EMULSION LIQUID MEMBRANE EXTRACTION

This chapter presents the results of the batch extraction of the Type II or Facilitated extraction of single component of phenylanine and tryptophan solution and also mixture solution of phenyalanine and tryptophan. The pH of the external feed phase was acidic pH. The cation carrier D2EHPA, and emulsifying agent Span 80, and n-dodecane were components in the organic membrane phase.

Experimental Materials and Methods.

1. Extraction of Single Amino Acid Component.

All chemicals used were previously described in chapter 4. 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 condition of emulsion liquid membrane system were as follows:

a) External Phase.

The external or feed phase are 0.006 M phenylalanine and 0.001 and 0.006 M tryptophan solutions at pH 2, 3 and 5. The pH of the solution was adjusted with HCl solution.

b) Membrane Phase.

Solvent	: n-Dodecane	176 ml
Carrier	: D2EHPA	10 ml
Surfactant	: Span80	4 ml
		190 ml

c) Internal Phase.

1.0 N HCl solution.

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 amino acid solution.

The vessel was 9 cm in diameter and was equipped with a six-blade 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 and stirred at the speed of 250 rpm. After each extraction, all solution was removed from the vessel. Then, the emulsion phase and external phases were separatedd after being allowed to settle. The volume of each phase were measured and amino acid concentrations in the external phase were measured by using UV Spectrophotometer at 257.7 nm for phenylalanine and 277.0 nm for tryptophan. The pH of the external phase solutions were also measured by a pH meter. The experiment data are shown in the Appendix . The schematic diagram of experimental apparatus is shown in Figure 5.1.

2. Extraction of Amino Acid Mixture Solution.

The membrane component and the method of extraction was the same as described above. The external phase was the mixture solution of 0.006 M phenylalanine and 0.006 M tryptophan or the mixture solution of 0.006 M phenylalanine and 0.001 M tryptophan at pH 2, 3 and 5. The concentrations of amino acid mixture in the external phase were measured by using Liquid Chromatography at wavelength of 278.0 nm. The experimental data are shown in the Appendix.

3. Calculation of Swelling in the Internal Phase.

The measurement of emulsion swelling can be done by measuring the volume of emulsion phase and external phase after each extraction experiment. The internal phase volume can be calculated by mass balances. The percentage of emulsion swelling can be calculated by the following equation:

$$\% \text{ Swelling} = \frac{(\text{Internal Phase Volume} - \text{Initial Internal Phase Volume}) \times 100}{\text{Initial Internal Phase Volume}}$$

$$\% \text{ Swelling } = \frac{(\text{Internal Phase Volume } - 25 \text{ ml}) \times 100}{25 \text{ ml}}$$

4. Calculation of Amino Acid Concentration in the Internal Phase.

In the experiment, concentrations of amino acid in the external phase were measured. The concentrations of amino acid in the internal phase were calculated by material balance based on the assumption that there was no accumulation of amino acid in the membrane phase. The calculation of amino acid concentration in the internal phase is shown in the following example:

Initial Conc. of amino acid in the external phase or $[A]_{f0} = 0.006$ M

From experimental result, the conc. of amino acid in the external phase after 1 min. extraction = 0.00525 M

Therefore, amino acid that penetrated into the internal phase
$$= 0.006 \text{ M} - 0.00525 \text{ M}$$
$$= 0.00075 \text{ M}$$

Since the volume of the external phase = 350 ml

From experimental result, the volume of the internal phase at 1 min
$$= 25 \text{ ml}$$

