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Selective Transport of a Peptide from Its Mixture with an Amino Acid Using a Supported Liquid Membrane Process

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ABSTRACT

Some experimental results on the separation of a peptide from a mixture of peptide and amino acid through a continuous supported liquid membrane process are described. As a model system a mixture of tryptophan (Trp, an amino acid) and tryptophan-leucine (Trp-Leu, a dipeptide) was chosen. The liquid membrane contained an anionic surfactant, sodium di-2-ethylhexyl sulfosuccinate (Aerosol OT/AOT), as a carrier dissolved in oleyl alcohol, as an organic solvent, supported on a commercial grade support (Celgard 2500). The liquid–liquid extraction experiments were carried out to study the influence of feed pH, feed and strip flow rates, and feed and carrier concentrations on the selective transport of Trp-Leu from its mixture with Trp. At pH 4–5 the transport rate of Trp-Leu was significantly higher than that of Trp. The increase in flow rate up to a value of 40 mL/h did not effect the selective removal of Trp-Leu. The flux rate increased with an increase of the carrier concentration up to 20% AOT, but the selectivity for Trp-Leu was highest at 10% AOT. An increase of Trp concentration up to 10-fold had little effect on the flux rate of Trp-Leu. The stability of SLM system in continuous removal of Trp-Leu from a single component system and from a binary mixture with Trp was studied. The effect of a competitive component did not alter significantly the flux rate and long-time performance in continuous operation. A procedure for regenerating SLM was examined, and the regenerated SLM performed as good as the freshly prepared one.

INTRODUCTION

The liquid membrane processes are efficient techniques for the selective separation and concentration of the target solute from dilute solutions

often encountered in food, dairy, and biochemical processing. Although they have been mostly applied to recover metals from wastewaters (16) and multicomponent mixtures (11, 15), recently their application has been gaining considerable interest to the processing and separation of bioproducts (2, 9, 12, 29, 32).

Liquid membranes, in the separation of amino acids and peptides, take the advantage of enhanced transport by using a selective carrier of anionic or cationic form dissolved in an organic solvent (20). The carriers used for this purpose include commercial quaternary ammonium salts, Aliquat 336, Adogen 464, di(2-ethylhexyl) phosphoric acid (D2EHPA), and sodium di-2-ethylhexyl sulfosuccinate (AOT) (4, 5, 9, 11). For the organic phase of the liquid membrane, oleyl alcohol, decanol, paraffin, and organic acids have been tried (13, 14).

Batch extraction of amino acids such as tryptophan (Trp) and phenylalanine (Phe) was performed by Teramoto et al. (28) with emulsion liquid membranes. The extraction equilibria, the extraction rate, and the distribution behaviour were explained with the aid of a permeation model. A two-film model was used to describe the stripping behavior of Phe from the carrier–amino acid complex in the organic phase by Uddin et al. (30). The underivatized amino acids were separated into optical isomers by means of supported chiral liquid membranes (3). They separated the following amino acids: alanine (Ala), leucine (Leu), phenylalanine (Phe), methionine (Met), serine (Ser), threonine (Thr), and glutamic acid (Glu) through liquid chiral alcohols immobilized in pores of ultrafiltration membranes. Transport of dipeptides and phosphono dipeptides through a 1-decanol solution supported in a porous polyethylene hollow fiber ultrafiltration membranes was reported by Wieczorek et al. (31). The separation and concentration of peptides through a supported liquid membrane was examined by Wong et al. (32). They reported the effect of hydrophobicity of the amino acid side chains on the transport rate in batch-scale experiments. Although these studies indicate that various amino acids and peptides can be transported through organic liquid membranes containing suitable carriers, only a few studies (23) have been devoted to the selective transport of amino acid/peptide from their mixtures. However, many food and biochemical industry streams contain several amino acids and peptides and require removal of specific components to increase their value and quality. Therefore, a systematic investigation has been undertaken to study and establish a stable membrane system for the processing of industrial streams.

