

Separation and Concentration of L-Phenylalanine using a Supported Liquid Membrane

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The separation and concentration of L-phenylalanine (L-Phe) using a supported liquid membrane (SLM) is investigated. A cation complex agent, di-2-ethylhexyl phosphoric acid (D₂EHPA), is used as a carrier in the SLM with n-Heptane as a solvent. The reaction order and equilibrium constant in the formation reaction of L-Phe-carrier complex are obtained from the extraction experiment. A mathematical model for a carrier mediated counter transport process is proposed to estimate the diffusion coefficient of L-Phe-carrier complex in the liquid membrane. Permeation experiments of L-Phe using a SLM are performed under various operating conditions and optimum conditions for the transport of L-Phe are obtained. Concentration of L-Phe in the strip phase against its concentration is observed. Transport rate of glucose through liquid membrane is less than that of L-Phe in the competitive transport of L-Phe and glucose. And the existence of glucose reduced the transport rate of L-Phe. The performance of separation with continuous strip phase is increased due to the dilution effect in the strip phase.

Key words: supported liquid membrane, facilitated transport, L-Phe, glucose, D₂EHPA

INTRODUCTION

L-phenylalanine (L-Phe) is an important amino acid and essential for human nutrient and component of amino acid infusions for medical purpose [1]. Especially, it has been used as an intermediate of the diet sweetener (aspartame) with its commercial significance. Prevalently, the downstream processes of L-Phe consisted of cell removal, ion-exchange, evaporation and crystallization. But these processes generate a lot of waste liquor for regeneration and much energy is wasted in the evaporation process. Thus the separation technique which is conventionally simple and saves the energy has been needed.

Separation technique using liquid membrane is the technique which can separate and concentrate materials without phase transition. Liquid membranes have gained increasing significance in combination with the facilitated transport which utilizes selective "carriers" transporting certain components such as metal ions selectively and at a relatively high transport rate across the liquid membrane [2, 3].

Liquid membranes are realized principally in two physical forms. In the first case, the selective liquid barrier material is stabilized as a thin film by a surfactant in an emulsion-type mixture [4]. This is called as a liquid emulsion membrane (LEM). The second configuration is generally referred to as a supported liquid membrane (SLM). To make a liquid membranes in SLM, a microporous polymer structure is filled with the liquid membrane phase [5]. In this configuration,

the microporous structure provides the mechanical strength and the liquid-filled pores are the separation barrier. SLM has some advantages than LEM because it is one step separation process and doesn't have swelling problem at high concentration [6].

SLM combines the advantages of classical membrane processes such as the cross-flow filtration (low operating cost and single-step operation) and the extraction process (flexibility and high selectivity). The simultaneous extraction and stripping stage require the reduced amount of organic phase. The study of supported liquid membrane (SLM) was started in 1980s and has been actively used in gas separation [7], metal ion separation [2, 3, 8] and purification [5] in recent years. The separation of L-valine from fermentation broth by SLM was performed using an anion carrier, Aliquant 336 [5]. The separation of L-Phe by LEM was performed using D₂EHPA as a cation carrier [8]. But the separation of L-Phe using SLM has not been performed. Also the effect of amino acid concentration and the dilution effect of strip phase on the L-Phe transport in SLM have not been done.

In this work, the separation of L-Phe using an SLM in pure L-Phe solution and in mixed solution of L-Phe and glucose are investigated. The effects on L-Phe transport of various operating parameters such as pH in the feed phase, the carrier type, carrier concentration and hydrochloric acid concentration in the strip phase have been examined and the optimal conditions were investigated. In the separation with the continuous strip phase, the dilution effect of strip phase on L-Phe transport has been carried out.

MATERIALS AND METHOD

Apparatus and Experimental Conditions

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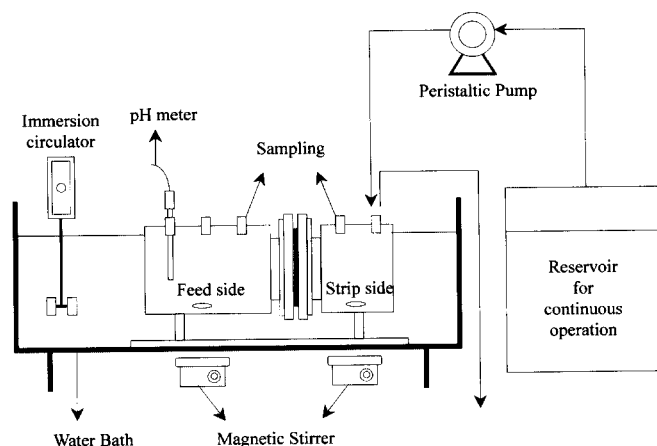


Fig. 1. Schematic diagram of experimental apparatus.