Therefore, conc. of amino acid in the internal phase or $[A]_i$
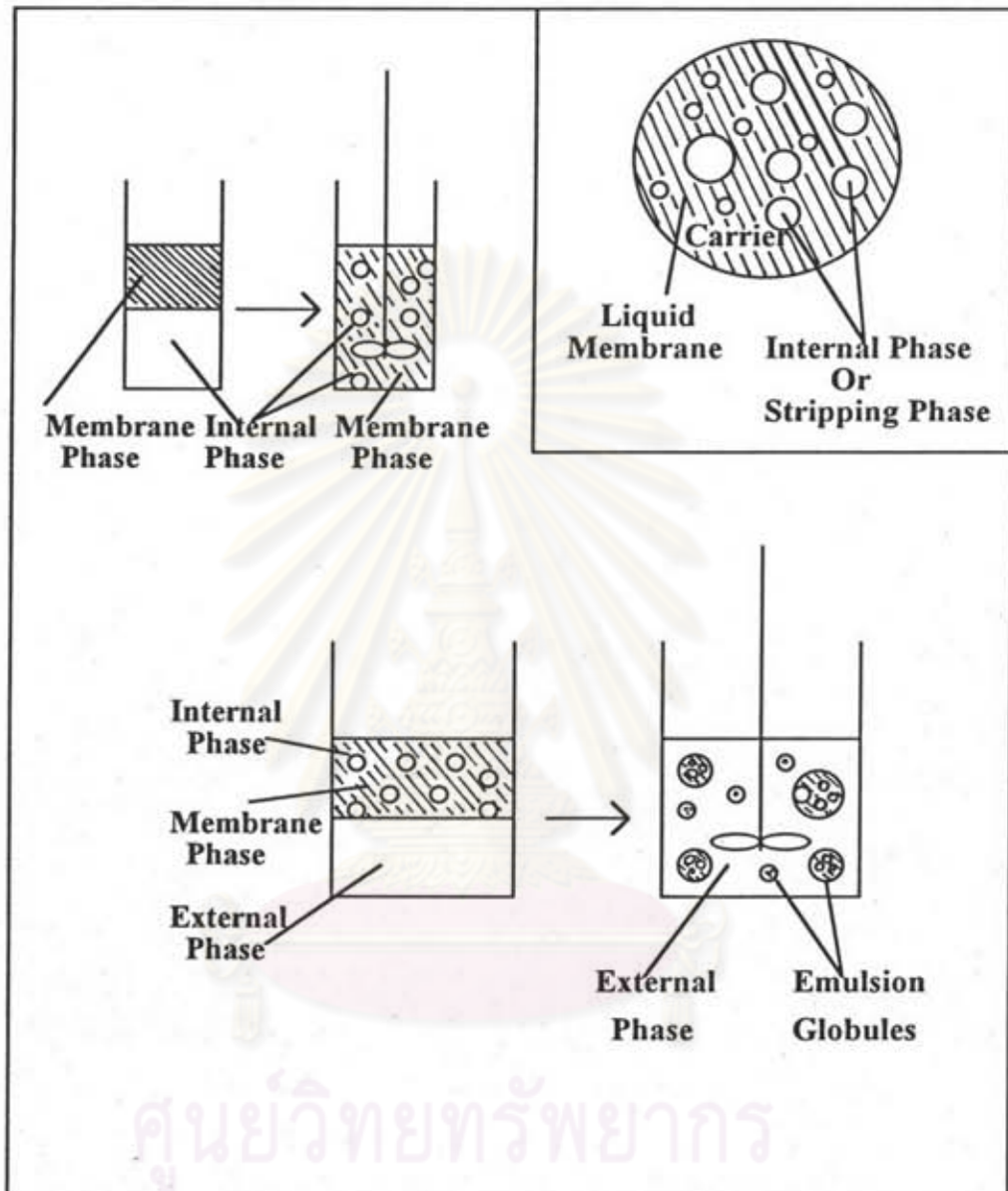$$= \frac{0.00075 \times 350}{25}$$
$$= 0.0105 \text{ M}$$

Figure 5.1 Schematic Diagram of Experimental Apparatus.

Results and Discussions.

1. Permeation of Single Amino Acid.

As shown in chapter 3 the ionic structure of phenylalanine and tryptophan changes significantly with changes in pH. In this study, a cation carrier was used, therefore phenylalanine and tryptophan must exist in cation . In this system, an acidic pH is desirable.

In order to separate and concentrate more phenylalanine and tryptophan into the internal phase, a large difference of H^+ between the internal and the external phase must be established (Itoh et.al., 1990a). That is a low pH in the internal phase and a high pH in the external phase are desirable. There is a possibility that due to the high pH in the external phase, phenylalanine and tryptophan 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.