In this paper some experimental aspects on the separation and recovery of peptides from a mixture of amino acid and peptide, using anionic complexing agents in a supported liquid membrane placed in a spiral module

system, are presented. The aim was to determine the operating conditions that give a SLM to process feed streams with high flux and sufficient stability. Separation experiments were carried out by using a mixture of Trp (a model amino acid) and tryptophan-leucine (Trp-Leu, a model dipeptide) to study the effects of the following variables:

pH of a 1:1 feed solution
Flow rates of feed and strip phases
Ratio of amino acid to peptide in feed
Concentration of carrier in SLM
Stability and regeneration of SLM

PROCESS MECHANISM

The overall transport process from the bulk feed side to the strip side can be described by the following physicochemical events:

1. Transport of peptide (and amino acid) from the feed solution to the aqueous boundary layer and diffusion through it to the feed–membrane interface
2. Reaction at the interface to form a complex with the carrier
3. Diffusion of the solute–carrier complexes through the membrane
4. Decomplexation reaction at the strip–membrane interface with the liberation of peptide (and amino acid)
5. Diffusion of ion-loaded carrier back to the feed–membrane interface
6. The released peptide (and amino acid) diffuses through the second aqueous boundary layer to the bulk strip solution where it is extracted from the system

The reaction system can be described by using the example of Trp and AOT. The carrier is anionic and can carry possitively charged ions from one side to the other side of the membrane. This is achieved by the carrier's association with either Na^+ or positively charged amino acid (or peptides). Each simple amino acid has two groups [carboxylic (pK_1) and amino (pK_2)], and can be represented by $NH_2CHRCOOH$. These groups are affected by the pH, and to ensure that these molecules are positively charged, the pH is chosen such that $pH < (pK_1 + pK_2)/2$. In the case of Trp where $pK_1 = 2.4$ and $pK_2 = 9.4$, the acidity of the solution required for favorable interactions with the carrier should be such that $pH \ll 5.7$. This should also be the case for the peptide Trp-Leu.

The mechanism for the transfer of Trp^+ from the feed solution (subscript "f") through membrane–feed side (subscript "mf"), through the

membrane–strip side (subscript "ms"), to the strip solution (subscript "s") can be represented by the following set of equations.

$$Trp_f^+ + (Na^+ AOT^-)_{mf} \rightleftarrows Na_f^+ + (Trp^+ AOT^-)_{mf} \quad \text{extraction} \quad (1)$$

$$(Trp^+ AOT^-)_{mf} \rightleftarrows (Trp^+ AOT^-)_{ms} \quad \text{membrane transport} \quad (2)$$

$$Na_s^+ + (Trp^+ AOT^-)_{mf} \rightleftarrows (Na^+ AOT^-)_{ms} + Trp_s^+ \quad \text{stripping} \quad (3)$$

By maintaining $Na_s^+ > Na_f^+$ and $Trp_s^+ < Trp_f^+$, Eqs. (1) to (3) remain out of equilibrium and each reaction proceeds to the right. The total effect of this shuttle mechanism is the transport of Trp from the feed solution to the strip solution and Na^+ from the strip solution to the feed solution

MATERIALS AND METHODS

The feed L-tryptophan or tryptophan-leucine was obtained from Sigma Chemical Co., USA. The chemicals—Aerosol OT, sodium dihydrogen orthophosphate, and disodium hydrogen orthophosphate—were purchased from B.D.H. Co., England. The membrane solvent oleyl alcohol (Aldrich Co., USA) and the phosphate buffer materials—sodium acetate and 85% orthophosphoric acid—were from Ajax Chemicals, Australia. Absolute alcohol was from Rhone-Poluene Lab Products (Australia), and sodium chloride (Regular) was from Prolabo (France). The polymeric support, Celgard 2500, was a gift from Celanese Separation Products, Charlotte, NC, USA.

Preparation of Supported Liquid Membrane

The SLMs were prepared by soaking the Celgard 2500 support in 10% AOT solution in oleyl alcohol for 5–10 minutes followed by placing the contents under vacuum for about 30 minutes. The membrane was then rinsed with deionized water and gently blotted with a tissue paper.

For regeneration, the SLMs were washed in 20% ethyl alcohol solution for 5–10 minutes followed by soaking in 100% ethyl alcohol solution for about 20 minutes. They were contacted with AOT solution as mentioned above. They were rinsed with deionized water and blotted with paper. The membrane was impregnated with 10% AOT solution as described above.