Permeation experiments were carried out in the apparatus illustrated in Fig. 1. The two half cells (volume of the one is 760 ml and the other is 360 ml) are joined with an O-ring joint. A membrane (SLM) is placed in the O-ring joint to separate the two cells. Solutions are poured into the cells through the sampling ports. The solutions are stirred with magnetic stirrer, and both feed and strip solutions are stirred at the same speed. In continuous strip phase operations, stripping phase solutions are circulated by a peristaltic pump from reservoir into the permeation cell. To maintain constant temperature, the cells are suspended in thermally regulated waterbath. All experiments have been performed at 20 ± 2 . Details of experimental conditions are illustrated in Tables 1 and 2.

Reagent and Membrane

PVDF-Polyvinylidene fluoride (Milipore Co.) with the thickness of 100 and the pore size of 0.45 is used to constitute the liquid membrane support. n-Heptane is used as the solvent. The carrier molecule is D2EHPA (di(2-ethylhexyl) phosphoric acid, Tokyo Kasei Kogyo co.) used as received. Filling the pores of this dry support polymers with the carrier solution is accomplished by immersing the membrane in the organic phase for at least 6-8 hr before use. The pores are immediately and quantitatively filled with the carrier solution by capillary action. This type of SLM polymeric support eliminates the transport of water through the membrane and is free of osmotic effects.

Table 1. Operating conditions of permeation experiment

Operating Parameters	Conditions	Typical Conditions
Initial pH in the feed phase	2.0, 3.0, 4.0, 5.0	3.0
Carrier concentration	10, 20, 30, 50% (V/V)	30% (V/V)
HCl concentration in the strip phase	1.0, 2.0, 3.0 N	2 N
L-Phe concentration in the feed phase	1 g/L	
Glucose concentration in the mixed solution	3 g/L	

Table 2. Permeator volume

Kind of operation	Feed phase volume	Strip phase volume	Flow rate
batch	760 mL	360 mL	-
continuous strip phase	760 mL	360 mL	2 mL/min (strip)
strip phase concentration	360 mL	760 mL	-
mixed solution of L-Phe and glucose	360 mL	760 mL	-

The L-Phe-carrier complex can diffuse across the supported membrane through the microporous structure. The feed phase is filled with L-Phe and stripping phase a molar solution of hydrochloric acid.

Liquid-Liquid Extraction Experiments

The stoichiometric coefficient of reaction and extraction equilibrium constant are determined at 20 after contacting organic and aqueous solutions until equilibrium. Initially the aqueous phase contained L-Phe with pH=2.0 and the organic phase was prepared by diluting D₂EHPA with heptane. The concentration of dimmeric D₂EHPA in the oil phase was varied from 5 to 15 volume percent.

Analysis

The L-Phe concentration is measured by UV-visible spectrophotometer (Shimadzu 240) at a wavelength of 257.3 nm. Glucose concentration is analyzed by the glucose sensor (Accutrend, Boehringer Mannheim).

MODEL

The complex formation reaction at the aqueous-organic interface is



where B and C are the carrier and carrier-complex, respectively, as shown in Fig. 2. Mass transfer resistance can be neglected in an aqueous phase due to the slow diffusion of the carrier-complex in the organic phase.

At steady-state, the flux is

$$J = -D_e \frac{dC_C}{dz} \quad (2)$$

The concentration gradient of carriers and complexes can be expressed linearly [2].

$$C_B = a_1 z + b_1 \quad (3)$$

$$C_C = -a_2 z + b_2 \quad (4)$$

To obtain unknown values (a_1 , a_2 , b_1 , b_2) in the above equations, the following limited assumptions are used.

1. L-Phe ions are not dissolved by themselves in the membrane phase. Thus the following stoichiometric relationship is maintained at steady-state.

$$J_{\bar{B}} = -nJ_{\bar{C}} \quad (5)$$

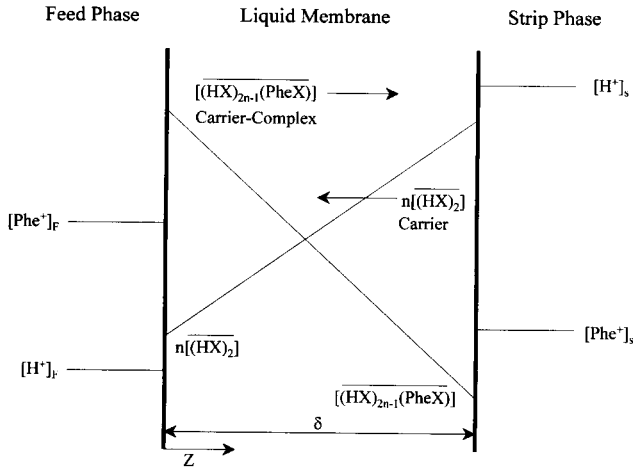


Fig. 2. The counter transport mechanism of L-Phe.

