CHAPTER 4

EXTRACTION EQUILIBRIUM

This chapter presents the study of extraction equilibrium experiments of L-lysine from aqueous solution using D2EHPA as a carrier and n-dodecane as an organic solvent.

Materials and Methods for Extraction Equilibrium Experimental.

1. Materials

Amino acids	:	L-lysine	(Sigma Chemical Co.)
Organic solvent	:	n-Dodecane	(Fluka Chemika)
Surfactant	:	Span 80	(Fluka Chemika)
Carrier	:	Di-(2-ethylhexyl)-phosphoric acid (D2EHPA)	(Sigma Chemical Co.)

2. Methods

Experiments on the extraction equilibrium of L-lysine was carried out by mixing 50 ml of the organic membrane phase and 50 ml of the aqueous phase and then shaking at speed 240 rpm for 48 hours in shaking incubator which control temperature at 25 ^{o}C. The organic membrane phase was prepared by dissolving D2EHPA and Span 80 in n-Dodecane. The acidity (pH) in the aqueous phase was adjusted to pH 2, 3, 4, 5, and 6 with Sulfuric acid. The membrane phase contains 3 v/v % (0.0465 mol/dm^3) to 10 v/v% (0.2325 mol/dm^3) D2EHPA and the aqueous

phase was 0.01 M L-lysine. The two phases were separated after being allowed to settle. The concentration of amino acids and the pH in the aqueous phase were then measured. The concentration of amino acids in the membrane phase were determined by the difference of amino acid concentrations between initial and final stages. L-lysine concentration was measured by Ninhydrin method using a Spectrophotometer (Spectronic 20D, Milton Roy Company) at wavelength of 570 nm.

The experimental conditions for liquid-liquid extraction are summarized as follows:

Table 4-1. Experimental Conditions for Liquid-Liquid Extraction.

Organic Phase (50 ml)	Aqueous Phase (50ml)	Temperature	Time
Solvent : n-Dodecane Carrier : 3%v/v to 5%v/v D2EHPA	0.01 M L-lysine (adjusted pH as 2, 3, 4, 5, and 6 by using H_2SO_4)	25 °C	48 hours

Results and Discussions.

Extraction Equilibrium of L-lysine.

For the liquid equilibrium extraction of L-lysine in the presence of D2EHPA, It was found that Lys^+ or A^+ form a complex with D2EHPA in the oil phase which exists in the monomeric meric form as follow (Yagodin et.al, 1986; Boyadzhiev and Atanassova, 1991):

$$A^{m+} + m(\overline{HR}) \longleftrightarrow \overline{AR_m} + mH^+ \qquad \text{..... (4.1)}$$

$$K_{ex} = \frac{\overline{[AR_m]}_{eq}\,[H^+]^m_{eq}}{[A^+]_{eq}\,\overline{[HR]}^m_{eq}} \qquad \cdots\cdots\cdots (4.2)$$

where $\overline{(HR)}$ is the monomer of D2EHPA in the membrane phase and m is the stoichiometric coefficient and $\overline{AR_m}$ is the carrier/Lys complex in the membrane phase. Since the proton concentration is much higher than K_2, the dissociation constant of amino group, the formation of $A^{\pm}$ can be neglected. Then the total amino acid concentration (A_T) is expressed by :

$$[A_T] = [A^{2+}] + [A^+] \qquad \cdots\cdots\cdots (4.3)$$

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

$$D^+ = \frac{\overline{[AR_m]}_{eq}}{[A^{2+}]_{eq}} \qquad \cdots\cdots\cdots (4.4)$$

From equation (4.2),

$$\frac{K_{ex}\overline{[(HR)]}^m_{eq}}{[H^+]^m_{eq}} = \frac{\overline{[AR_m]}_{eq}}{[A^{2+}]_{eq}} \qquad \cdots\cdots\cdots (4.5)$$