1.1 Extraction of 0.006M Phenylalanine Solution.

As shown in Figure 5.2, the permeation rate of phenylalanine increases with pH of the external feed phase. There is a significant different in permeation rate between pH 2 and pH 3, but not much different between pH 3 and pH 5. This due to the fact that at higher pH, phenylalanine is less in positively charge form, even though, there is a big driving force for H^+ concentration between internal and external phase.

Figure 5.3 shows the concentration of phenylalanine in the internal phase. It can be seen that in this study, phenylalanine were concentrated up to 4-6 times within 4 min of extraction time. However, at 30 min of the extraction time, phenylalanine in the internal phase were less concentrated than at 4 min due to the swelling effect of w/o emulsion globules.

The Effect of pH on swelling is shown in Figure 5.4. At pH 2 and 3, the swelling started at about 4 min after extraction whereas at pH 5, the swelling started at about 1 min. However, at the end of the extraction time, the swelling effect was 80% which was the same for all pH values.

Figure 5.5 shows the change of pH value in the feed phase during the extraction. According to the transport mechanism for

phenylalanine, Phe^+ is exchanged for H^+, therefore the pH in the external feed phase decreased during the extraction.

1.2 Extraction of 0.006 M and 0.001 M Tryptophan Solution.

The permeation rates of 0.006 M and 0.001 M tryptophan are shown in Figure 5.6 and 5.10, respectively. The effect of pH on the permeation rate was the same as that have been observed from permeation of 0.006 M phenylalanine solution.

Figure 5.7 and 5.11 shows the concentration of 0.006 M and 0.001 M tryptophan in the internal phase during the extraction. In this study tryptophan can be concentrated up to 4-8 time within about 4 min. The effect of pH on swelling for both extraction experiments are shown in Figure 5.8 and 5.12. Generally, the swelling started at about 2 min ofter extraction and there were 100% swell at all pH values of the feed phase at 30 min.

Figure 5.9 and 5.13 shows the change of pH value in the feed phase during the extraction. The same trend of pH change in case of phenylalanine extraction was also observed.

2. Permeation of Binary Mixture of Amino Acid.

When investigating the use of emulsion liquid membrane as a bioseparation operation, the issue of selectivity shall be examined. There are various kinds of impurities in fermentation broths eg. glucose, lactic acid and tryptophan etc. In this study, tryptophan is selected as an impurities in the study of selectivity effect on ELM extraction.

Figure 5.14 and 5.15 show the concentration profile of the extraction of the binary mixture solution of 0.006 M phenylalanine and 0.006 M tryptophan and the binary mixture solution of 0.006 M phenylalanine and 0.001 M tryptophan, respectively. It can be seen that there was not much difference in the transport rate of phenylalanine and tryptophan from single component system.

Figure 5.16, 5.17 and 5.18 show the comparision of phenylamine and tryptophan tronsport in ELM at pH 2, 3 and 5, respectively. In case of single component system or in the absence of competitor, it was found that trypophan had a higher flux than phenylalanine. This can be attributed to the higher hydrophobicity of tryptophan than phenylalane (Thein et. al.,1988). It was found that the transport of phenylalanine was little effected by the presence of

