SEPARATION AND CONCENTRATION OF TRYPTOPHAN BY EMULSION LIQUID MEMBRANE

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ABSTRACT

Extraction equilibrium and batch extraction of L-tryptophan (Trp) by emulsion liquid membrane systems were studied. The membrane phase consists of cation carrier, di (2-ethylhexyl) phosphoric acid (D2EHPA) and the surfactant Span 80 dissolved in n-dodecane and aqueous solution of 1N HC1 solution in the internal phase. Both the effect of initial external phase pH on the extraction efficiency and the effect of pH in the feed solution on the distribution coefficient of L-tryptophan (D^+) from the extraction equilibrium are reported. The average K_{ex} for Tryptophan for this system is 0.11 dm^3/mol.

INTRODUCTION

Solvent extraction is one separation technique of quite wide applicability in the process industries but it often suffers from the cost of the material and equipment requirement for the extraction and subsequent recovery operations. Li [1-2] showed that some of the limitations of solvent extraction could be removed by using liquid membrane extraction or emulsion liquid membrane (ELM) technique in which the extraction and product stripping operations are combined in one stage. This has led to considerable interest in developing novel and more efficient processes. Many researchers have actively applied this technique to the separation of heavy metals, phenols and organic acids [3-5]. Recently the applications of ELM in bioseparations and biomedical use have become active. Liquid membrane extraction could have great potential for applications in biotechnology and for recovery of fermentation products [5-6].

This article presents, firstly, the equilibrium extraction of L- tryptophan (Trp) with D2EHPA and secondly, batch extraction of Trp by an emulsion liquid membrane system.

The Emulsion Liquid Membrane Systems

There are 2 types of emulsion liquid membrane systems; water/oil/water (w/o/w) and oil/water/oil (o/w/o). In case of w/o/w system, the oil phase acts like a membrane between the internal aqueous phase and the external aqueous phase. The external phase is the feed solution and the internal phase is the concentrated product solution. Emulsion liquid membrane is usually prepared by first forming an emulsion between two immiscible phase, and then dispersing this emulsion in a third or continuous phase by mild agitation. In general the internal phase

droplets are small, having diameters of 1-10 μm, whereas the emulsion, globules are usually about 0.1-2 mm in diameter. The size depending on the physical properties of the continuous and dispersed phases and the agitation speed (Figure 1).

The separation of a mixture or removal of a solute from the external continuous phase can be effected in two different ways [7]. The first one is called Type I transport which is a simple diffusion process in which the solute partitions into the membrane phase from the exterior phase, diffuses across the membrane to the dispersed interior phase droplets, and partitions into the interior phase. A reaction takes place in the internal phase that converts the solute into a species which is incapable of partition back into the membrane phase. The second type of the transport process is Type II or facilitated transport that is applied for membrane insoluble material such as charged species, e.g. metal ions., organic acids and zwitterions. This mechanism is commonly known as a carrier-mediated transport. By introducing a "carrier" molecule into the membrane phase, the solute solubility is increased by the reversible formation of a membrane carrier-solute complex. This results in faster mass transfer rates. The mass transfer mechanism of amino acid extraction by emulsion liquid membrane system is Type II or facilitated transport.

At the end of an extraction run, the emulsion and aqueous feed phases are separated. The reacted internal reagent phase can be recovered, if desired, by breaking the emulsion. One disadvantage of this system is swelling due to the transportation of water into the internal phase that results in a decrease in the degree of concentration of the solute inside the membrane [8].

MODEL SYSTEM

The model solute in this study is L-tryptophan (Figure 2). Like all α-amino acid, L-tryptophan is a zwitterion and its zwitterionic character imparts unique acid/base characteristics to the species. The charge of amino acid will be changed depending on pH as follow:

$$\underset{\substack{NH_3^+ \\ (A^+)}}{R\text{-}CHCOOH} \xrightarrow[pH<3]{K_1} \underset{\substack{NH_3^+ \\ (A^\pm)}}{R\text{-}CHCOO^-} + H^+ \quad \text{—— (a)}$$

$$\underset{\substack{NH_3^+ \\ (A^\pm)}}{R\text{-}CHCOO^-} \xrightarrow[pH<9]{K_2} \underset{\substack{NH2 \\ (A^-)}}{R\text{-}CHCOO^-} + H^+ \quad \text{—— (b)}$$

$$K_1 = \frac{[A^\pm]\,[H^+]}{[A^+]} \quad \text{—— (c)}$$

(Phe : $K_1 = 10^{-1.83}$ mol/dm^3,
Trp : $K_1 = 10^{-2.38}$ mol/dm^3)

$$K_2 = \frac{[A\text{-}]\,[H^+]}{[A^\pm]} \quad \text{—— (d)}$$

(Phe : $K_2 = 10^{-9.13}$ mol/dm^3,
Trp : $K_2 = 10^{-9.39}$ mol/dm^3)

Where A^+, $A^\pm$ and A are the cation, zwitterion and anion of amino acid respectively, K_1 and K_2 are the dissociation constants of amino acid.

