CHAPTER 4

EXTRACTION EQUILIBRIUM

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

Experimental Materials and Methods.

1. Materials.

All chemical used were obtained from Wako Pure Chemica Industries , Japan, except di (2-ethylhexyl) phosphoric acid or D2EHPA was obtained from TCI company, Japan. All the chemicals are analytical grade.

Amino acids	:	L-phenylalamine and L-tryptophan
Organic solvent	:	n-Dodecane
Surfactant	:	Span 80
Carrier	:	Di-2 ethylhexyl phosphoric acid (D2EHPA)

2. Method.

Experiments on the extraction equilibrium of phenylalanine and tryptophan were carried out by mixing 20 ml of the organic membrane phase and 20 ml of the aqueous phase and then shaking for 48 hours at a temperature of 25°C in a thermostatic shaking water bath (Model T-225, Thomas Kaguher, Co. Ltd.). The organic membrane phase was prepared by dissolving D2EHPA and Span 80 in n-dodecane. The pH in the aqueous phase were adjusted to pH 2, 3 and 5 with hydrochloric acid. The membrane phases contain 0.072M- 0.36 M D2EHPA and the aqueous phase were 0.006 M phenylalanine and 0.006 M tryptophan. 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. Phenylalanine and

tryptophan concentrations were measured by a UV-Spectrophotometer (Hitachi 320) at wavelength of 257.7 mm. and 277.0 nm, respectively.

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

Table 4.1 Experimental Conditions for Liquid-Liquid Extraction.

Oil Phase (20 ml)	Aqueous Phase (20 ml)	Temperature and Time
Solvent: n-Dodecane Carrier : 0.0721 M- 0.361 M D2EHPA (dimeric form).	0.006 M Phe or 0.006 M Trp adjusted to pH 2-5 with HCl.	Shaking for 48 hrs at 25°C.

Results and Discussions.

1. Extraction Equilibrium of Phenylalanine.

For the liquid equilibrium extraction of phenylalanine in the presence of D2EHPA, it is assumed that Phe^+ or A^+ form a complex with D2EHPA in the oil phase which exists in the dimeric form as follow :

$$A^+ + m\,\overline{(HR)_2} \overset{K_{ex}}{\Leftrightarrow} \overline{AR(HR)_{2m-1}} + H^+ \quad \text{..... (4.1)}$$

$$K_{ex} = \frac{[\overline{AR(HR)_{2m-1}}]_{eq}\,[H^+]_{eq}}{[A^+]_{eq}[\overline{(HR)_2}]^m_{eq}} \quad \text{..... (4.2)}$$

Where $\overline{(HR)_2}$ is the dimer of D2EHPA in the membrane phase and m is the stoichiometric coefficient and $\overline{AR(HR)_{2m-1}}$ is the carrier/Phe 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:

$$[A_T] = [A^+] + [A^\pm] \quad \text{..... (4.3)}$$

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

$$D^+ = \frac{\overline{[AR(HR)_{2m-1}]}_{eq}}{[A^+]_{eq}} \quad \text{..... (4.4)}$$

From equation (4.2),

$$\frac{K_{ex}\,\overline{[(HR)_2]}^{m}_{eq}}{[H^+]_{eq}} = \frac{\overline{[AR(HR)_{2m-1}]}_{eq}}{[A^+]_{eq}} \quad \text{..... (4.5)}$$

From equation (4.4) and (4.5),

$$D^+ = \frac{K_{ex}\overline{[(HR)_2]}^{m}_{eq}}{[H^+]_{eq}} \quad \text{..... (4.6)}$$

$$\log D^+ = \log K_{ex}\overline{[(HR)_2]}^{m}_{eq} - \log [H^+]_{eq} \quad \text{..... (4.7)}$$

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

$$\log (D^+[H^+]_{eq}) = \log k_{ex} + m \log \overline{[(HR)_2]}_{eq} \quad \text{..... (4.8)}$$

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;

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

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:

$$[\overline{(HR_2)}]_{eq} = [\overline{(HR)_2}]_i - 2([A_T]_i - [A_T]_{eq}) \quad \text{..... (4.10)}$$

$$[\overline{AR(HR)_{2m-1}}]_{eq} = [A_T]_i - [A_T]_{eq} \quad \text{..... (4.11)}$$

From the above equations, the values of K_{ex}, D^+ and $[(HR)_2]_{eq}$ can be calculated. Figure 4.1 shows the relationship between distribution coefficient of Phe^+ (D^+) and $[H^+]_{eq}$. As can be seen from the equation, the slope of the graph is -1 that is corresponding to quation (4.7).