2. Since the carrier is not dissolved at all in the aqueous phase, the total concentration of carrier $(HX)_2$, is conserved.

$$\frac{1}{\delta} \int_0^{\delta} (C_{\bar{B}} + nC_{\bar{C}}) dz = \frac{1}{2} C_{\bar{B}} \quad (6)$$

where d is the thickness of the liquid membrane (100 mm) and the subscript, o , denotes the initial concentration.

3. Reaction equilibrates at the membrane interface due to the fast formation reaction of carrier-complex.

$$Ke = \frac{C_{\bar{C}} \times C_{H^+}}{C_{Phe^+} \times C_{\bar{B}}^n} \quad (7)$$

By combining Eqs. (2) ~ (7),

$$\frac{1}{n(1-\gamma)} [\alpha n(1+\gamma)b_1^n + 2b_1 - C_{\bar{B}}] + \beta \left[\frac{\gamma}{1-\gamma} \left(\frac{\gamma-1}{\gamma} b_1 + 2n\alpha b_1^n - C_{\bar{B}} \right) \right]^n = 0 \quad (8)$$

$$\alpha = Ke \frac{C_{Phe^+}}{C_{H^+}} \quad (9)$$

$$\beta = Ke \frac{C_{Phe^+}}{C_{H^+}^n} \quad (10)$$

$$\gamma = \frac{D_{\bar{C}}}{D_{\bar{B}}} \quad (11)$$

Equations could be reduced as $f(b_1, \alpha, \beta, \gamma, n, C_{\bar{B}_0})$ in Eq. (8). a and b can be determined from the operating conditions. γ is diffusivity ratio of the free carrier to the complex in the membrane phase. Unknown values (a_1, a_2, b_1, b_2) could be determined by iteration of Eq. (8) using Newton-Rapson method [9] and through Eqs. (5)~(10). Using the fluxes obtained by the permeation experiment at various operating conditions, effective diffusivity of carrier complex, D_e , in the SLM can be estimated.

RESULTS AND DISCUSSION

Extraction Experiment

To know the unknown values of variable (n, Ke),

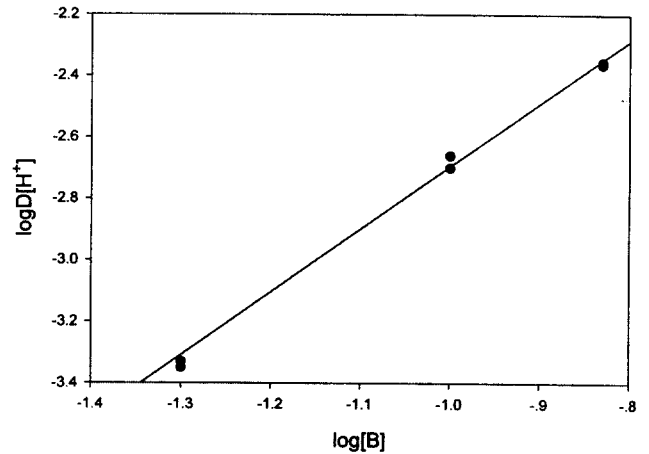
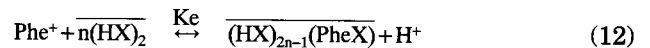


Fig. 3. effective diffusivity estimation by adjusting the model to the experimental data at various pH in the feed phase. $[(HX)_2]_0 = 906 \text{ mol/m}^3$ (30 volume % carrier), $[H^+]_0 = 2N$, initial $[Phe]_0 = 1 \text{ g/L}$; experiment (\circ), estimation (\bullet).

following methods were used. At the aqueous-organic interface, the equilibrium relation is



The equilibrium constant Ke is expressed as

$$so Ke = \frac{[(HX)_{2n-1}(PheX)][H^+]}{[Phe^+][HX]_2^n} \quad (13)$$

Let define the distribution coefficient as

$$D = \frac{[(HX)_{2n-1}(PheX)]}{[Phe^+]} \quad (14)$$

Then,

$$Ke = \frac{D[H^+]}{[HX]_2^n} \quad (15)$$

By taking the logarithm

$$\log D[H^+] = \log Ke + n \log [HX]_2 \quad (16)$$

By plotting $[H^+]$ vs. $[HX]_2$ with the extraction experimental result, n and Ke could be obtained as shown in Fig. 3. Result shows that the reaction stoichiometric coefficient (n) is 2, and the extraction equilibrium constant (Ke) is 0.224 at 293 K. Under the conditions