Since tryptophan is insoluble in the oil phase, an ion exchange carrier must be added to the membrane phase in order to solubilize tryptophan into the oil or membrane phase and transport it to the internal phase. The cation carrier D2EHPA (Figure 3) is solubilized in the membrane phase and its aqueous solubility is extremely low. As shown schematically in Figure 4, the D2EHPA first exists as a carrier/proton complex. When the carrier reaches the interface between the external and membrane phases, an ion exchange reaction takes place and the carrier makes a complex with Trp^+. Although the actual structure of the complex may be complicated, a simplified structure is shown in Figure 4.

The carrier/Trp complex then diffuses though the membrane to the interface between the internal and the membrane phases. Another ion exchange reaction takes place, the cation/Trp complex must release the Trp^+ and the carrier is immediately protonated. These processes are repeated and the Trp is thus separated and concentrated in the internal phase.

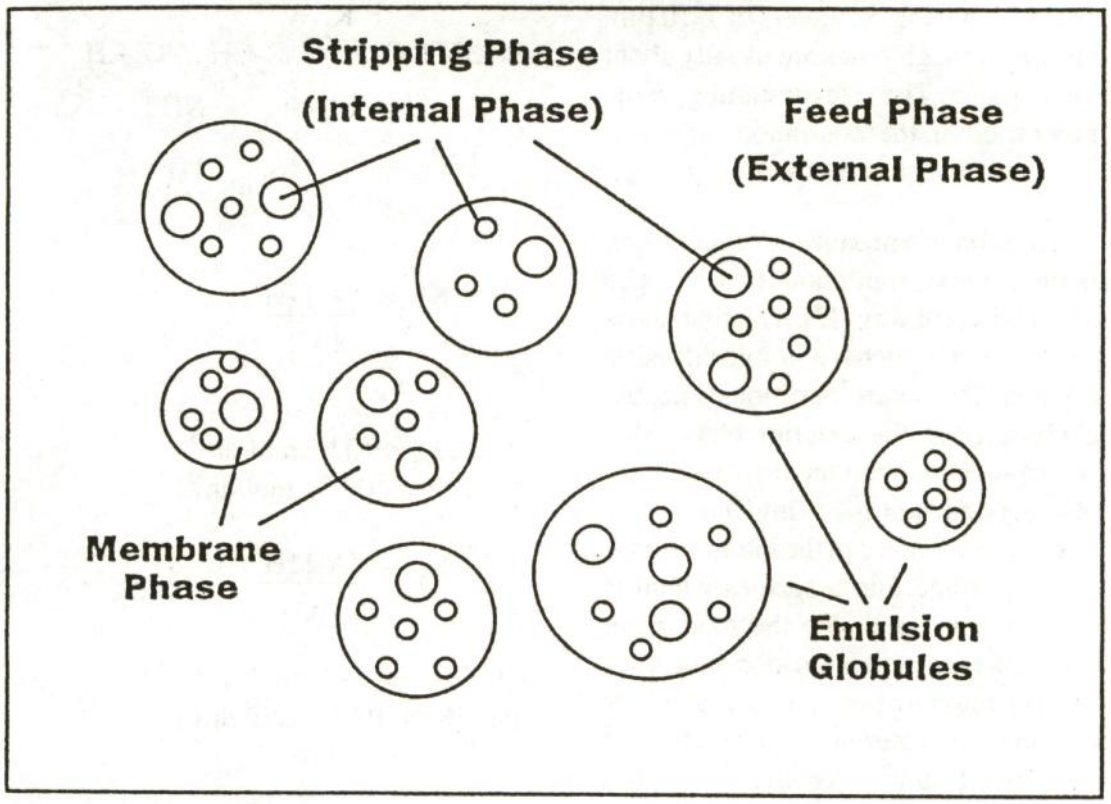

Figure 1 Schematic diagram of emulsion liquid membrane.

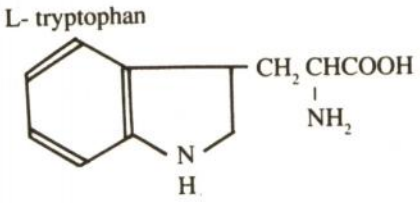

Figure 2 Chemical structure of L-tryptophan.

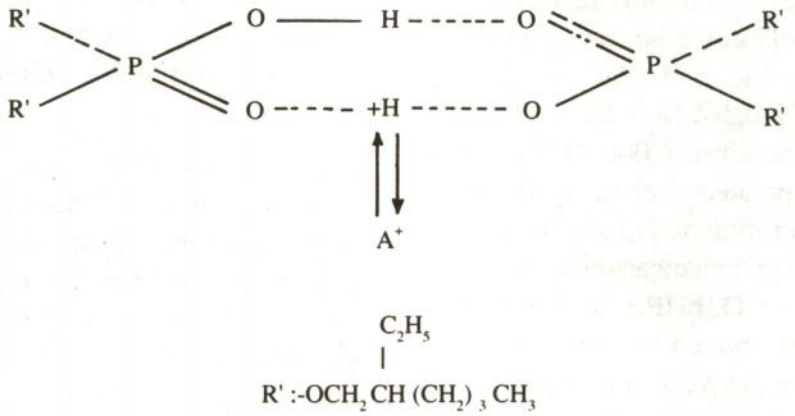

Figure 3 Di (2-ethylhexyl) phosphoric acid (D2EHPA).