Separation Equipment

The apparatus for separation experiments was designed and fabricated according to the literature (24). It consists of two half-cell faceplates between which the SLM is placed. The faceplates are about 10 cm in diameter and contain spiral machined channels through which the feed and strip solutions flow. The entire length of channel is 94 cm with a depth of 0.16 cm and a width of 0.32 cm. The volume of channel is 4.75 cm^3, and the effective membrane area for transport is 29.7 cm^2. The feed solution was pumped into one of the channels from a feed reservoir by a Bio-Rad Econo pump with digital control. The strip solution was similarly pumped from a strip reservoir through the obverse channel. A flow diagram of the continuous membrane module is shown in Fig. 1.

The pHs of the feed and strip solutions were measured periodically with a PHM-64 Research pH meter (Radiometer Co., Copenhagen). The conductivity of the strip solution at the cell exit was measured using a Bio-Rad Econo Gradient Monitor.

Transport Measurements

The transport of Trp-Leu and Trp across the membrane was monitored by measuring the change in concentration of the initially peptide-free strip solution. At regular time intervals samples of strip solution were collected in a fraction collector (Bio-Rad Model 2110 fraction collector). The absorbance of strip solution was measured spectrophotometrically at 280 nm

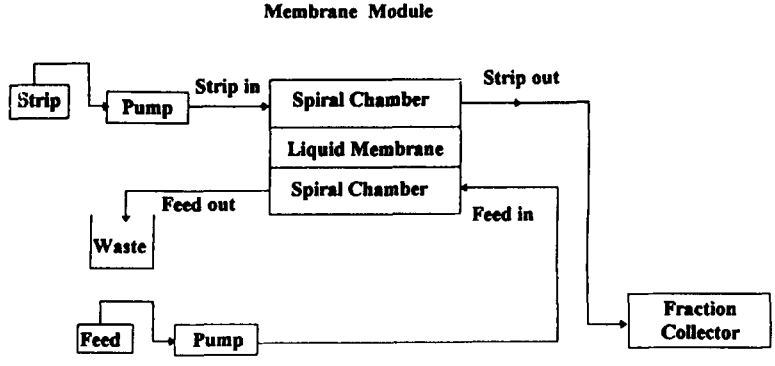

FIG. 1 Schematic of the experimental setup of a continuous supported liquid membrane process.

using a UV-visible spectrophotometer (Shimadzu UV-160). The feed samples were diluted and their absorbance values were also measured spectrophotometrically at 280 nm.

HPLC of the Strip Solution

The samples of strip solution were analyzed for Trp and Trp-Leu using a Chemstation method on an HPLC system (HP-Ti Series 1050). The column size was 25 × 0.46 cm packed with Nucleosil C18 5μ particles. The elution system was made up of a combination of two solvents: Solvent A was 0.05% aqueous trifluoroacetic acid, pH 1.6; Solvent B was 0.05% trifluoroacetic acid in water/MeCN, 10/90. The solvent gradient was 0–2 min 0% B, 11–14 min 19% B, 26 min 80% B, and 28 min 0% B. An operating temperature of 20°C, a flow rate of 1 mL/min, and a sample volume of 300 μL were used for detection at 280 nm.

Calculation of Flux Rate and Selectivity

The flux rate of Trp-Leu (and Trp) was calculated by obtaining the concentration values from the absorbance measurements in the strip solution at the inlet and outlet of the membrane module. The following equation was used

$$J_P = \frac{V(C_{P,o} - C_{P,i})}{A} \tag{4}$$

where J_P is the flux rate of Trp-Leu in mmol/(m^2·h); V is the strip flow rate in mL/h; $C_{P,i}$ and $C_{P,o}$ are the inlet and outlet concentrations of the solute Trp-Leu (mmol/ml); and A is the effective surface area of the membrane (m^2). The flux rate of Trp, J_A, was calculated using Eq. (4), replacing the concentrations by $C_{A,i}$ and $C_{A,o}$, the concentrations for Trp.

The selectivity, S, of SLM for mass transport is defined as

$$S = \frac{J_P/(C_F)_P}{J_A/(C_F)_A} \tag{5}$$

where $(C_F)_P$ and $(C_F)_A$ are the feed concentrations of Trp-Leu and Trp, respectively.