tryptophan in both high and low concentrate of tryptophan. It can be concluded that this system can not purify phenylalanine from a mixture solution of phenylalanine and tryptophan but tryptophan does not have a significant effect on the phaenylalanine transport rate.
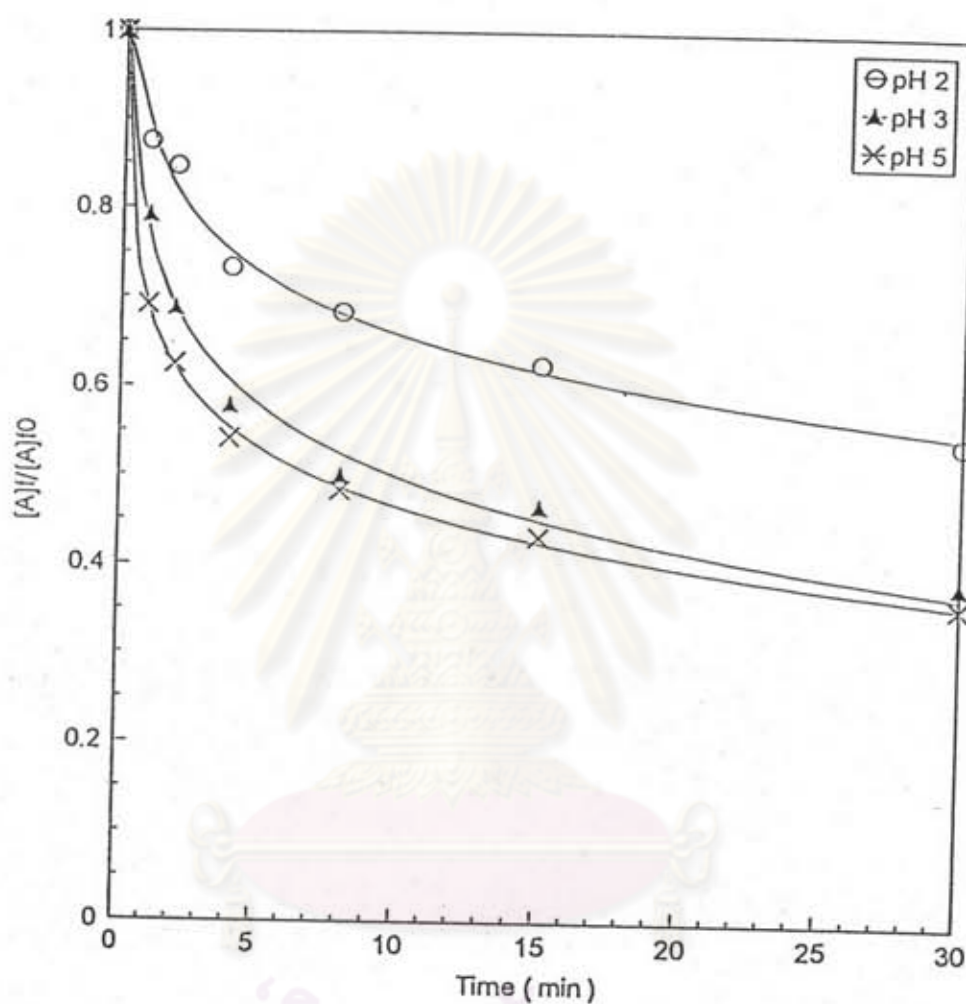
Figure 5.2   Extraction of 0.006 M phenylalanine by emulsion liquid membrane.

[A]f = Amino acid concentration in the feed phase.