The graph of $\log (D^+[H^+]_{eq})$ vs. $\log [\overline{(HR)_2}]_{eq}$ is shown in Figure 4.2. According to equation (4.8) the slope of the graph of $\log (D^+[H^+]_{eq})$ vs. $\log [\overline{(HR)_2}]_{eq}$ is *m*. In this case, the value of slope or *m* is equal 2. By using the value of dissociation constant of phenylalanine (K_1) of $10^{-1.83}$ mol/dm^3 or 1.479×10^{-2} mol/dm^3. The calculated value of extraction equilibrium constant or K_{ex} for phenylalanine is 0.167 dm^3/mol. Figure 4.3 showed the calculated value of individual experiment data points. The scattering points show a small change of the values of K_{ex} with respect to the values of $[\overline{(HR)_2}]_{eq}$. This change may be the result of experimental errors.

2. Extraction Equilibrium of Tryptophan.

For the study of liquid-liquid equilibrium extraction of tryptophan in the presence of D2EHPA as a carrier, the complex formation between ammino acid and carrier and the equations involved are the same as for phenylalanine. Therefore, equation (4.1) to (4.11) can be applied.

The value of K_{ex}, D^+ and $[\overline{(HR)_2}]_{eq}$ for tryptophan system can be calculated. Figure 4.4 shows the relationship between distribution coefficient of Trp^+ (D^+) and $[H^+]_{eq}$. The slope of this curve is also -1 which is corresponding to equation (4.7).

According to equation (4.8), *m* is the value of slope of the graph of $\log (D^+[H^+]_{eq})$ vs. $\log [\overline{(HR)_2}]_{eq}$. As shown in Figure 4.5 , that slope of this graph is 2.2, indicating that the value for *m* is 2.2. By using the the value of $K_1 = 10^{-2.38}$ or 4.169×10^{-3} mol/dm^3 the calculated value of K_{ex} for tryptophan is 0.11 dm^3/mol. Figure 4.6 showed the calculated value of individual experiment data points. The scattering points show a small change of the values of K_{ex} with respect to the values of $[\overline{(HR)_2}]_{eq}$. This change may be the result of experimental errors.