The strip flow rate and the membrane area being the same, the expression for S reduces to

$$S = \frac{(C_{P,o} - C_{P,i})}{(C_{A,o} - C_{A,i})} \times C_R \tag{6}$$

where C_R is the ratio of the feed concentration of Trp to that of Trp-Leu.

RESULTS AND DISCUSSION

The transport experiments were carried out to determine the conditions where the transport of Trp-Leu is faster than that of Trp. The SLM characteristics and the experimental operating conditions are listed in Table 1. Once the optimum pH was determined, the experiments with the mixture were conducted at that feed solution pH. All the experiments (except for stability experiments) were carried out until steady state was reached, which was confirmed by the little change in absorbance values over a period of time.

It is noted that the UV absorbance measured was the total value for the two solutes: Trp-Leu and Trp. This was converted to the individual absorbance using the percentage area distribution in HPLC analysis. The corresponding concentration value was obtained using an absorbance–concentration plot.

The effects of various parameters on the transport of Trp-Leu and Trp are presented below. Unless otherwise mentioned, the conditions were the same as those in Table 1.

TABLE 1
Membrane Characteristics Supported on Celgard 2500 and Experimental Conditions for Trp-Leu and Trp Transport from a 1:1 Solution

(a) Supported Liquid Membrane Characteristics	
Support	Celgard 2500
Porosity	37–48%
Pore dimensions	0.05 × 0.19 μm
Thickness	20 μm
Liquid membrane	10% AOT solution in oleyl alcohol
(b) Experimental Conditions for Trp-Leu and Trp Transport	
Feed phase:	
1×10^{-3} mmol/ml tryptophan or tryptophan-leucine in 0.1 M acetate-phosphate buffer solution	
pH range	4.5
Flow rate	10 mL/h
Strip phase:	
1 M sodium chloride in 0.1 M phosphate solution	
pH range	4.5
Flow rate	10 mL/h
Temperature of both phases	293 K

Effect of Feed pH

The final concentrations of Trp-Leu and Trp in the strip solution for transport at various pH values are presented in Table 2. Figure 2a shows the flux rate of Trp across the SLM for pH 3.5, 4.0, 4.5, and 5. The system reached a steady state in about 60 minutes after the experimental runs began. The flux rate increased with time until the steady state was reached. The maximum flux rate decreased with the feed solution pH, and at pH $\geq$ 5.0 the decrease was remarkably low. This is due to the decrease in concentration of Trp^+ as pH approaches p*I* value (5.7 for Trp). Figure 2b shows the flux rate of Trp-Leu across the SLM for pH values the same as in Fig. 2a. The flux rate increased with time, reaching a steady state in about 60–90 minutes. The effect of feed pH on the flux rate is less sensitive than to that of Trp, i.e., less decrease in steady state value with the increase of feed pH. This suggests that the available concentration of Trp-Leu and its transport is significantly higher than that of Trp. This is expected because p*K* values of dipeptides are higher than their corresponding amino acids (9) and the permeability of Trp-Leu is greater than that of Trp (32). The extraction behavior of amino acid we present in these figures is similar to those of Furusaki and Kishi (8), Takeshima et al. (23), and Hatton (10). For dipeptide (Trp-Leu) we did not observe any sharp maximum peak as was reported by Wieczorek et al. (31) for the transport of L-alanyl-L-valine through a 1-deconol membrane containing Kryptofix 222.

TABLE 2
Final Concentrations in the Strip Solution for Transport through 10% AOT-Oleyl Alcohol Membrane from a 1:1 (Trp-Leu:Trp) Feed Solution at Different pH Values

	Final concentration values in the strip solution $\times 10^3$ (mmol/mL)[a]	
pH of feed solution	Trp-Leu	Trp
3.0	0.491	0.32
3.5	0.50	0.25
4.0	0.45	0.13
4.5	0.43	0.04
5.0	0.25	0.01
5.5	0.18	0.0

[a] Taken after 60 minutes of experiment.