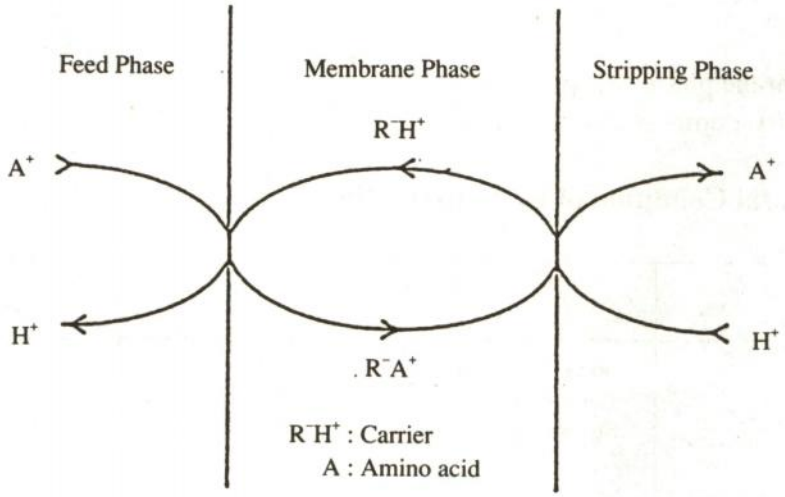

Figure 4 Schematic diagram of the transport mechanism for amino acid .

1. Experimental

1.1 Extraction equilibrium

Experiment on the extraction equilibrium of tryptophan were carried out by mixing 20 ml of organic membrane phase and 20 ml of aqueous phase and then shaking for 48 hr at 25°C by Thermostatic shaking water bath (Model T-225, Thomas Kaguker, Co. Ltd.) Organic membrane phase was prepared by dissolving D2EHPA in n-dodecane. The pH in the aqueous were adjusted with hydrochloric acid. The membrane phase contained 0.072-0.361 M of D2EHPA and the aqueous phase contained 0.006 M Trp. The two phases were separated after being allowed to settle. The concentration of Trp and the pH in the aqueous phase were then measured. The concentration of Trp and D2EHPA in the membrane phase were determined by the difference of Trp concentrations between initial and final external phase. Trp were measured by a UV-Spectrophotometer (Hitachi 320) at wavelength of 277.0 nm.

1.2 Extraction with emulsion liquid membranes

The membrane phase was prepared by blending all necessary components in advance. The organic liquid membrane solution consisted of n-dodecane, D2EHPA and Span 80. The experimental conditions of emulsion liquid membrane system were shown in table 1.

The emulsion was prepared by homogenizing an equal volume of 60 ml of internal phase and 60 ml of membrane phase with high speed homogenizer. About 360 ml of emulsion was prepared at each set of experiment. The W/O emulsions (50 ml) thus prepared were poured and dispersed in a baffled vessel containing a measured volume of external phase (350 ml) of 0.006 M tryptophan solution.

The vessel was 9 cm in diameter and was equipped with a six-bladed turbine stirrer. There were 6 samples for one set of experiment. The extraction time for each sample was started from the time that emulsion was poured into the external phase. After each extraction, all solution was removed from the vessel. Then, the emulsion phase and external phases were separated after being allowed to settle. The volumes of each phase were measured and tryptophan concentration in the external phase were measured by UV-spectrophotometer at wavelength of 277.0 nm.

Table 1 Experimental Condition of ELM Extraction

External Phase (Feed Phase)	Membrane Phase		Internal Phase (Stripping Phase)
0.006 M Trp adjusted pH to 2.0, 3.0 and 5.0 with HC1	Solvent: n-Dodecane Carrier: D2EHPA Surfactant: Span 80	176 ml 10 ml 4 ml 190 ml	1.0 N HC1

RESULTS AND DISCUSSIONS

1. Extraction Equilibrium

In this case it is assumed that Trp+ or A+ (in the equation) form a complex with D2EHPA which exist as the dimer form as follow:

$$A^+ + {}_m(\overline{HR})_2 \rightleftharpoons \overline{AR(HR)_{2m-1}} + H^+ \quad \text{——(1)}$$

$$K_{ex} = \frac{[A^+]_{eq}\,[(\overline{HR})_2]^m{}_{eq}}{[AR(\overline{HR})_{2m-1}]_{eq}\,[H^+]_{eq}} \quad \text{—— (2)}$$

when $(HR)_2$ is the dimer of D2EHPA in the menbrane phase and $_m$ is the stoichiometric

coefficient and $\overline{AR(HR)}_{2m-1}$ is the carrier/Trp complex in the membrane phase, since the proton concentration is much higher than K_2, the dissociation constant of amino group. The formation of A^- can be neglected. Then the total amino acid concentration (A_T) is expressed by