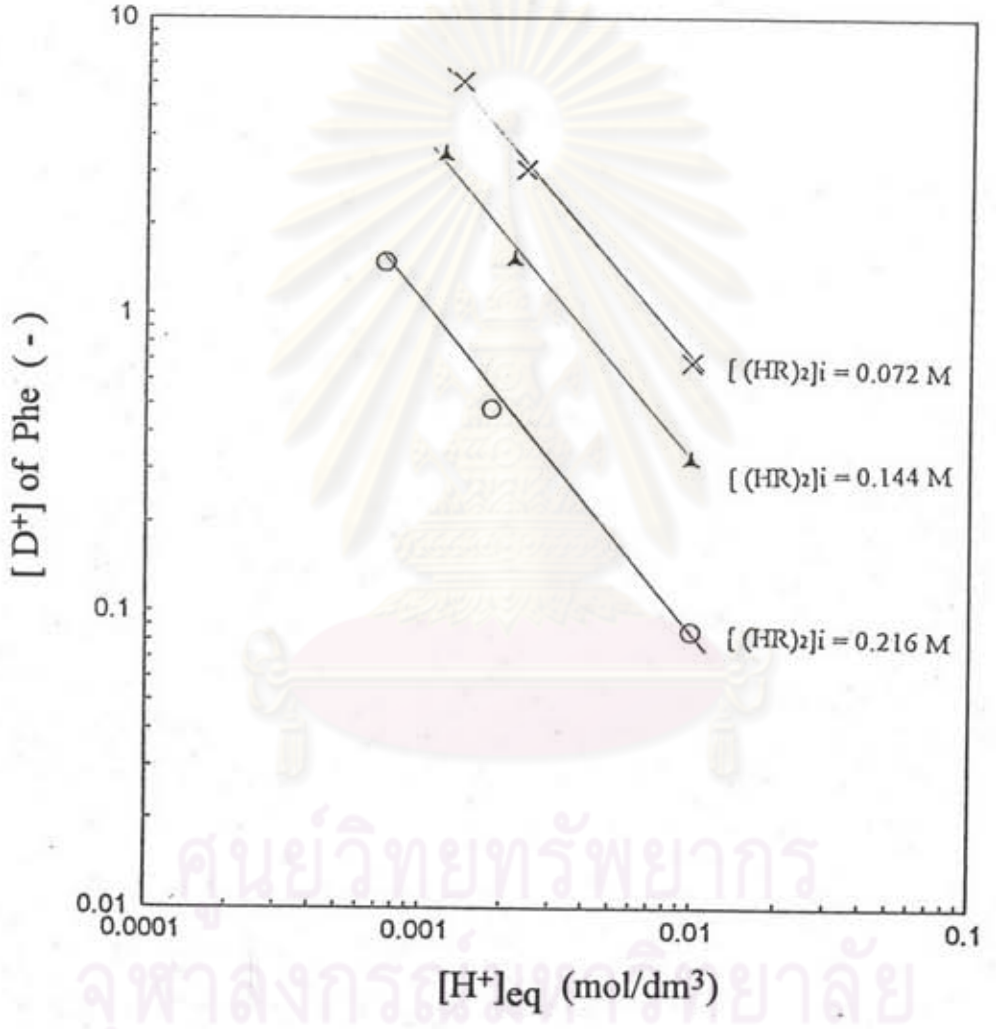

Figure 4.1 Distribution Coefficient of Phe^{+} vs. $[H^{+}]_{eq}$.