From equation (4.4) and (4.5),

$$D^+ = \frac{K_{ex}\overline{[(HR)]}^m_{eq}}{[H^+]^m_{eq}} \qquad \cdots\cdots\cdots (4.6)$$

$$\log D^+ = \log K_{ex}\overline{[(HR)]}^m_{eq} - \log [H^+]^m_{eq} \qquad \cdots\cdots\cdots (4.7)$$

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

$$\log (D^{+} [H^{+}]^{m}_{eq}) = \log K_{ex} + m \log[\overline{(HR)}]_{eq} \quad \text{..... (4.8)}$$

From equation (4.3) and the equation of dissociation constant of amino acid as shown in equation (3.3), the equation (4.9) can be obtained;

$$[A^{2+}]_{eq} = \frac{[H^{+}]_{eq} [A]_{eq}}{[H^{+}]_{eq}+K_1} \quad \text{..... (4.9)}$$

Based on the assumption that L-lysine reacted with D2EHPA, The following mass balance equation can be obtained :

$$[\overline{(HR)}]_{eq} = [\overline{(HR)}]_i - m([A_T]_i - [A_T])_{eq} \quad \text{.....(4.10)}$$

$$[\overline{AR_m}]_{eq} = [A_T]_i - [A_T]_{eq} \quad \text{.....(4.11)}$$

From the above equations, the values of K_{ex}, D^{+} and $[\overline{(HR)}]_{eq}$ can be calculated. Figure 4-1 shows the relationship between distribution coefficient of Lys^{+} (D^{+}) and $[H^{+}]^{m}_{eq}$. It was found that graph between pH 2.0 to 4.0 shown the straight line which slope equal to -0.9931, it was nearly -1.0, as can be seen from the equation 4.7, the slope of the graph is -1 that is corresponding to equation. It was found that , when $[H^{+}]_{eq}$ was decreased, the $[D^{+}]_{eq}$ was increased. From equation 4.9, $[A^{2+}]$ was decreased at low pH but from appendix A the carrier concentration and Lys/carrier complex was not significantly difference. The graph of log $(D^{+} [H^{+}]^{m})_{eq}$ versus log $[(HR)]_{eq}$ is shown in figure 4-2. according to equation (4.8) the slope of graph of log $(D^{+} [H^{+}]^{m})_{eq}$ versus log $[(HR)]_{eq}$ is m. In this case, the value of slope or m is equal 0.12, it was found that 12 moles of Lys^{2+} reacted with one mole of D2EHPA to form complex in the membane phase. By using the value of

dissociation constant of L-lysine (K_1) of $10^{-2.18}$mol/dm^3 or 6.607×10^3mol/dm^3. The calculated value of extraction equilibrium constant or K_{ex} for L-lysine is 0.2047 dm^3/mol. Figure 4-3 showed the calculated value of individual experiment data points show a change of the values of K_{ex} with respect to the values of $[(HR)]_{eq}$. It was found that, the change of K_{ex} values seem to be constant.

Itoh et.al. (1990) and Noppaporn Panich (1994) study the mechanism of mass transfer in phenylalanine, they have been found that one mole of phenylalanine formed complex with two moles of D2EHPA (in the dimeric form). Their result was difference from L-lysine study because L-lysine has a long chain in one molecule so it might be polymerized and this polymer will be form complex with carrier at the interphase.