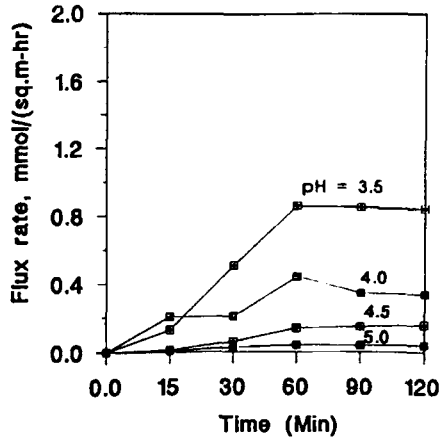

FIG. 2a Time course of Trp flux for pH = 3.5, 4, 4.5, and 5 from a 1:1 Trp and Trp-Leu solution.

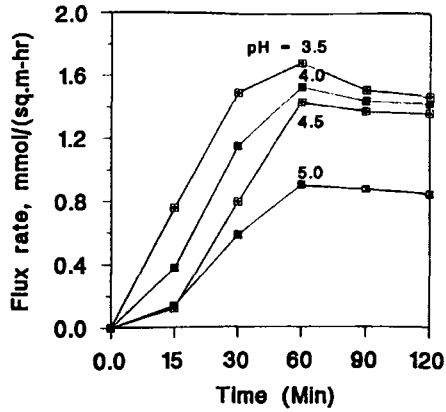

FIG. 2b Time course of Trp-Leu flux for pH = 3.5, 4, 4.5, and 5 from a 1:1 Trp and Trp-Leu solution.

The selectivity of the membrane system was calculated using Eq. (6), and is plotted for pH 3, 3.5, 4, 4.5 and 5 in Fig. 3a. The selectivity reached a constant value after 60–90 minutes. The selectivity increased with pH, and at pH 4.5 the value was about 10. The selectivity for pH 5 was higher, but the flux rate of Trp-Leu was comparatively lower than that at pH 4.5.

The flux rates of Trp-Leu and Trp at various pH values are compared in Fig. 3b. The difference between the transport rates was maximum at

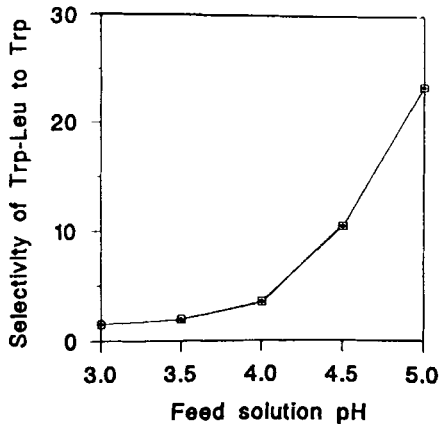

FIG. 3a Effect of pH on the selectivity of SLM.

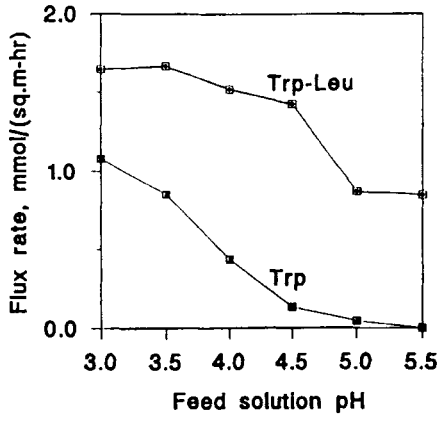

FIG. 3b Effect of pH on the transport rates of Trp and Trp-Leu through the SLM.

pH 4.5 with a value of about 1.3 mmol/m^2·h. Therefore this pH was chosen as the optimum and used in the following experiments for selective removal of Trp-Leu from its mixture with Trp. An equimolar feed concentration was used in all the experiments except for those conducted to study the effect of feed concentration ratio.

Effect of Flow Rate

The effect of flow rate on the transport of Trp-Leu and Trp was studied by varying the flow rates of both feed and strip solutions as 10, 15, 20, 30, and 40 mL/h and maintaining a constant ratio of 1.

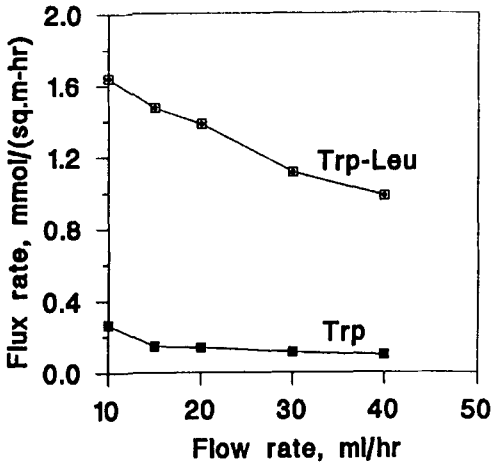

FIG. 4 Effect of the flow rate on the transport of Trp-Leu and Trp at a constant ratio of feed and strip phase flow.