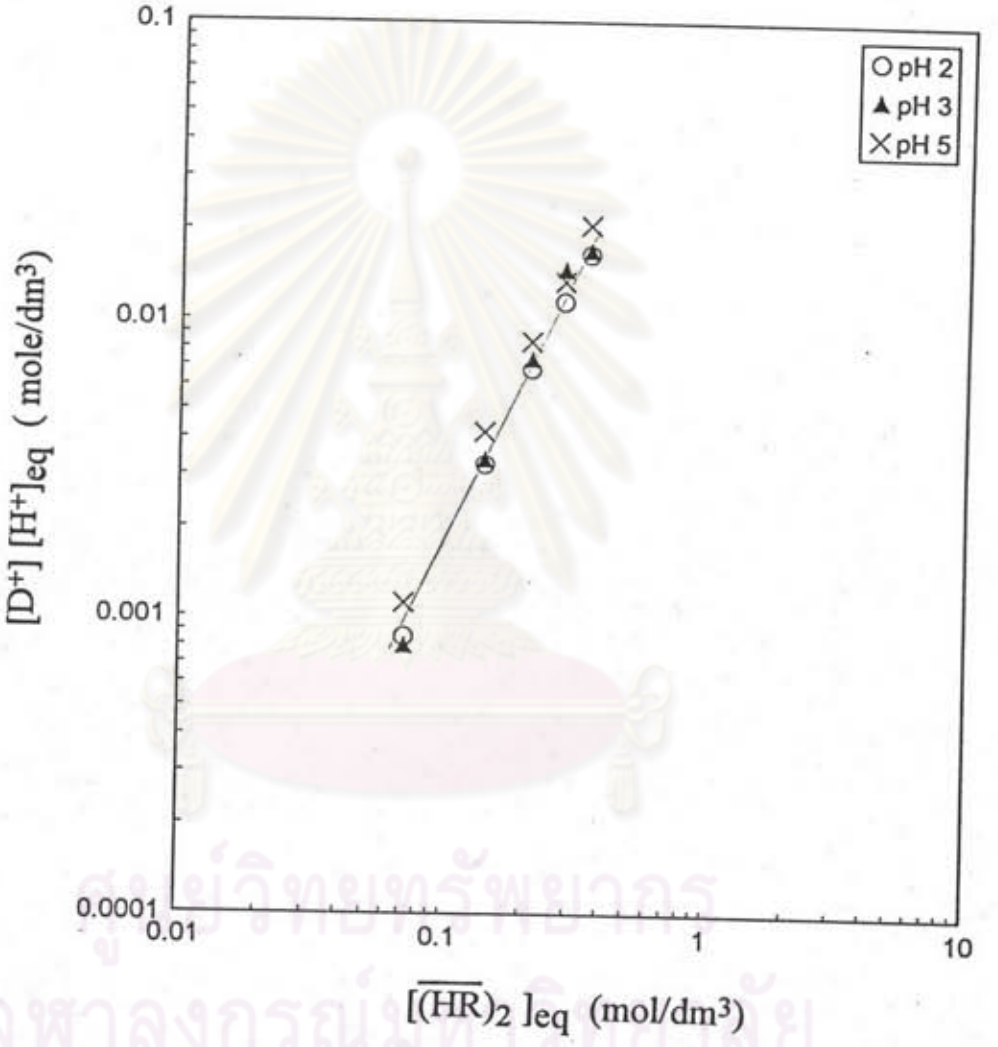

Figure 4.2 $[D^+]\,[H^+]_{eq}$ vs. $[\overline{(HR)}_2]_{eq}$ of Phenylalanine.