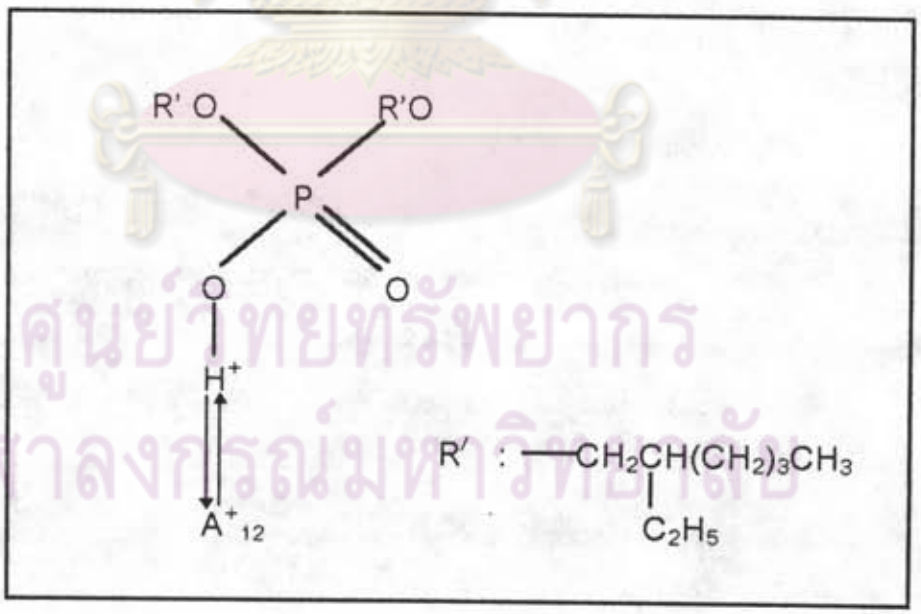

Figure 4-1. Proposed Transport Mechanism of L-lysine reacted with D2EHPA monomer.