$$[A_T]=[A^+]+[A^\pm] \quad \text{——} \quad (3)$$

The distribution coefficient of amino acid in the cationic form is expressed by:

$$D^+ = \frac{[\overline{AR(HR)_{2m-1}}]_{eq}}{[A^+]_{eq}} \quad \text{——} \quad (4)$$

from equation (2),

$$\frac{K_{ex}[\overline{(HR)_2}]^m_{eq}}{[H^+]_{eq}} = \frac{[\overline{AR(HR)_{2m-1}}]_{eq}}{[A^+]_{eq}} \text{——} (5)$$

from equation (4) and (5),

$$D^+ = \frac{K_{ex}\,[\overline{(HR)_2}]^m_{eq}}{[H^+]_{eq}} \quad \text{——} \quad (6)$$

$$\log D^+ = \log K_{ex}\,\overline{[(HR)_2]}^m_{eq} - \log [H^+]_{eq} \text{——} (7)$$

$$\log D^+[H^+]_{eq} = \log K_{ex} + m \log \overline{[(HR)_2]}_{eq} \text{——} (8)$$

equation (9) can be obtained from equation (b) and (3) as follows:

$$[A^+]_{eq} = \frac{[H^+]_{eq}\,[A_T]_{eq}}{[H^+]_{eq} + K_1} \quad \text{——} \quad (9)$$

based on the assumption that one mole of tryptophan reacted with two moles of dimeric form of D2EHPA, the following mass balance equation can be obtained;

$$[(HR)_2]_{eq} = [(HR)_2]_i - 2([A_T]_i - [A_T]_{eq}) \text{——} (10)$$

$$[AR(HR)_{2m-1}]_{eq} = [A_T]_i - [A_T]_{eq} \quad \text{——} \quad (11)$$

From the above equations, the value of K_{ex}, D^+ and $[(HR)_2]_{eq}$ can be calculated. Figure 5 shows the relationship between distribution coefficient of Trp^+ (D^+) and $[H^+]_{eq}$. As can be seen from the equation, the slope of the graph is -1 which is corresponding to equation (7).

According to equation (8), ***m*** is the value of slope of the graph of $\log D^+[H^+]_{eq}$ vs. $\log [(HR)_2]_{eq}$. As shown in Figure 6, the slope of this graph is **2.2** indicating that the value for ***m*** is **2.2**. By using the value of $K_1 = 10^{-2.38}$ or 4.169×10^{-3} mol/dm^3, the calculated value of K_{ex} for Trp is 0.11 dm^3/mol. The value of K_{ex} that have been reported by Teramoto et. al. (9) is 0.055 dm^3/mol. In their study, the concentration of tryptophan is 0.005 M.

2. Permeation of Tryptophan

It's been known that the ionic structure of Trp changes significantly with changes in pH. As long as a cation carrier is used, Trp must exist as cation to be separable. Therefore, in this system, a low pH is desirable. However, in order to separate and concentrate more Trp into the internal phase, a large difference of $[H^+]$ at initial stage between internal and external phases must be established. That is a low pH in the internal phase and a high pH in the external phase are desirable. These is a possibility that due to the high pH in the external phase, the Trp will not be able to dissociate as cation. On the other hand, if the pH in the external phase is too low, the carrier will become protonated and thus unable to transport other ions. Since pH in the external phase is thus important, the experiments under various pH values in the external phase were carried out. The results are shown in Figure 7, As the pH of the feed solution is increased, the