The steady-state flux rates of Trp-Leu and Trp are plotted in Fig. 4. The flux rates of both solutes decreased slightly with the increase of flow rate. This can be expected in a continuous processing device where an increase in flow means a decrease in residence time. The flow rate of 10 mL/h means a residence time of 28 minutes in each chamber, and the variation of flow from 10 to 40 mL/h means a decrease in residence time from 28 to 7 minutes. This could have affected the decline in flux rates, assuming the process is predominantly limited by the diffusion of solute–carrier complex through the membrane (27).

Effect of the Concentration Ratio of Trp/Trp-Leu in Feed

The concentrations of Trp (maintaining the Trp-Leu concentration constant) in the feed mixture was varied from 1 to 10, and the effects on the flux rates of Trp and Trp-Leu are shown in Fig. 5a. The flux rate of Trp-Leu was more or less unchanged whereas that of Trp increased linearly with the increase in concentration ratio. This could be explained by the saturation capacity of the carrier and the availability of more transportable ions. The SLM prepared with 10% AOT was still unsaturated and could transport more within the residence time allowed if the concentration of positively changed ions were increased and/or competition between the solutes were decreased. Increasing the ratio of Trp to Trp-Leu in the feed at constant pH provided higher concentration and less intense competi-

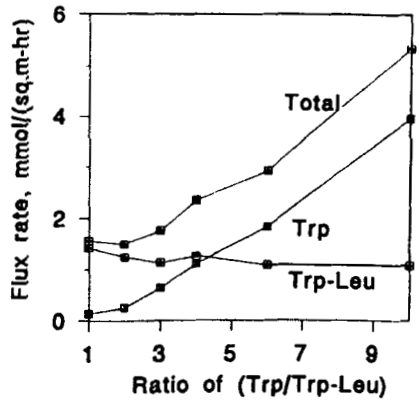

FIG. 5a Effect of the concentration ratio of Trp to Trp-Leu in the feed solution on the transport of the solutes.

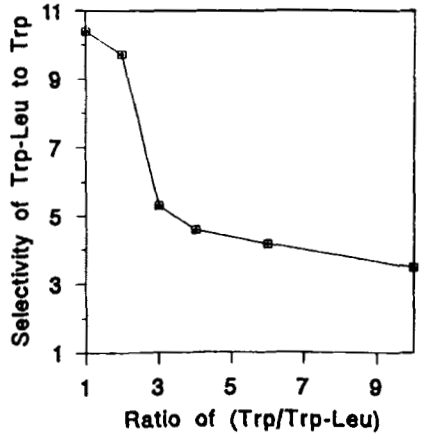

FIG. 5b Effect of concentration ratios on the selectivity of SLM.

tion; as a result, more Trp was transported. At a ratio of about 4, the flux rates of both the solutes were identical, suggesting a higher permeability for Trp-Leu. For low values of concentrations ratio of Trp to Trp-Leu (i.e., ratio of 1–2), the selectivity of transport decreased slightly; it dropped sharply in the range 2–3 and finally down to a constant value at high values of concentration ratio (Fig. 5b). Therefore selective removal of Trp-Leu could be achieved better for a feed stream with comparable values of concentration.

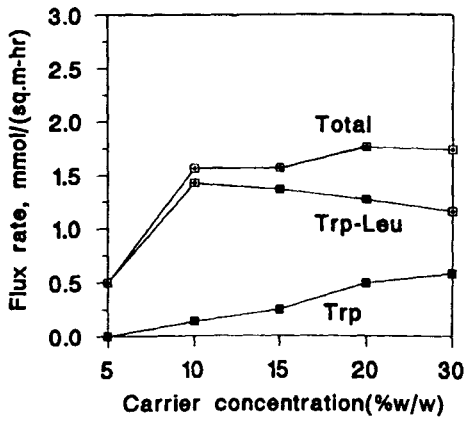

FIG. 6a Effect of the carrier concentration (% AOT in oleyl alcohol) on the transport of Trp-Leu and Trp from 1:1 feed solution.