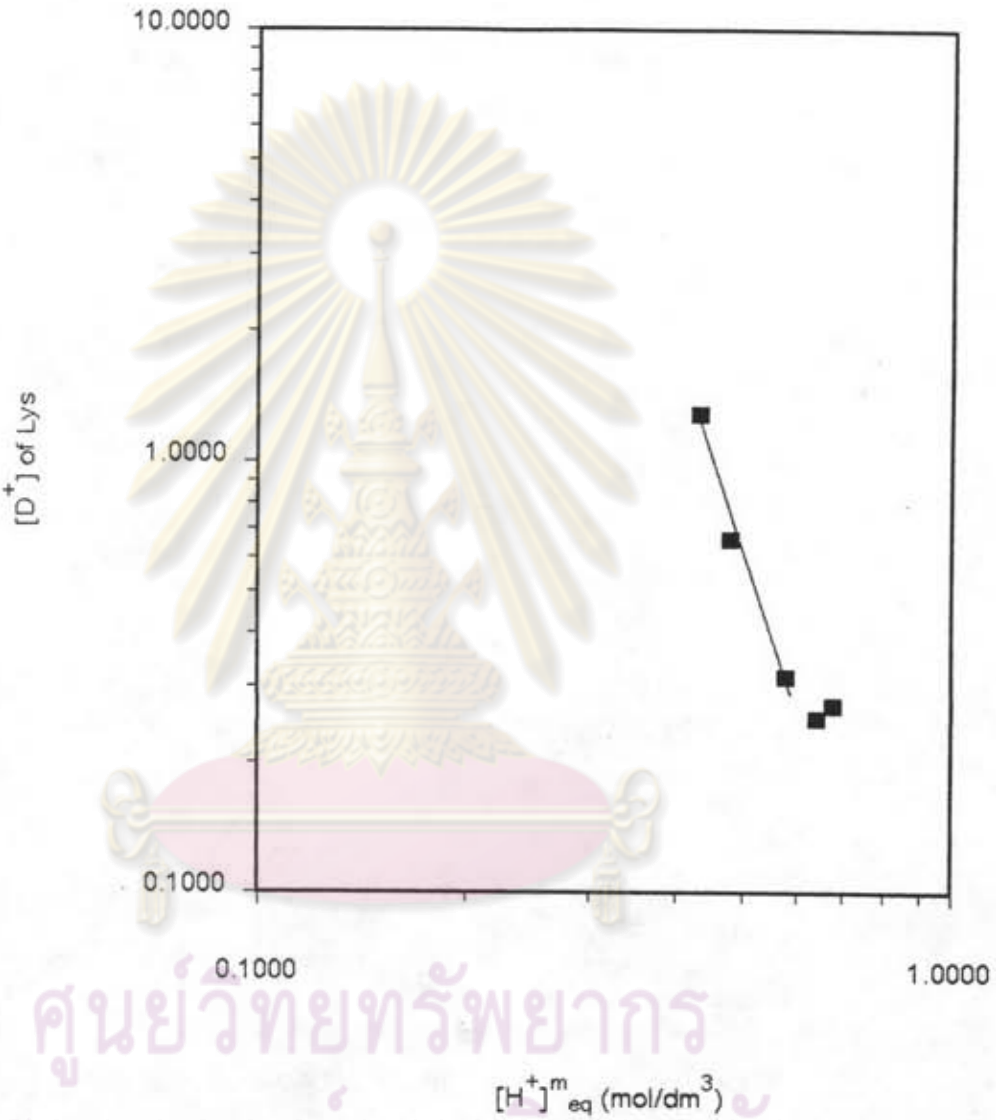

Figure 4-2 Distribution Coefficient of Lys vs. $[H+]^m$

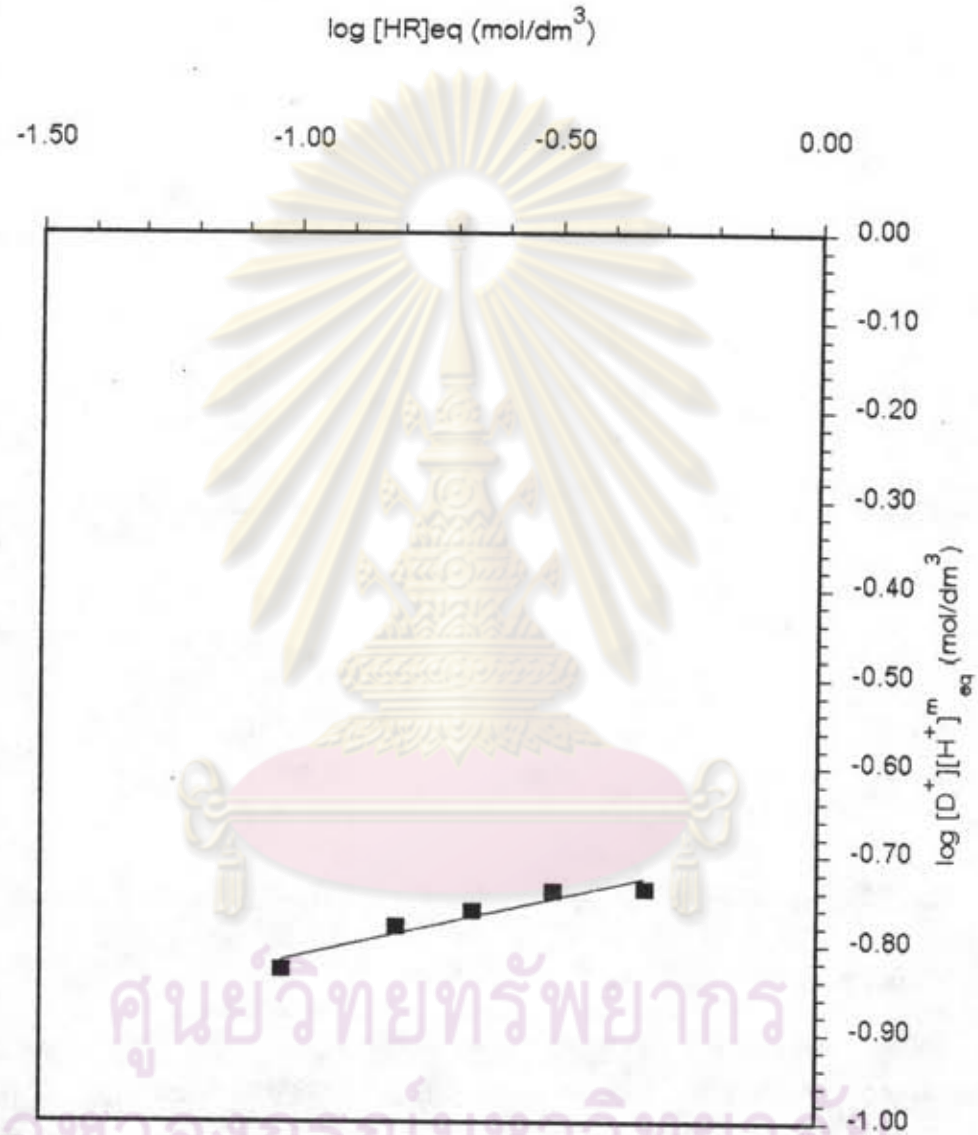

Figure 4-3 $[D^+][H^+]^m_{eq}$ vs. $[HR]_{eq}$ of L-lysine.

