Aliquat 336 (1.5 g), in 25 mL of tetrahydrofuran (THF). The solution mixture was stirred with a homogenizer and poured into a petri-dish. After evaporating the THF for 24 h at room temperature, the resultant PIM was typically obtained with an average film thickness of 321 ± 23 μm using a micrometer (Digimatic, Mitsutoyo, Tokyo).

2.3 Permeation experiment

The PIM was sandwiched between two cells of the apparatus shown in Figure 1. A membrane with an effective area, *A*, of 12.56 cm^2 was fixed in the apparatus. The feed solution was 5 $mmol/dm^3$ of an aqueous tryptophan solution without pH adjustment. Receiving solutions included 0.1 mol/dm^3 of aqueous sodium carbonate solution. The transport experiment was initiated by adding 100 mL of each solution at 297 ± 1 K to their respective compartments. The stirring speed of the magnetic bar in each cell was controlled at 300 rpm. Samples from both solutions were withdrawn at regular time intervals. All experiments were duplicated and were reproducible with 5% as relative standard deviation. The tryptophan concentrations were analyzed via HPLC using *o*-phtalaldehyde (OPA) pre-column derivatization method [13]. The samples were automatically derivatized with OPA, and their concentrations were determined using a HPLC (LC-20AB, Shimadzu Corp., Kyoto, Japan) with a fluorescence detector (RF-20A, Shimadzu Corp., Kyoto, Japan). The analysis was performed using a Shimadzu AMINO-NA column.

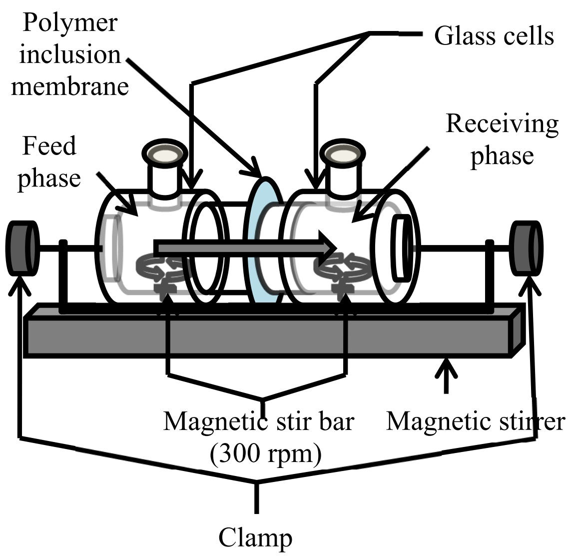

Figure 1. Experimental apparatus for tryptophan permeation through polymer inclusion membrane.

3. Results and Discussion

In the previous studies on the permeation of amino acids through the bulk and supported liquid membranes containing Aliquat 336, it has been reported that the amino acids permeated when pH values of the feed solution were within 10-14 and the receiving phase was the sodium chloride solution, hydrochloric acid, or distilled water [8-11]. In our previous study [7] on the phenylalanine permeation through PIM containing bi-functional ionic liquid, [A336][D2EHPA], phenylalanine successfully permeated against its concentration gradient when feed solution was distilled water and receiving solutions included 0.1 mol/dm^3 of aqueous sodium carbonate solution. First, we examined our previous feed and receiving solutions for PIM containing Aliquat 336. Figure 2 shows the results of the time courses of tryptophan and phenylalanine [7] concentrations in the feed and receiving solutions when feed solution was distilled water and receiving solutions included 0.1 mol/dm^3 of aqueous sodium carbonate solution. Tryptophan as well as phenylalanine was successfully permeated to the receiving phase, but the permeation of tryptophan against concentration gradient was not observed. Considering that carbonate-exchanged Aliquat 336 was a better anion-exchanger than original Aliquat 336 [14,15], a possible permeation mechanism is shown in Figure 3. In Figure 2,