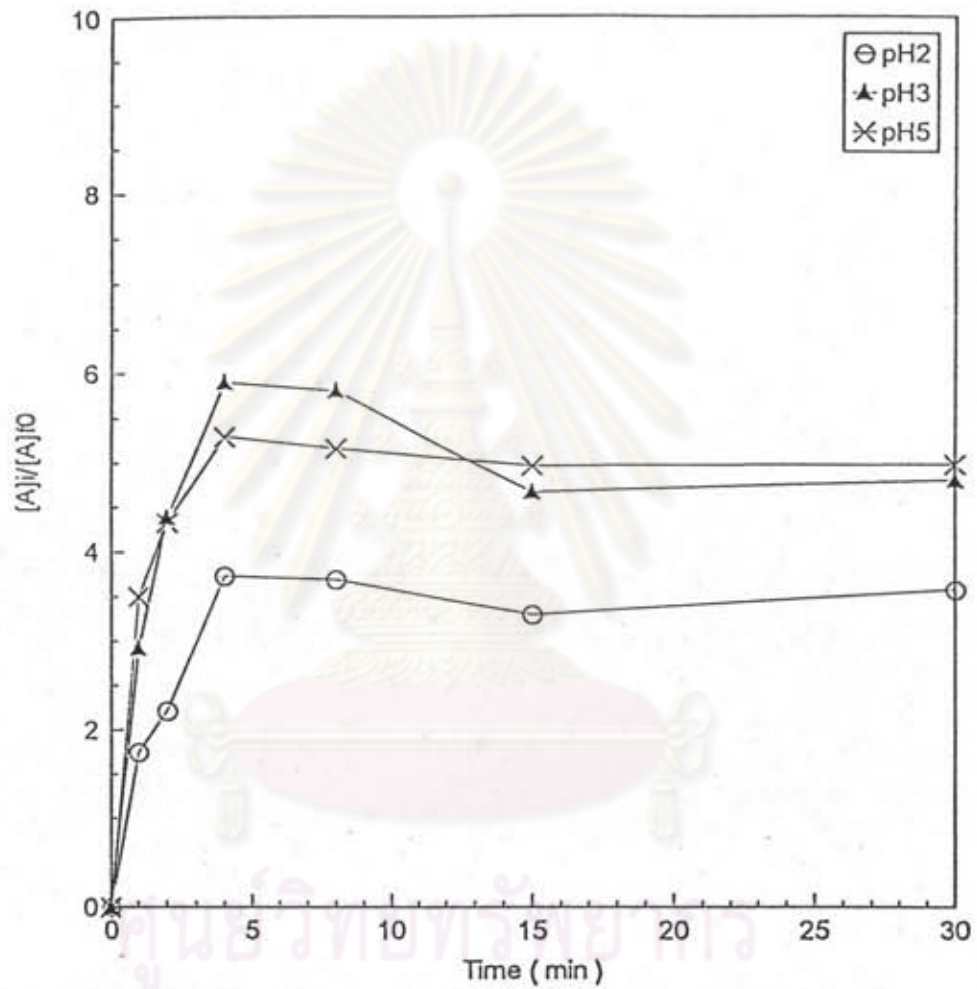