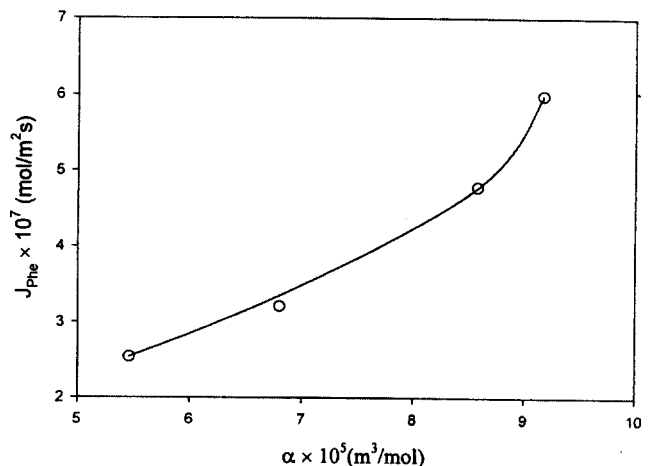


Fig. 4. Result of extraction experiment.

where the initial carrier concentration, C_{B_0} , is known and n and K_e are calculated, the effective diffusivity, D_e , could be calculated from Eq. (4) to Eq. (11). The α and β are determined at the operating conditions. At 30 volume percent carrier concentration, C_{B_0} is 906 mol/m^3 . g is determined as 0.937 based on the Wilke-Chang relation [10]. Permeation experiments of L-Phe is done and L-Phe fluxes are calculated. The values of effective diffusivity (D_e) was estimated as shown in Fig. 4 by comparing the model prediction with the experimental data obtained for two different pH values in the feed solutions. The corresponding estimates of effective diffusivity of D_2 EHPA-Phe carrier complex was calculated as $3.39E-07 \text{ cm}^2/\text{sec}$. This is in agreement with the values found in the similar systems [11-13].

Permeation Experiment

Fig. 5 shows the effect of the pH in the feed solution on the separation performance in permeation experiments. In this system, the difference in hydrogen concentration between the feed and strip phases is the driving force for the transport of L-Phe. If the Donnan equilibrium relation is assumed to be effective [14], $[H]$ and $[Phe]$ ions can be transported across the membrane until equilibrium as follows.

$$\frac{[Phe]_s}{[Phe]_f} = \frac{[H]_{sf}}{[H]_{ff}} \quad (17)$$

To separate and concentrate more L-Phe into the

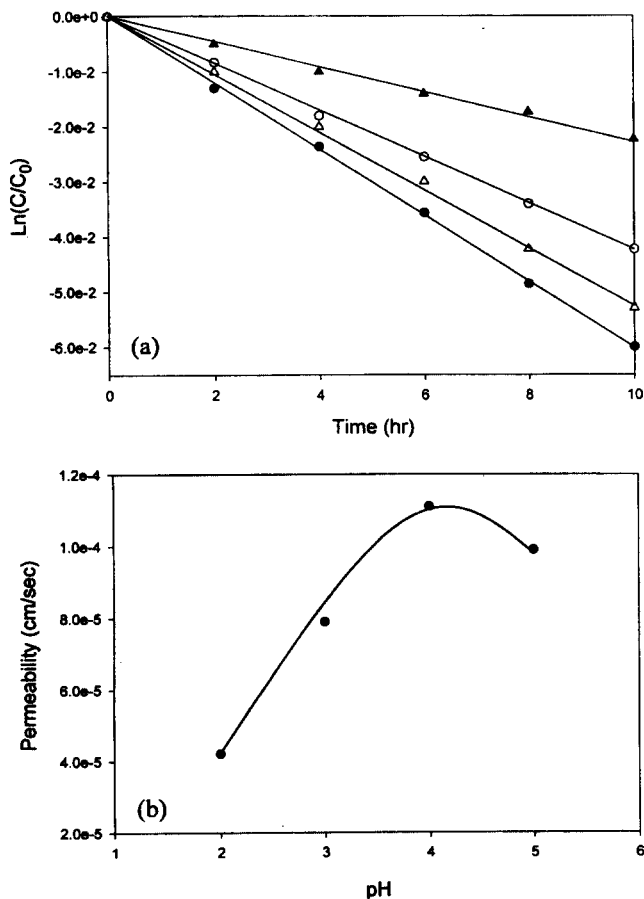


Fig. 5. Effect of pH in the feed phase on permeation of L-Phe; (a) pH=2 (\blacktriangle), pH=3 (\circ), pH=4 (\bullet), pH=5 (\triangle) (b) L-Phe permeability vs. pH in the feed phase.

strip phase, high HCl concentration in the strip phase and high pH in the feed phase are desirable.