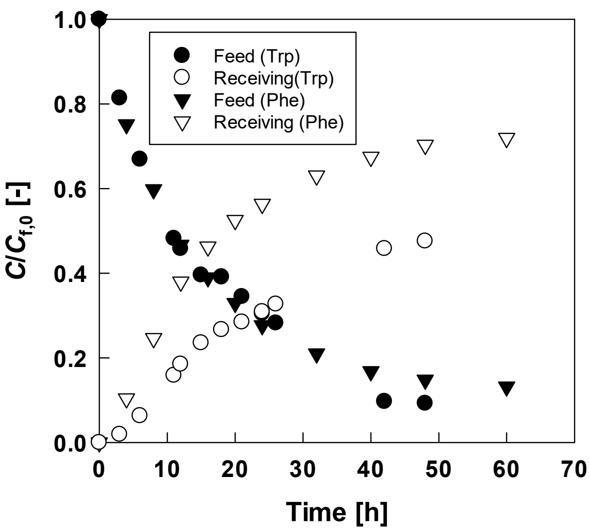

Figure 2. Time-courses of concentrations of Trp and Phe [7] in feed and receiving phases. (feed: without pH adjustment, receiving: 0.1 mol/dm^3 Na_2CO_3) (n=2).

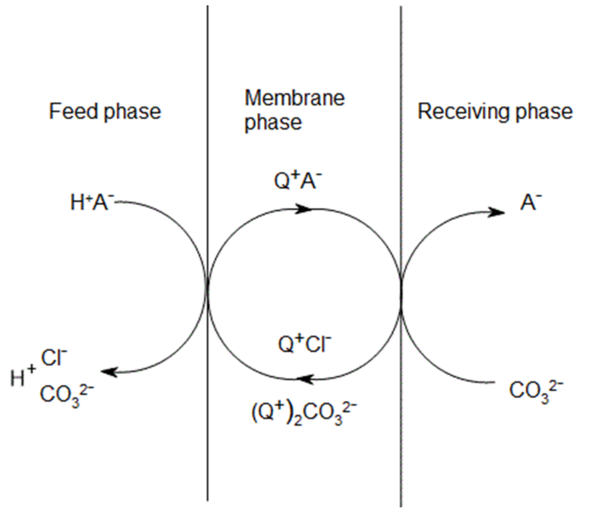

Figure 3. Permeation mechanism of amino acids through the PIM with Aliquat 336 as a carrier when feed solution is distilled water and receiving phase is sodium carbonate solution. (Q^+: quaternary ammonium cation, A^-: anionic amino acid).

although decrease rates in the phenylalanine and tryptophan concentrations in the feed phase were almost similar, the stripping rate of tryptophan was found to be lower than that of phenylalanine because of more hydrophobic character of tryptophan (values of log P for phenylalanine and tryptophan are 1.16 and 1.46, respectively [16]). To improve the recovery rate of tryptophan, we examined the effect of pH of feed on the permeation as shown in Figure 4. The pH of the feed was adjusted by adding 5 mol/dm^3 hydrochloric acid or sodium hydroxide solution. Lower pH of feed solution caused the decrease in the permeation rate. This confirmed that the anionic form of tryptophan was permeable by an anion exchange mechanism. Evidently, increase in the pH of the feed solution

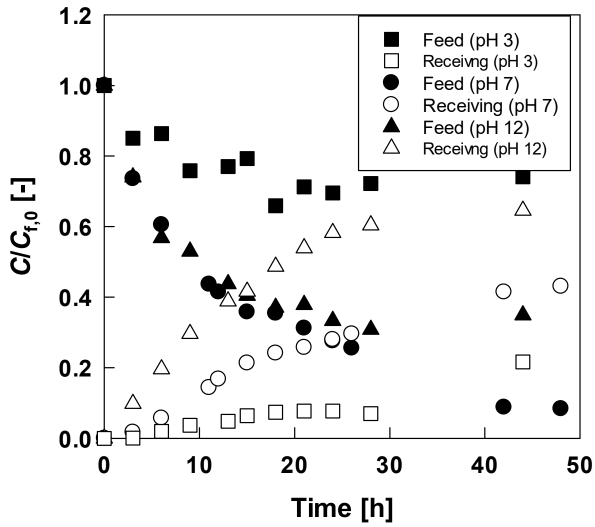

Figure 4. Effect of pH of feed solution on tryptophan concentration. (feed: pH adjustment with HCl and NaOH, receiving: 0.1 mol/dm^3 Na_2CO_3) (n=2).