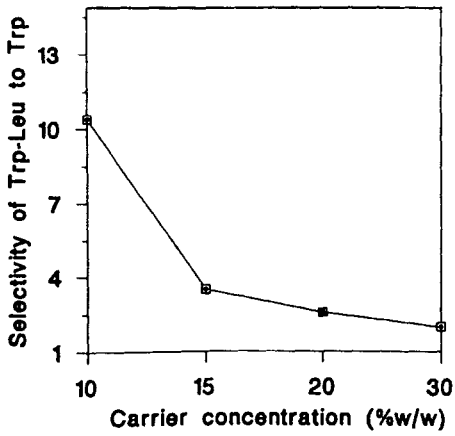

FIG. 6b Effect of carrier concentration (% AOT in oleyl alcohol) on the selectivity of SLM from 1:1 feed solution.

Effect of the Carrier Concentration

The carrier concentration (w/w% AOT in oleyl alcohol) was varied during SLM preparation, and the effect on the flux rates of the sclutes from a 1:1 feed mixture are shown in Fig. 6a. When a low concentration of carrier (i.e., 5%) was used, the flux rate of Trp-Leu was small and that of Trp was negligible. As the carrier concentration was increased, the

total flux rate increased (because of the increase of Trp flux rate) with a decrease in selectivity toward Trp-Leu. This increase in flux rate was observed up to 20% carrier concentration, after which the rate approached a constant value. One possible explanation is that at about 20% carrier concentration the SLMs were nearly saturated to their transport capacity and any increase in carrier concentration would not be effective. The progressive reduction in the flux rate could also be attributed to the negative effect of increased viscosity (at higher carrier concentration) on the diffusivity of the solute–carrier complex (7).

The selectivity of SLM for Trp-Leu transport decreased significantly with the increase of carrier concentration (Fig. 6b). The increase in carrier concentration decreases the diffusivity of solutes/solute–carrier complexes and this effect could be greater for Trp-Leu, which has a larger molecular weight than Trp. Thus the flux of Trp-Leu could be more adversely affected by the increase in carrier concentrations, resulting in a decrease in the selectivity. The SLMs prepared with 10% AOT provided the highest flux rate and selectivity for Trp-Leu, and therefore can be recommended for its selective removal from the mixture.

Operational Stability and Regeneration of SLM

The stability of SLM in continuous operation was studied for 1) a 1 mM Trp-Leu solution and 2) a 4:1 mixture of Trp and Trp-Leu.

1. The flux rates across a freshly prepared SLM and after regeneration for Trp-Leu is shown in Fig. 7. The flux rate decreased at a slow rate (about 1% of initial steady value every 2 hours), suggesting a reasonable performance over a period of a few days. After 72 hours of experiment when the flux rate reduced to about 40% of the initial flux rate, SLMs were regenerated and their performance was tested. The regeneration procedure was: wash with 20% ethanol followed by a wash of 100% ethanol to remove all the organic materials before contacted with AOT solution. The regenerated SLM recovered the flux rate, and its performance was as good as the original one over a period of 72 hours.
2. The flux rate of Trp-Leu and Trp across a fresh SLM and after regeneration are shown in Fig. 8. This experiment was performed with a higher feed concentration of Trp (the ratio of the concentration of Trp/Trp-Leu = 4) and with SLM prepared using 20% AOT in oleyl alcohol to obtain comparable flux rates. The flux rate of Trp-Leu decreased in the similar way to what it did in the case of a single component (Fig. 7). The flux rate of Trp remained at the initial level for about 20 hours and then drastically went down to a small value

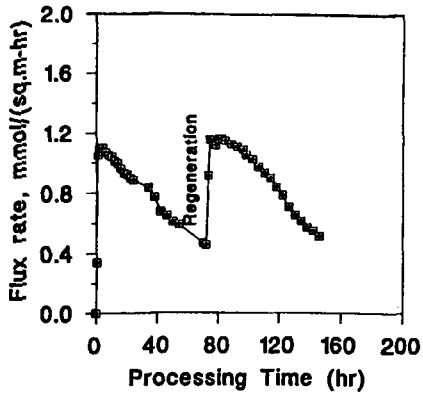

FIG. 7 Stability of the SLM in continuous processing of 1 mM Trp-Leu with fresh and regenerated membrane.