L-Phe is a zwitterion with $pK_1=1.83$, $pK_2=9.13$. In the acidic solution, the equilibrium could be expressed based on Hendersian Hasselbalch relation [15].



$$K_1 = \frac{[Phe^0][H^+]}{[Phe^+]} \quad (19)$$

By taking logarithm,

$$\log \frac{[Phe^0]}{[Phe^+]} = -pK_1 + pH \quad (20)$$

The total concentration of dissolved L-Phe, C , is

$$[Phe] = [Phe^\pm] + [Phe^+] \quad (21)$$

By combining Eqs. (20) and (21),

$$[Phe^+] = \frac{[Phe]}{1 + 10^{(pH - pK_1)}} \quad (22)$$

So the $[Phe^+]$ in the feed solution can be calculated. When a cation carrier is used, L-Phe should exist as a cation to be separable. As pH in the feed phase increases, i.e. the increase of driving force, the permeability of L-Phe should be increased. But the fraction of positively charged L-Phe which can make carrier-complex is decreased as pH increases. By these two competing effects, optimal pH which makes L-Phe transport rate to be fastest should exists in this system.

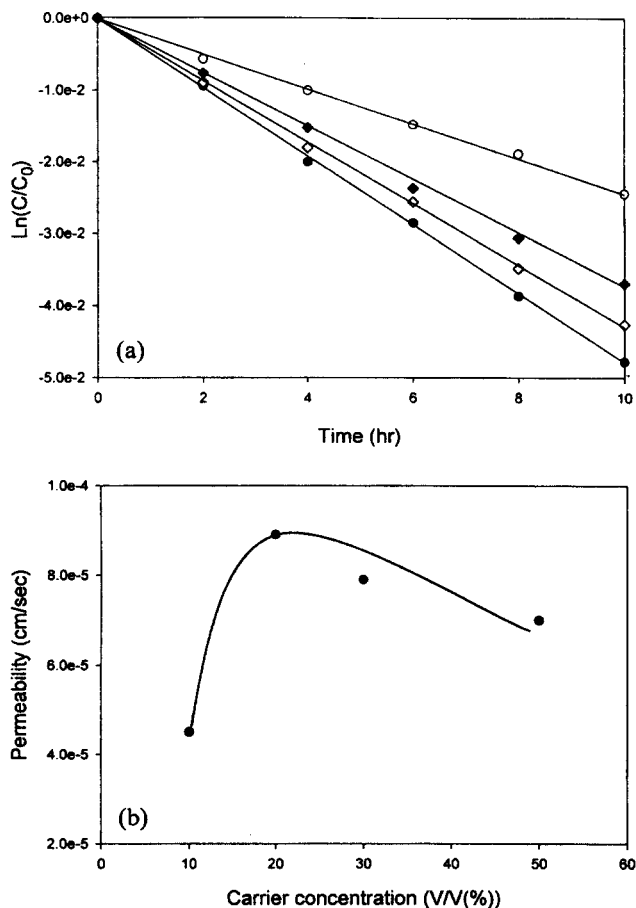


Fig. 6. Effect of carrier concentration on permeation of L-Phe; (a) 10% (\circ), 20% (\bullet), 30% (\diamond), 40% (\blacklozenge) (b) L-Phe permeability vs. carrier concentration.

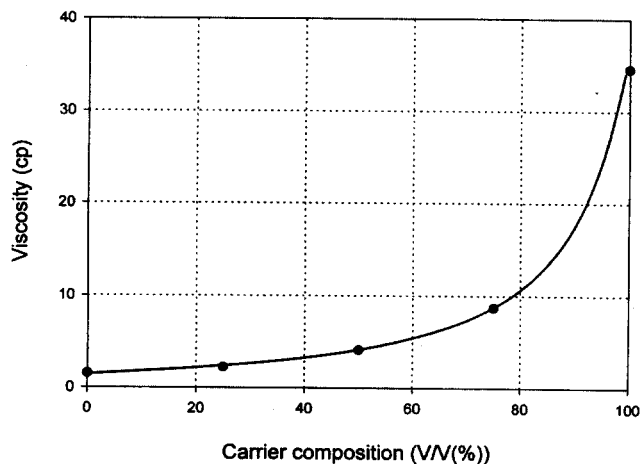


Fig. 7. Effect of carrier composition on viscosity.

Result shows that the transport rate of L-Phe is increased as pH increases up to 4.0. The separation performance was reduced above pH=4.0. Thus it could be determined that the initial pH 4.0 in the feed phase is optimal to enhance the separation of L-Phe.

The effect of carrier concentration on L-Phe transport rate is shown in Fig. 6. Based on Donnan equilibrium equation, the change of carrier concentration does not change the final equilibrium condition of the system but does affect how fast equilibrium is reached. It is expected that the transport rate of L-Phe would be increased as the carrier concentration increases because of the existence of large amount of carrier molecules. But the transport rate was decreased as carrier concentration increased more than 20% of carrier concentration. This behavior can be interpreted in terms of the viscosity of carrier solution. As the carrier concentration increases, the viscosity is increased exponentially as shown in Fig. 7. The organic phase viscosity is a controlling parameter for the optimal carrier concentration in a liquid membrane system [16]. High carrier concentration decreases mass transfer coefficient of carrier-complex due to the high viscosity. In these two combined effects, the existence of optimal carrier concentration is explained. It can be concluded that 20% carrier concentration seems to be optimal for this system as shown in Fig. 6.