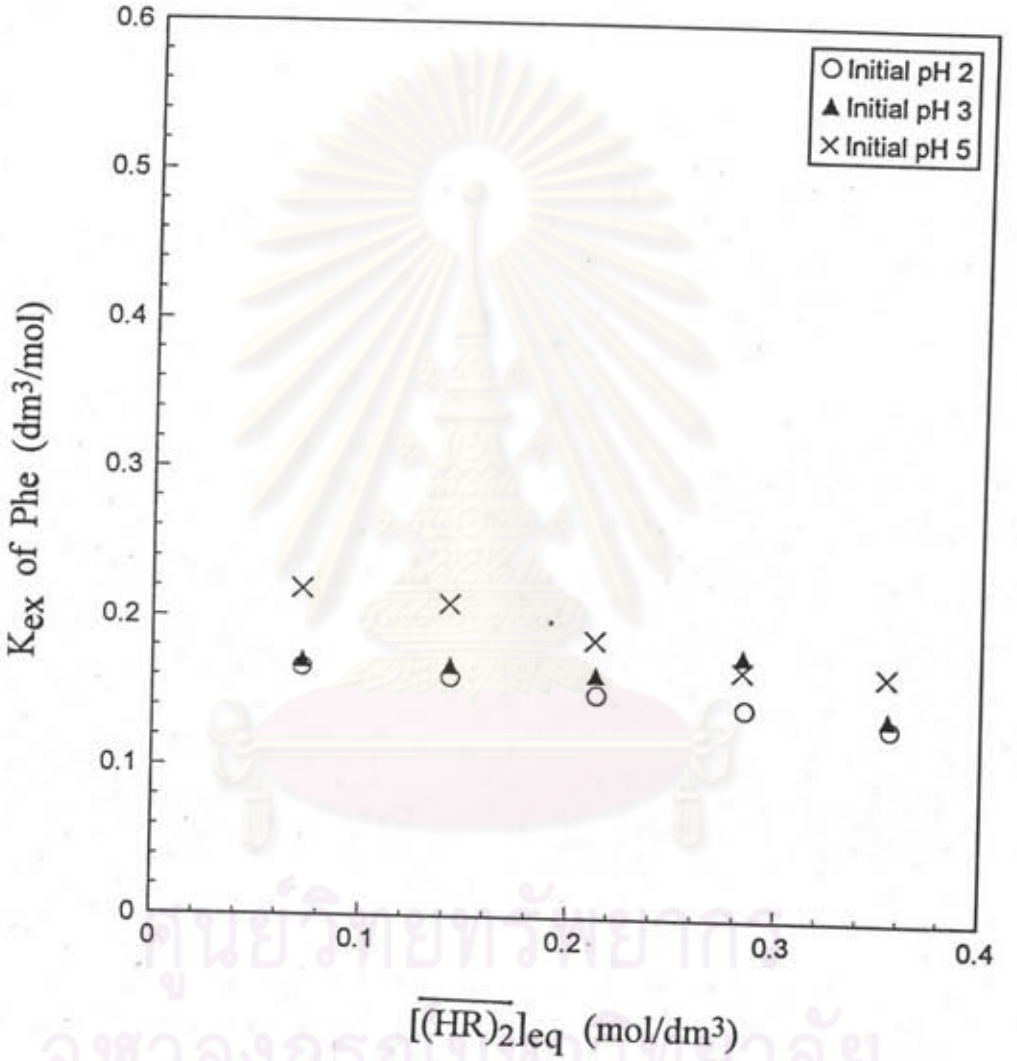

Figure 4.3 K_{ex} of Phe vs. $\overline{[(HR)_2]}_{eq}$.