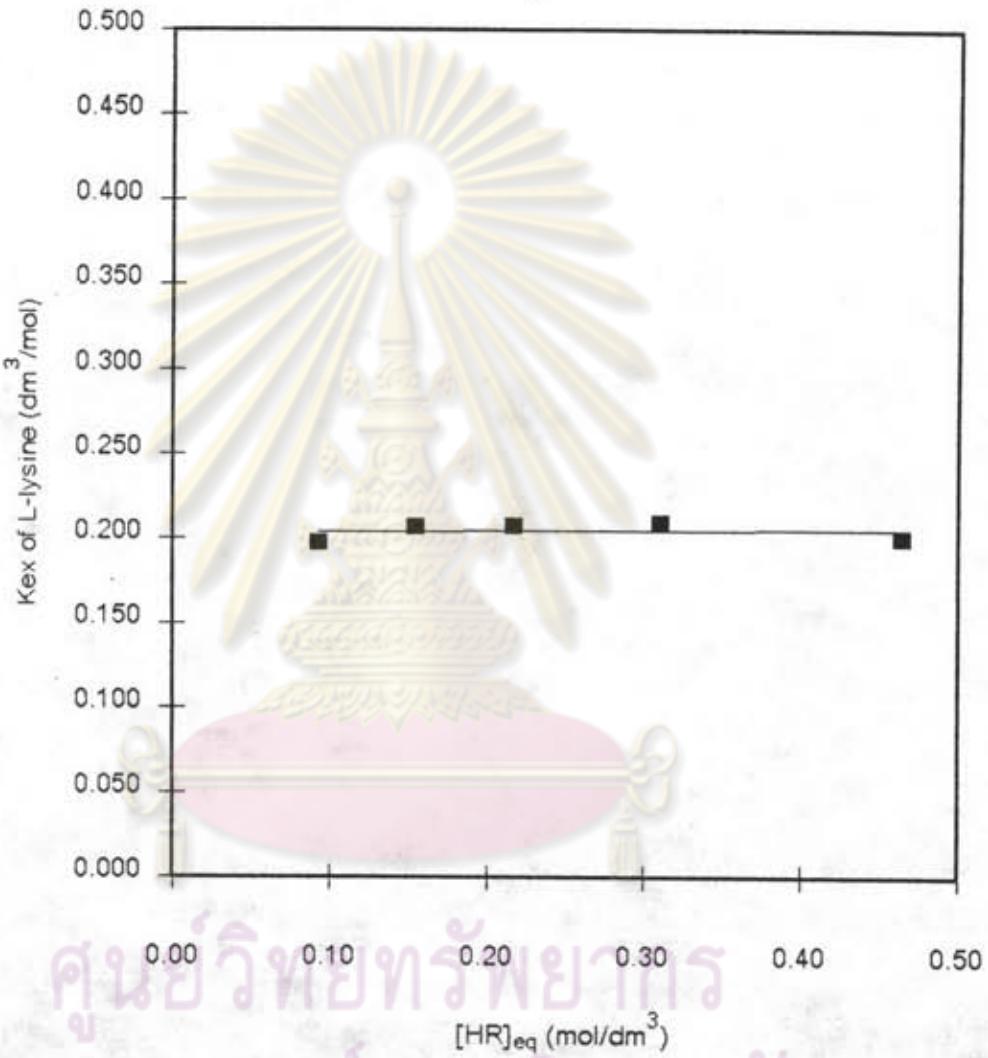

Figure 4-4 Kex of L-lysine vs. $[HR]_{eq}$