Fig. 8 shows the comparison of L-Phe transport rate

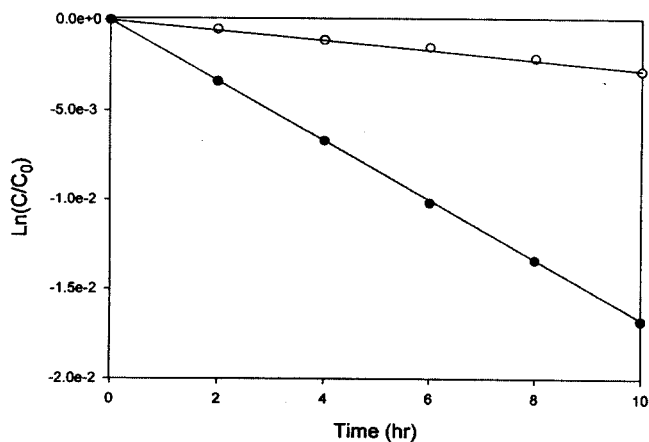


Fig. 8. Effect of carrier type on permeation of L-Phe; D₂EHPA (●), PC88A (○).

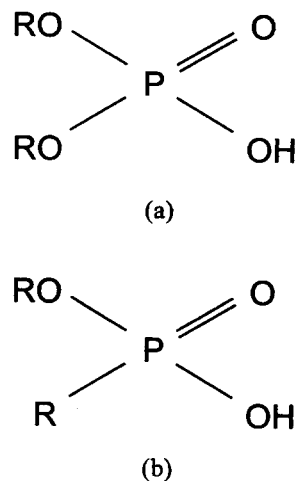


Fig. 9. Structure of carriers (R=C₄H₉CH(C₂H₅)CH₂); (a) D₂EHPA (b) PC88A.

using D₂EHPA and PC88A (HEH (EHP), 2-ethylhexyl hydrogen 2-ethylhexyl phosphate). The structure of D₂EHPA and PC88A are shown in Fig. 9. For the given same typical condition, D₂EHPA led to more than 10 times higher permeation rate than PC88A. It is expected that the difference of the chemical properties of carriers might lead to the difference of the distribution coefficient of L-Phe and carrier-complex.

The effect of HCl concentration in the strip phase on

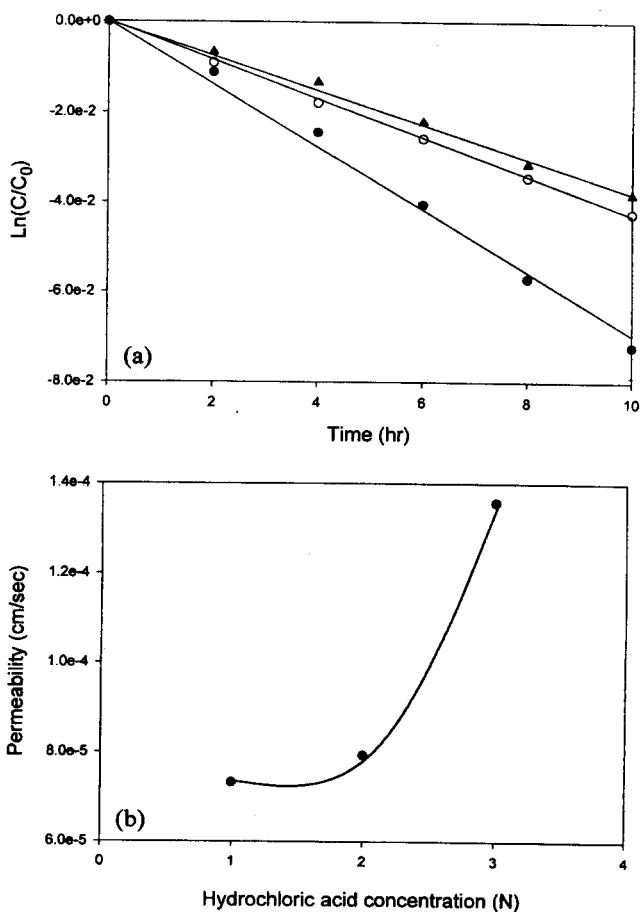


Fig. 10. Effect of HCl concentration in the strip phase on permeation of L-Phe; (a) 1N (▲), 2N (○), 3N (●) (b) L-Phe permeability vs. HCl concentration in the strip phase.