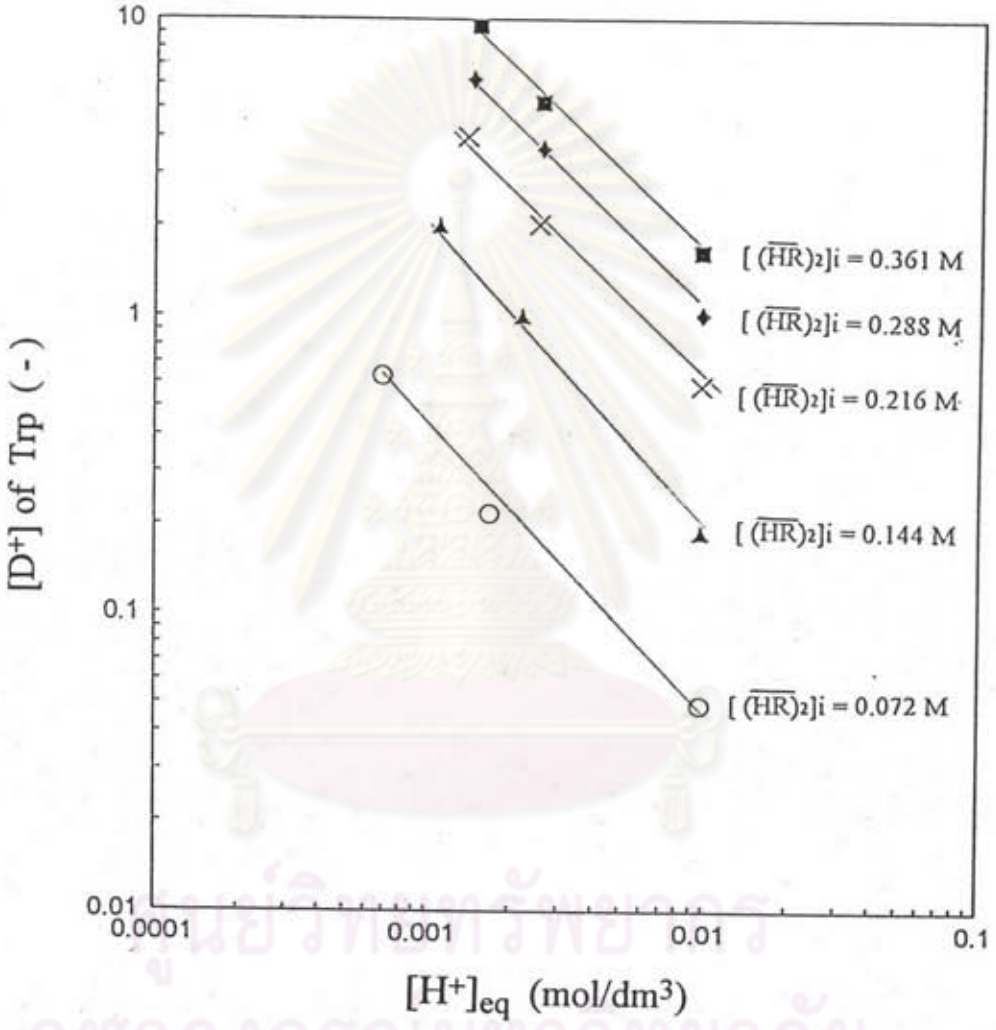

Figure 4.4 Distribution Coefficient of Trp^+ vs. $[H^+]_{eq}$.

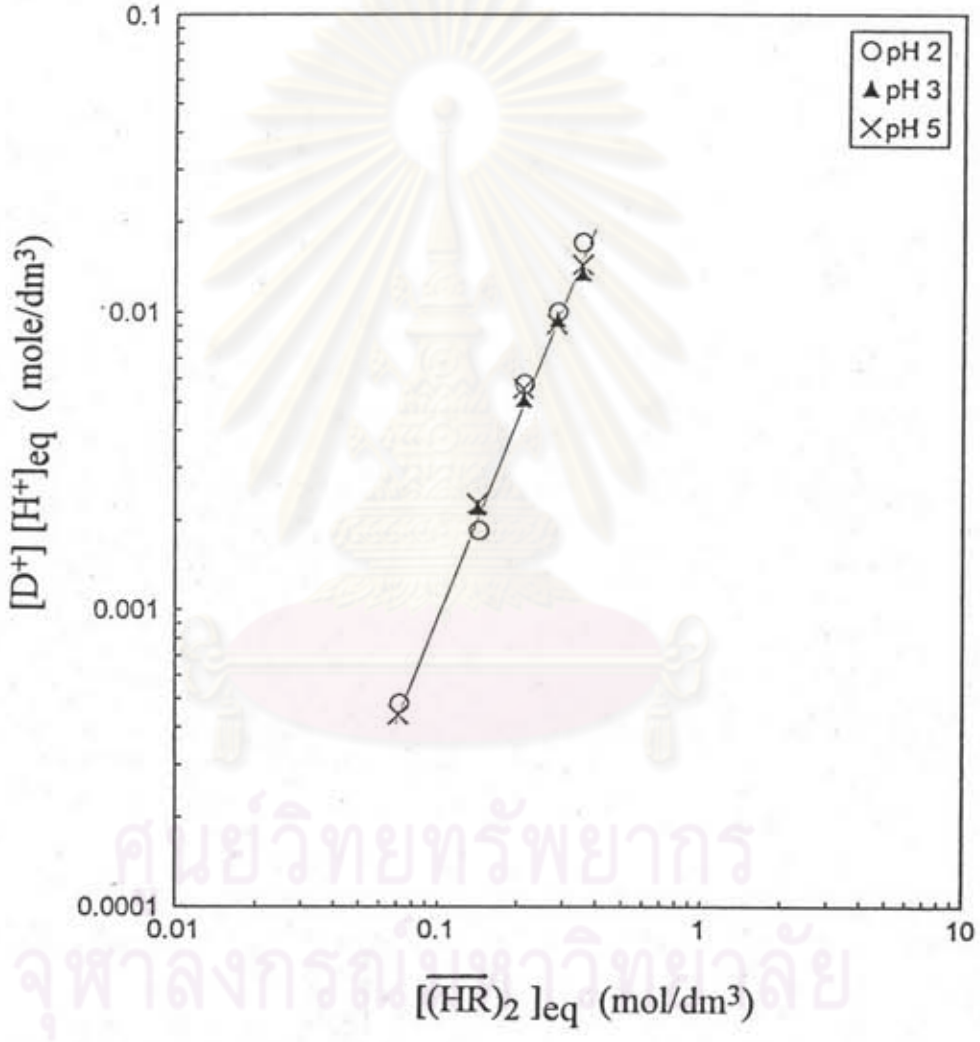

Figure 4.5 $[D^+]\,[H^+]_{eq}$ vs. $[\overline{(HR)_2}]_{eq}$ of Tryptophan.