enhanced the stripping rate of tryptophan, while decrease rates in the tryptophan concentrations in the feed phase in the initial stage are almost similar between pH 7 and 12. Moreover, in the second half of the experiment at pH 12, the tryptophan concentration of the feed phase tended to increase. So, we used sodium hydroxide solution in place of distilled water and hydrochloric acid in place of sodium carbonate solution as in the previous paper [8]. The feed solution of sodium hydroxide solution was used to form the anionic form of tryptophan. The receiving solution of hydrochloric acid played the roles of supplying source of counter (chloride) ion and keeping the driving force of permeation by ensuring the cationic form of tryptophan in the receiving phase as shown in Figure 5. When the feed and receiving solutions were prepared by 0.1 mol/dm^3 sodium hydroxide solution and hydrochloric acid, respectively, the permeation result was shown in Figure 6, along with the results of carbonate system of feed pH 12. The permeation against the concentration gradient and larger decrease rate of tryptophan in the feed phase in hydrochloric acid system were observed, and the final recovery of tryptophan was improved to more than 85%. When concentrations of NaOH and HCl were increased to 1 mol/dm^3, no further effects were observed.

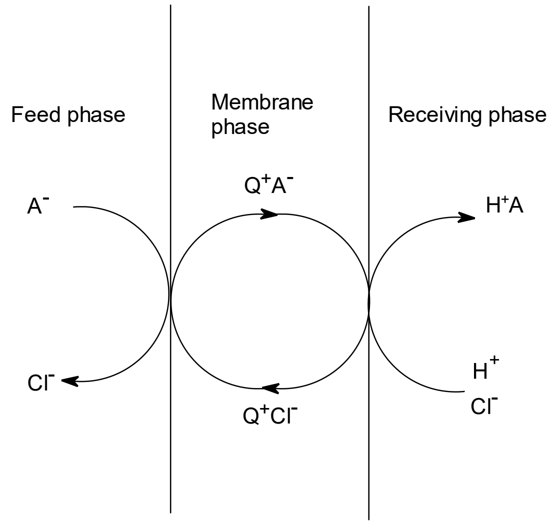

Figure 5. Permeation mechanism of amino acids through the PIM with Aliquat 336 as a carrier when feed solution is sodium hydroxide solution and receiving phase is hydrochloric acid. (Q^+: quaternary ammonium cation, A^-: anionic amino acid).

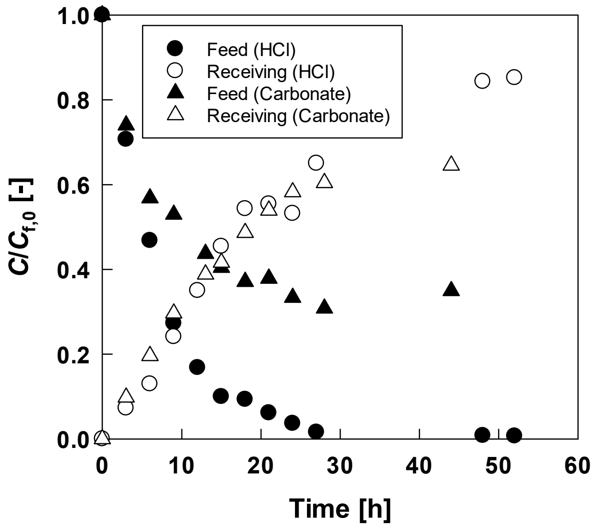

Figure 6. Time-courses of concentrations of tryptophan in feed and receiving phases (circle: feed 0.1 mol/dm^3 NaOH, receiving 0.1 mol/dm^3 HCl, triangle: feed without pH adjustment, receiving 0.1 mol/dm^3 Na_2CO_3) (*n*=2).

4. Conclusion

We conducted permeation of tryptophan through PVC-based membrane containing Aliquat 336 as a carrier. Tryptophan successfully permeated through PIMs when feed solution was distilled water and receiving solutions included 0.1 mol/dm^3 of aqueous sodium carbonate solution. However, the permeation against concentration gradient was not observed because of more hydrophobic character of tryptophan. When sodium hydroxide solution and hydrochloric acid in place of distilled water and sodium carbonate solution, respectively, were used, the up-hill transport of tryptophan were observed. The final recovery of tryptophan was improved to more than 85%. This technology may be useful for separation and purification of L-tryptophan produced by fermentation or enzymatic method.

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