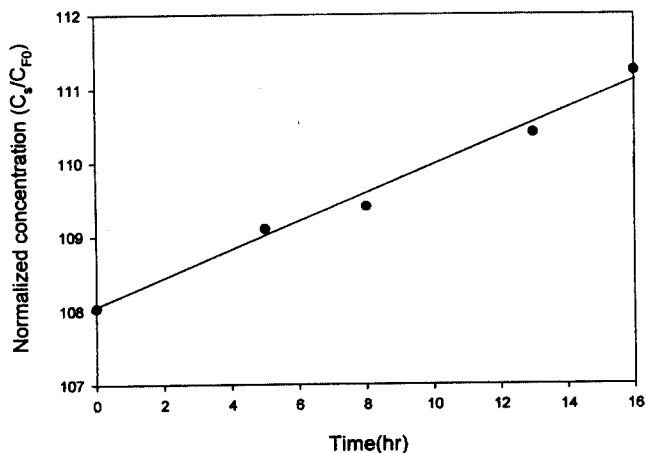


Fig. 11. Time course change of normalized L-Phe concentration in the strip phase.

L-Phe transport rate is shown in Fig. 10. As mentioned earlier, the difference in $[H^+]$ between the feed and strip phase is the driving force in the SLM process. The L-Phe transport rate is increased as HCl concentration increases because of the increase of the H^+ ion concentration gradient between feed and strip phase. In the LEM operation, the transport rate could not be increased over 1.6 N HCl concentration in the internal phase due to the osmotically induced swelling and breakage of LEMs [8]. But separation efficiency in SLM was enhanced up to 3 N HCl concentration in the strip phase without breakage of membrane as shown in Fig. 10.

Fig. 11. shows the result of concentration experiment in the SLM. Initial L-Phe concentrations in the feed and strip phase are 0.1 g/L and 10 g/L, respectively. Though the L-Phe concentration in the strip phase is 100 times higher than that in the feed phase, L-Phe was transported from feed to strip phase and then L-Phe concentration in the strip phase was increased. This is due to the H^+ ion concentration gradient between feed phase ($[H^+]=0.001$ N) and strip phase ($[H^+]=2$ N). Based on the Donnan equilibrium relation, L-Phe could be transported through the membrane against concentration gradient of L-Phe at this condition. As the feed phase volume becomes larger than that of strip phase and as HCl concentration in the strip phase becomes higher, it is expected that more concentrated L-Phe solution is obtained in the strip phase. In the LEM experiment, when the volume ratio of external to internal phase was 10, it was possible to separate and concentrate L-Phe in the internal phase eight times higher than the initial concentration of the external phase [8]. In the SLM process, more concentration of L-Phe to the strip phase would be possible than the LEM process because of no swelling and breakage of membrane phase at high H^+ concentration as shown in Fig. 11. In the stability of SLM, the separation performance of SLM does not change more than 50 hours of operation (Data are not shown). After 50 hours operation, leakage of membrane phase solution occurred. By re-immersing the membrane to the carrier solution, the liquid membranes could be reused.

Effect of Glucose

In the separation of amino acid in the fermentation

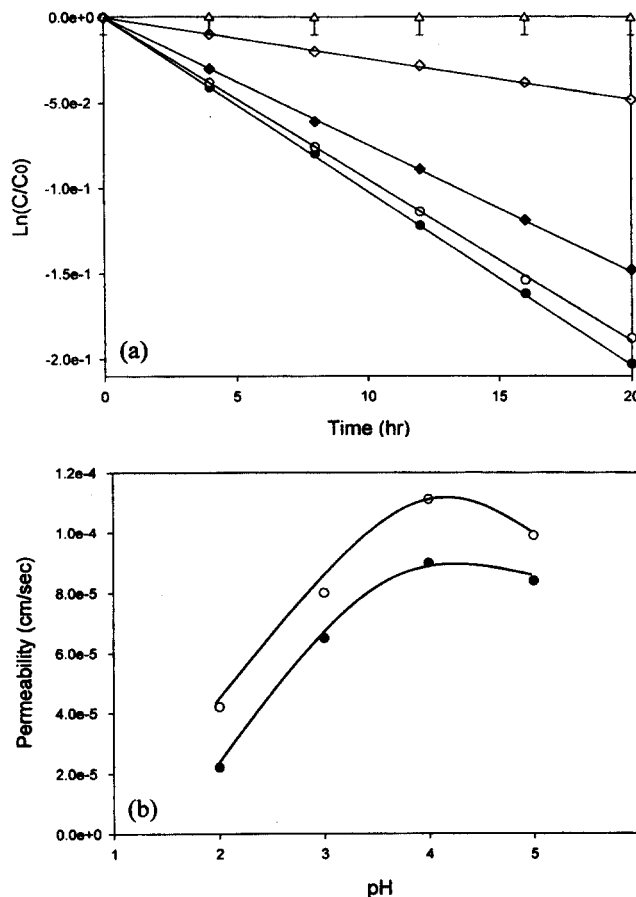


Fig. 12. Effect of pH in the feed phase on permeation of L-Phe in L-Phe and glucose mixed solution; (a) pH=2 (\diamond), pH=3 (\diamond), pH=4 (\bullet), pH=5 (\circ), glucose (\triangle) mixed permeability vs. pH in the feed phase, glucose mixed solution (\bullet), pure L-Phe solution (\circ).