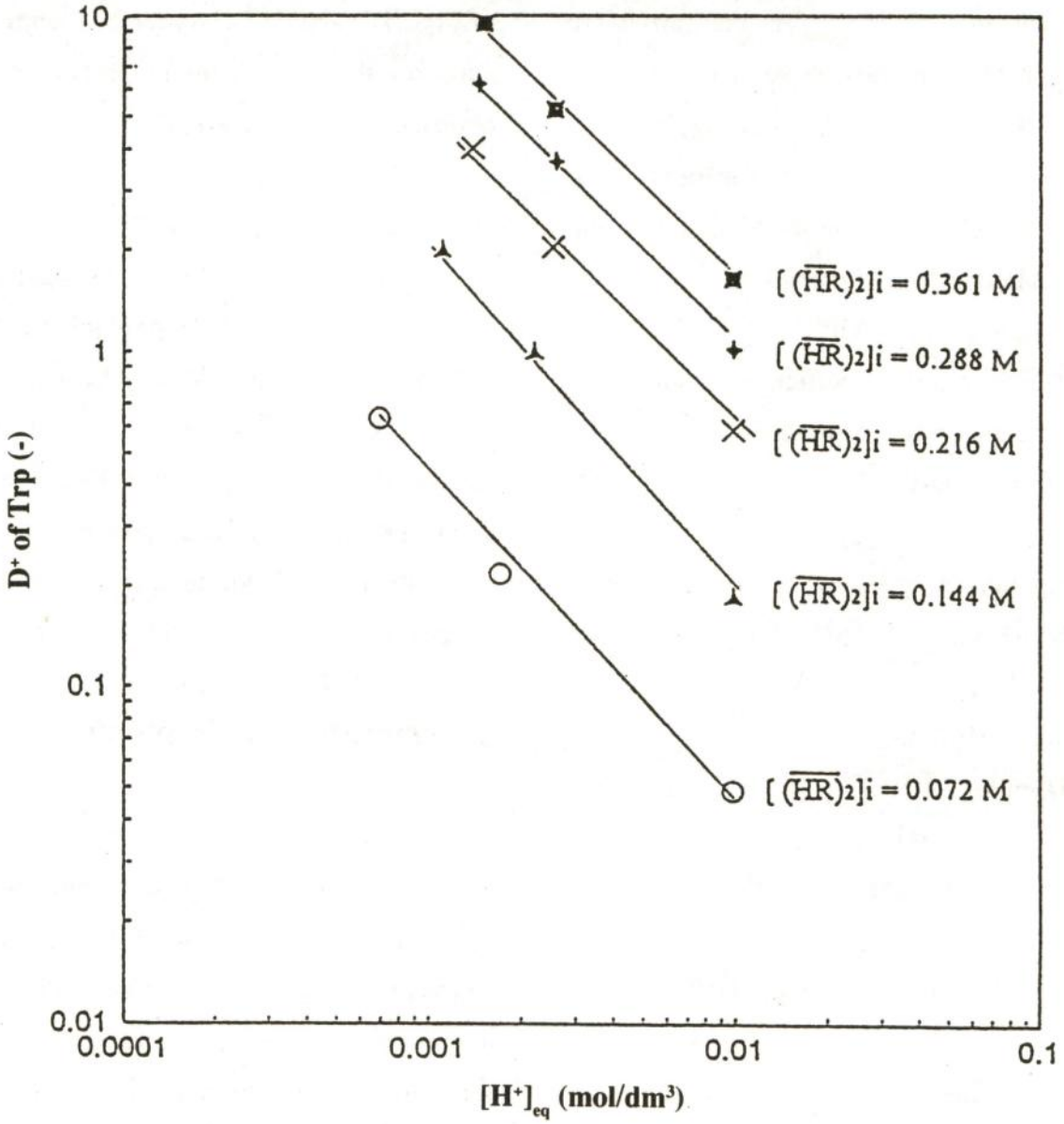

Figure 5 Distribution Coefficient of Trp^+ (D^+) vs. $[H^+]_{eq}$.