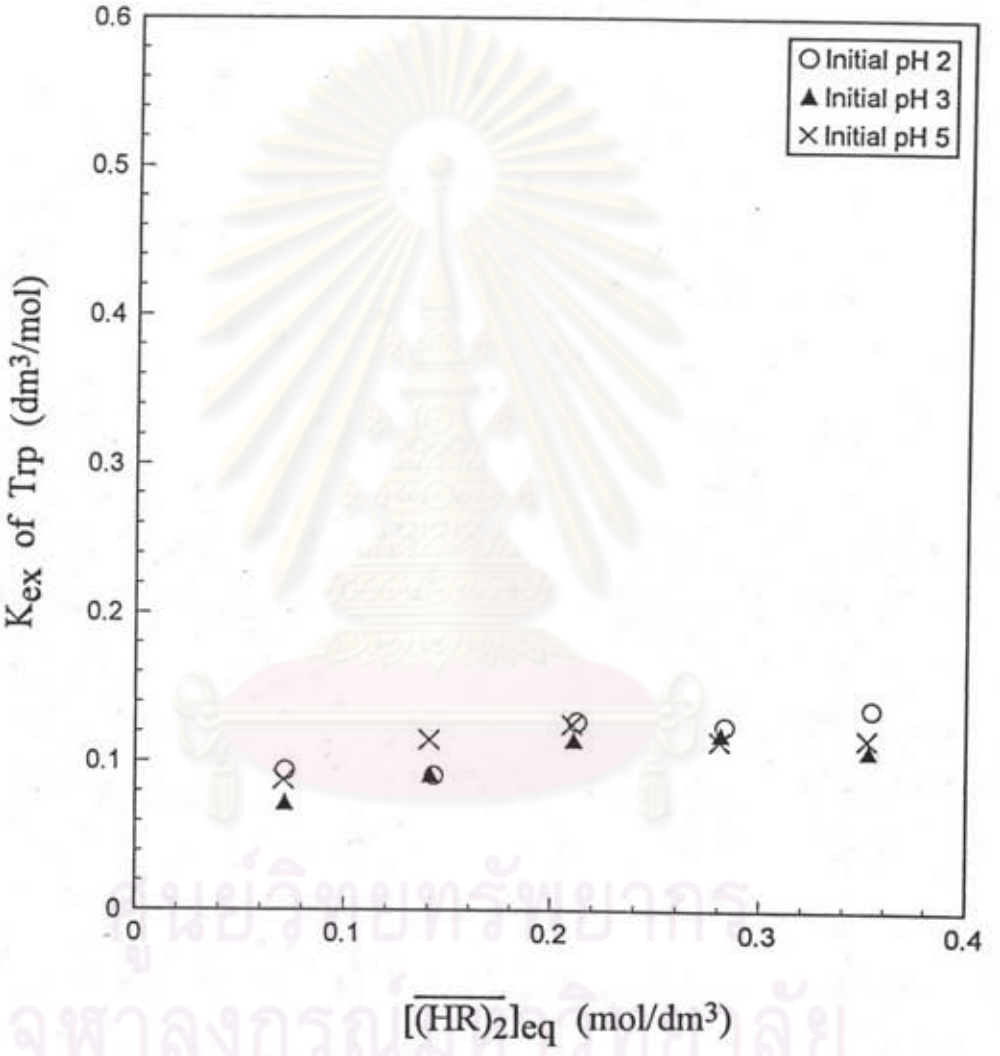

Figure 4.6 K_{ex} of Trp vs. $[\overline{(HR)_2}]_{eq}$.