process, there exist impurities including the substrate in the medium. These impurities are the main problems for separation and purification of amino acid. Since glucose is generally used as a substrate, it is the main impurity. Thus in this experiment, the selectivity of L-Phe toward glucose was examined and the results are presented in Fig. 12(a). The transport rate of L-Phe is highest at pH=4.0 as shown in Fig. 12(a). The overall trend and optimal condition of pH are similar to the results of single component permeation experiment in Fig. 5. The glucose permeation rate is negligible in comparison with L-Phe permeation rate but the existence of glucose decreased the L-Phe permeation rate about 30% as shown in Fig. 12(b). The reason is thought to be the effect of glucose on liquid-liquid extraction between L-Phe and L-Phe-carrier complex. When glucoses interact with the cation carrier (D 2EHPA) at the aqueous-organic interface, parts of the carrier molecules becoming unavailable. So the distribution coefficient of L-Phe would decrease.

Separation with Continuous Removal and Replenishment of Strip Phase

Fig. 13 illustrates the separation performance using two different types of operation. In operation with continuous strip phase, the strip phase concentration of L-Phe is maintained at low-level by continuous withdrawal of L-Phe permeated and supply of fresh HCl

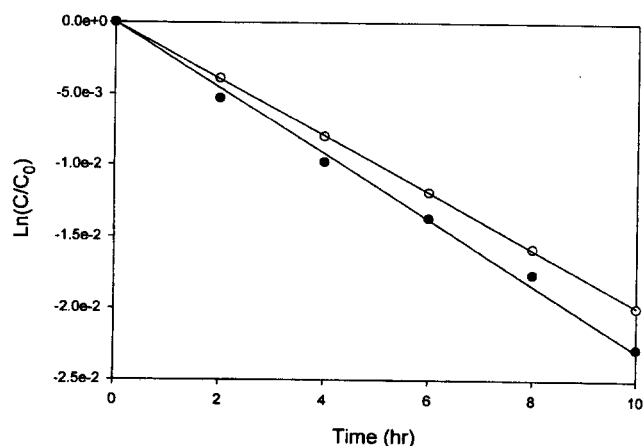


Fig. 13. Comparison of batch and continuous strip phase on permeation of L-Phe; batch strip phase (○), continuous strip phase (●).

solution. Since L-Phe concentration gradient between the feed and strip phases can be kept constant by the dilution effect of the strip phase, the permeation rate increased.

CONCLUSIONS

In this study, the carrier mediated SLM process is proposed to separate and concentrate the L-Phe having zwitterion characteristics from aqueous solutions. From the extraction experiment, the reaction stoichiometric coefficient is 2 and the extraction equilibrium constant is 0.224 at 293 K. A mathematical model of carrier mediated transport was proposed. Based on the proposed model, $3.39E-7$ cm²/sec was estimated as the effective diffusivity of carrier-complex through the SLM. The effects on L-Phe transport of various operating parameters such as pH in the feed phase, the carrier type and concentration and hydrochloric acid concentration in the strip phase have been examined and optimal conditions were found in permeation experiment. In the concentration experiment, it was found that the SLM process is more effectively satisfied Donnan equilibrium relation than the LEM due to no breakage and swelling of membrane phase. And the stability of SLM was maintained up to 50 hours operation. Separation of L-Phe experiments from glucose contained feed solution have been performed. The optimal operating conditions were not changed but the separation performance was lowered significantly due to the existence of the competitive material. Separation with continuous strip phase gives more enhanced permeation rate of L-Phe due to the continuous dilution effect of strip phase.

NOMENCLATURE

L-Phe: L-phenylalanine

$(HX)_2, \bar{B}$: D₂EHPA in the membrane phase (mol/m³)

$(HX)_{2n-1}PheX, \bar{C}$: carrier complex in the membrane phase (mol/m³)

D: diffusion coefficient (mol/m²sec)

$C_{\bar{B}}$: concentration of carrier (mol/m³)

$C_{\bar{C}}$: concentration of carrier complex (mol/m³)

n: reaction stoichiometric coefficient

H_{fr} : final feed phase hydrogen ion concentration (mol/

m³)
 H_{sf} : final strip phase hydrogen ion concentration (mol/m³)
 C: concentration (mol/m³)

Greek symbols

$$\alpha = Ke \frac{C_{Phe, f}}{C_{H^+, f}} \quad (\text{m}^3/\text{mol})$$

$$\beta = Ke \frac{C_{Phe, s}}{C_{H^+, s}} \quad (\text{m}^3/\text{mol})$$

$$\gamma = \frac{D_C}{D_B}$$

δ : thickness of liquid membrane (m)

Subscript

f: feed phase

s: strip phase

0: initial

Superscript

-: species in the organic phase

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