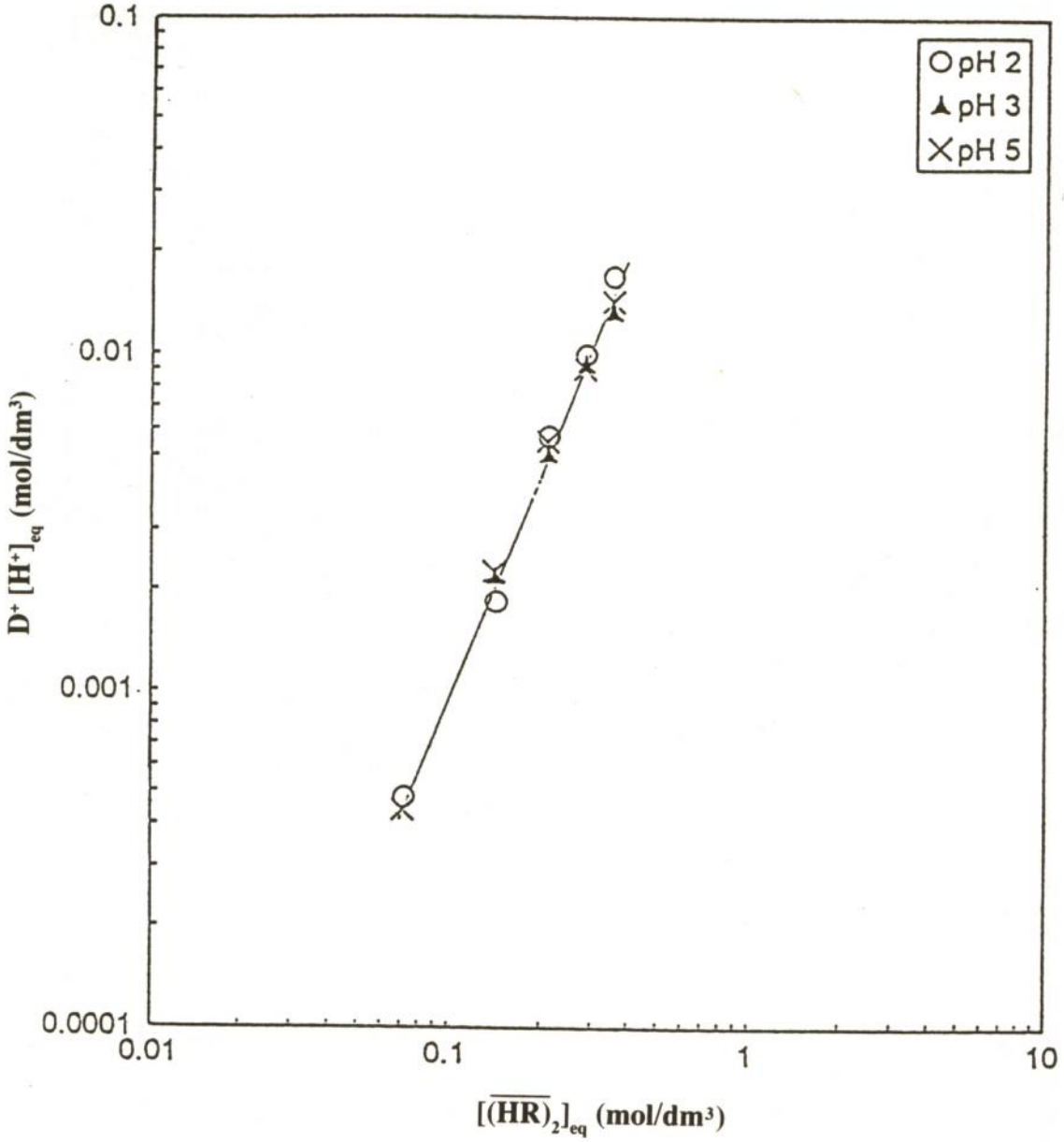

Figure 6 $D^+[H^+]_{eq}$ vs. $[(HR)_2]_{eq}$ of Tryptophan.

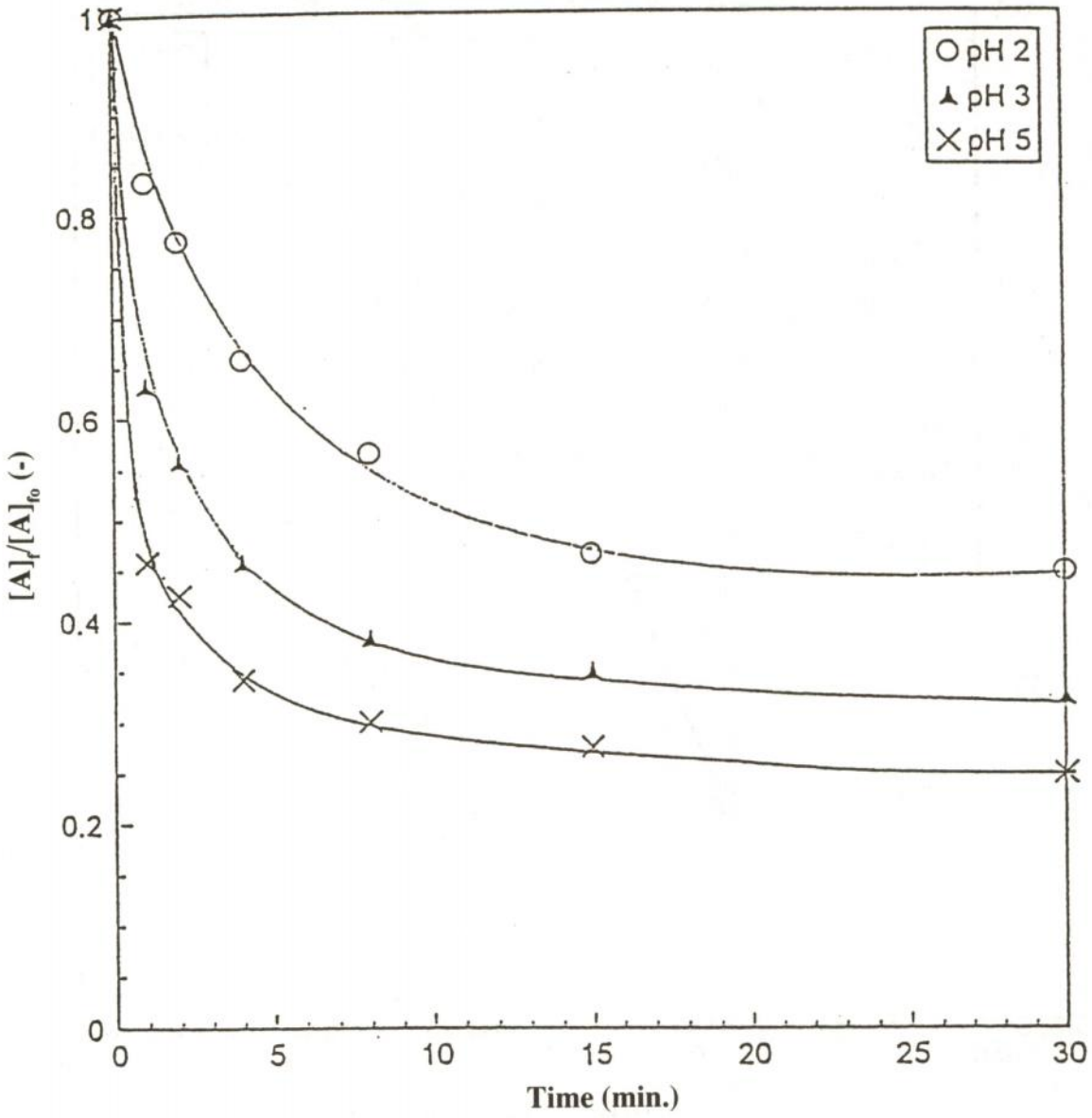

Figure 7 Extraction of 0.006M Tryptophan by Emulsion Liquid Membrane.
$[A]_f$ = Amino Acid Concentration in the Feed phase.
$[A]_{fo}$ = Initial Amino Acid Concentration in the Feed phase.