(about 28–30% of the initial value). Eventually it was close to zero at $t = 55$ hours when the flux rate of Trp-Leu was about 46% of its initial value. This behavior suggests that there was a loss of AOT and/or oleyl alcohol from the membrane phase after continuous operation of more than 20 hours. This had caused in a depletion of AOT concentration and resulted in a decline in the flux rates of Trp and Trp-Leu. This is consistent with the results shown in Fig. 6a, where the flux rate decreased with carrier concentration.

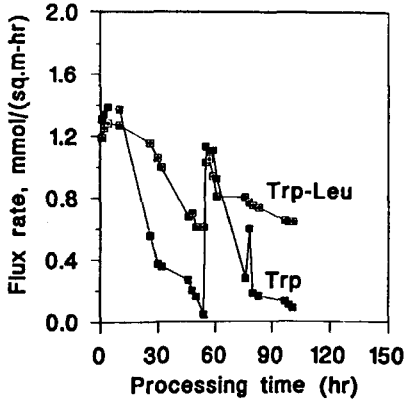

FIG. 8 Stability of the SLM in continuous separation of Trp-Leu from a 4:1 feed solution with fresh and regenerated membrane.

The decline in the flux rate could be related to the loss of carrier and/or oleyl alcohol from the membrane phase. Although oleyl alcohol and AOT exhibit low solubility in aqueous media, in the presence of low salt concentrations they could leak little by little (1, 13), especially when the feed and strip solutions are not saturated with these chemicals (as in our experiments). The loss of AOT and oleyl alcohol can be quantified by applying photometric tests based on ion-pair extraction (21, 25).

The loss in performance of SLM could also be due to the formation of small emulsion droplets when the feed solution is flowing along the surface (17–19). The stability of SLM can be improved by gelation of the liquid membrane phase (18, 19).

Experiments are underway to study quantitatively the stability of SLMs by 1) determining the leakage rate of solvent and carrier from the membrane phase and 2) applying a gel network in the pores of the support or applying a gel layer to the feed side. The results of these investigations will be published in a later communication.

CONCLUSIONS

A continuous flow membrane module with AOT-oleyl alcohol membrane supported on Celgard 2500 was capable of separating Trp-Leu from a solution of Trp-Leu and Trp. The process has the following attributes which could be useful for selective removal of the peptide.

- A high flux rate of Trp-Leu and a selectivity of about 10 at a feed pH of 4.5
- An increase of flow rate up to 40 mL/h does not change the selectivity of Trp-Leu transport
- A 10% carrier solution (i.e., 10% w/w of AOT in oleyl alcohol) produces SLM with reasonable selectivity and flux rate of Trp-Leu
- An increase in concentration ratio of (Trp/Trp-Leu) more than 2 significantly affects the selectivity of Trp-Leu transport
- The SLM preparation procedure uses food industry acceptable chemicals (a very low concentration of AOT in food is allowed by FDA)
- The SLM is stable for a period of a few days; it is regenerable, and the regenerated SLM performs as good as the freshly prepared one.

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SYMBOLS

A	surface area (m^2)
Ala	alanine
AOT	Aerosol OT, sodium di-2-ethylhexyl sulfosuccinate
C_A	concentration of (Trp) amino acid (mmol/mL)
C_F	concentration of Trp (and Trp-Leu) in feed solution (mmol/mL)
C_P	concentration of (Trp-Leu) peptide (mmol/mL)
C_R	concentration of Trp to Trp-Leu in feed solution
J	flux rate based on the area of the spiral chamber, defined in Eq. (4) (mmol/m^2/h)
Leu	leucine
Met	methionine
Na	sodium
Phe	phenylalanine
S	selectivity, defined in Eq. (5)
Ser	serine
Thr	threonine
Trp	tryptophan
Trp-Leu	tryptophan-leucine
V	strip solution flow rate (mL/min)

Subscripts

A	amino acid
f	feed solution
i	inlet of the membrane module
mf	membrane–feed side interface
ms	membrane–strip side interface
o	outlet of the membrane module
P	peptide

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