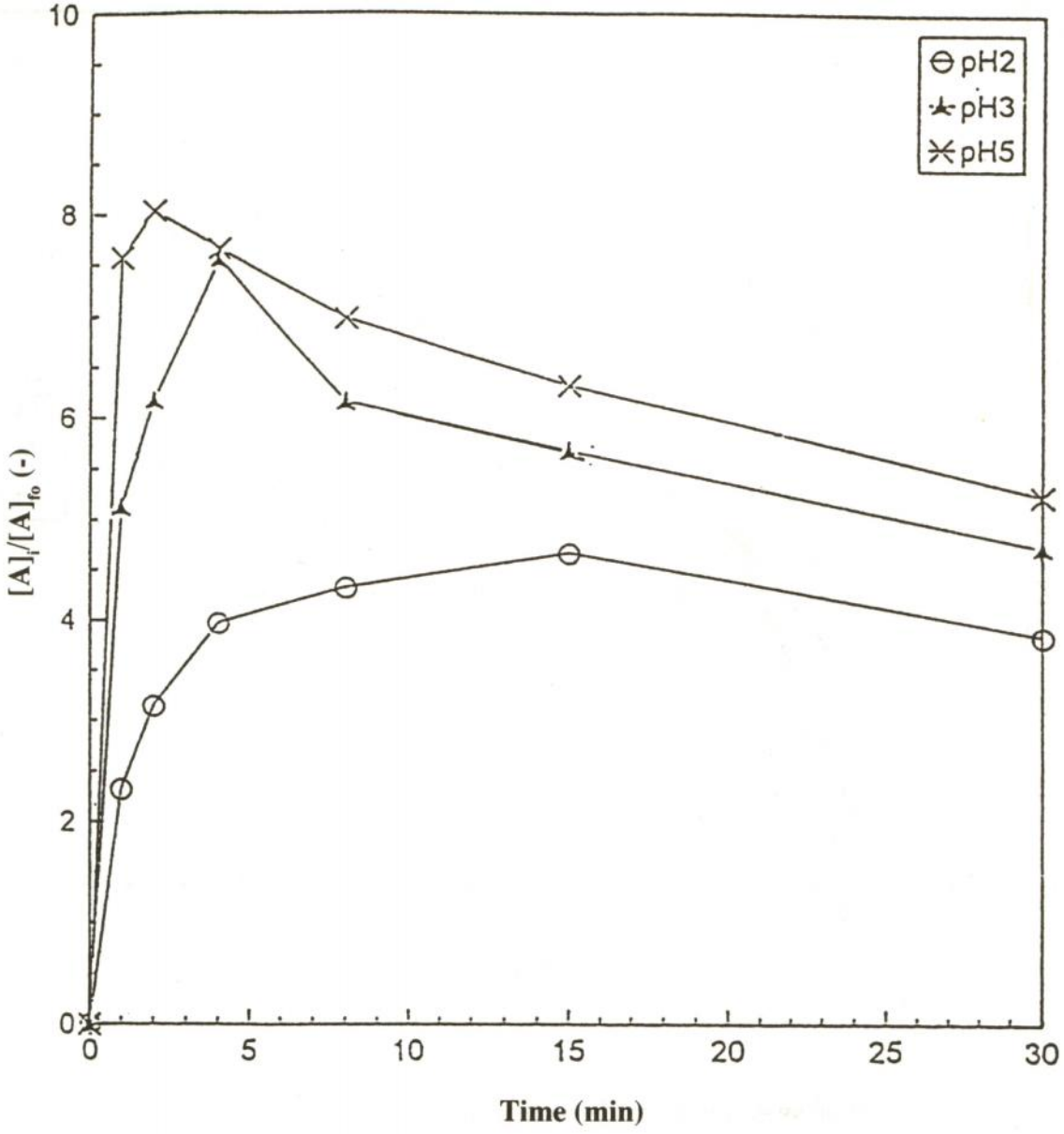

Figure 8 Concentration of 0.006M Tryptophan in the Internal phase.
$[A]_i$ = Amino Acid Concentration in the internal phase.
$[A]_{fo}$ = Initial Amino Acid Concentration in the Feed phase.

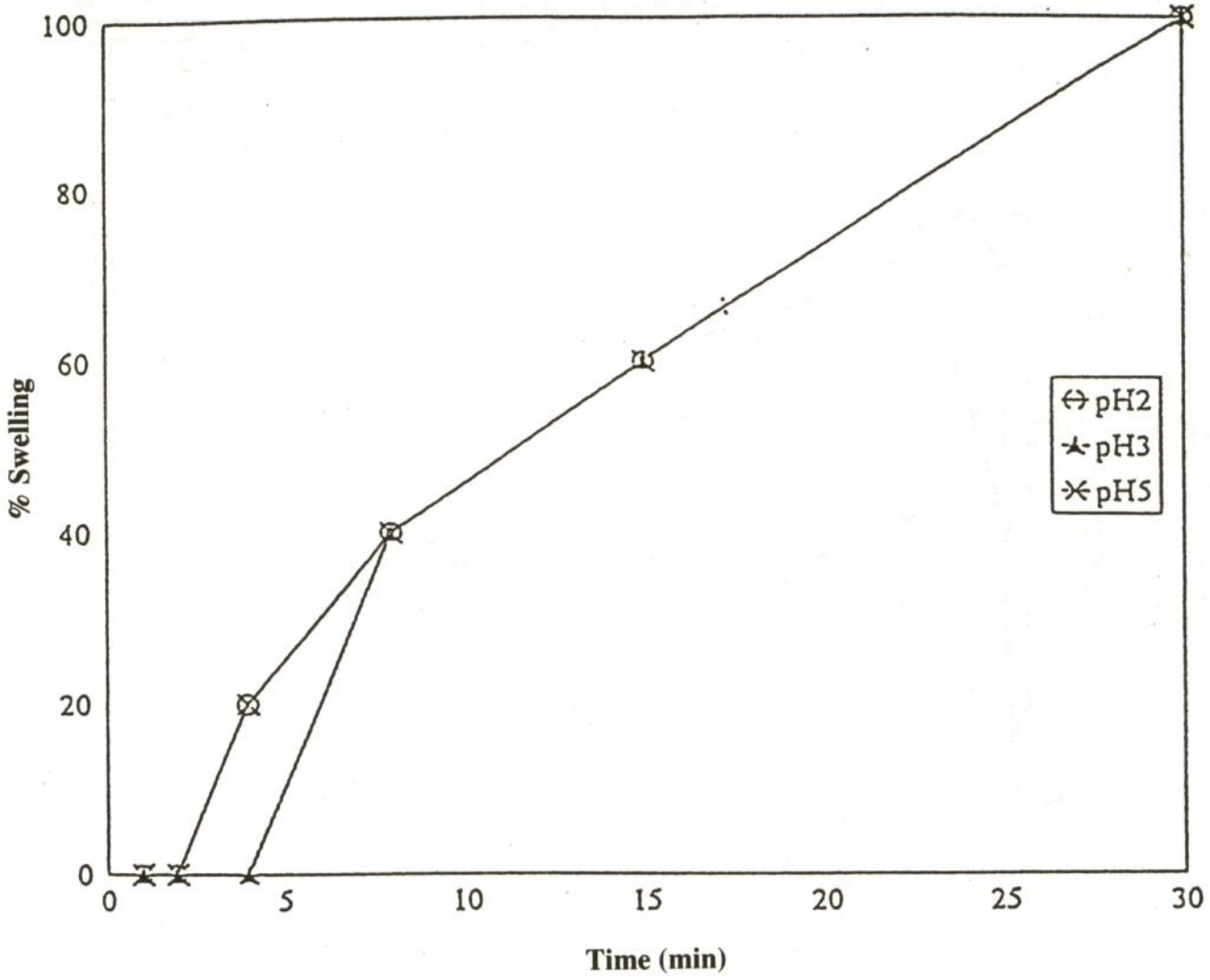

Figure 9 Effect of pH on swelling in emulsion liquid membrane extraction of 0.006M tryptophan solution.

distribution ratio of Trp increased, which resulted in an increase of the extraction rate.

As can be seen, the initial transport rate of Trp was high, gradually decreased as the driving force decreased. It should be noted that during the separation, swelling and breakage occurred at approximately the same time. Water and Trp are transported from the external phase into the internal phase while the emulsion gradually undergoes breakage. As a result, the swelling causes the dilution of the internal phase. The breakage causes the decrease of system efficiency, since Trp which was once separated in the internal phase was released back to the external phase. As shown in Figure 8, Trp concentration in the internal phase reached the maximum at about 2-4 min. of extraction time because at the beginning there was no swelling of the membrane. In this study, Trp can be concentrated up to 4-8 time within about 4 min. It was found that the volume of the internal aqueous phase at 30 min. became double of the initial volume or 100% swelling as shown in Figure 9.

CONCLUSIONS

1. The distribution coefficient of Trp^+ increased as the pH of the feed solution increased.

2. It was found that one mole of Trp^+ reacted with 2.2 moles of dimeric form of D2EHPA to form complex in the membrane phase.

3. Extraction equilibrium for 0.006 M tryptophan solution with 0.072 - 0.361 M dimeric form of D2EHPA solution were studied. It was found that average K_{ex} for tryptophan was 0.11 dm^3/mol.

4. From the emulsion liquid membrane extraction of tryptophan, the initial transport rate of tryptophan corresponded with initially high ions gradient. The flux gradually decreased as the driving force decreased.

5. Tryptophan can be separated and concentrated up to 4-8 times within 4 min. of extraction time by emulsion liquid membrane.

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