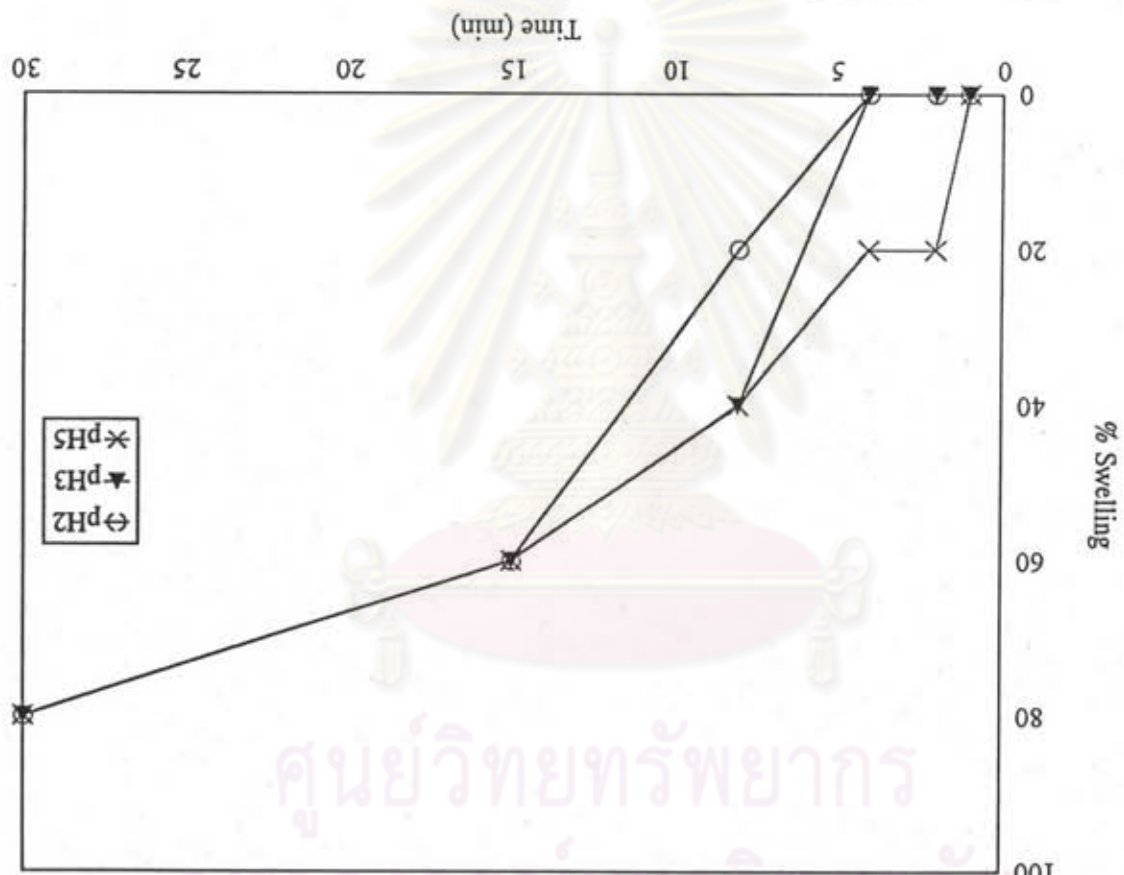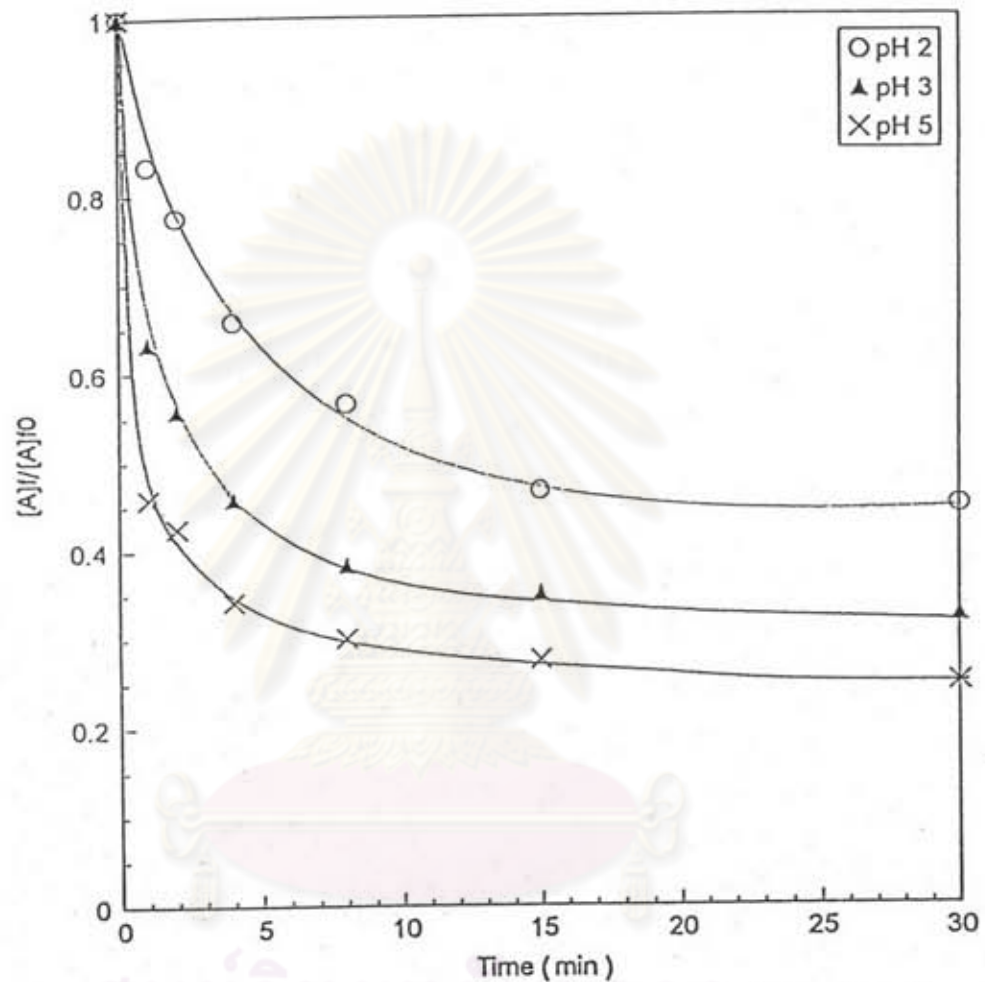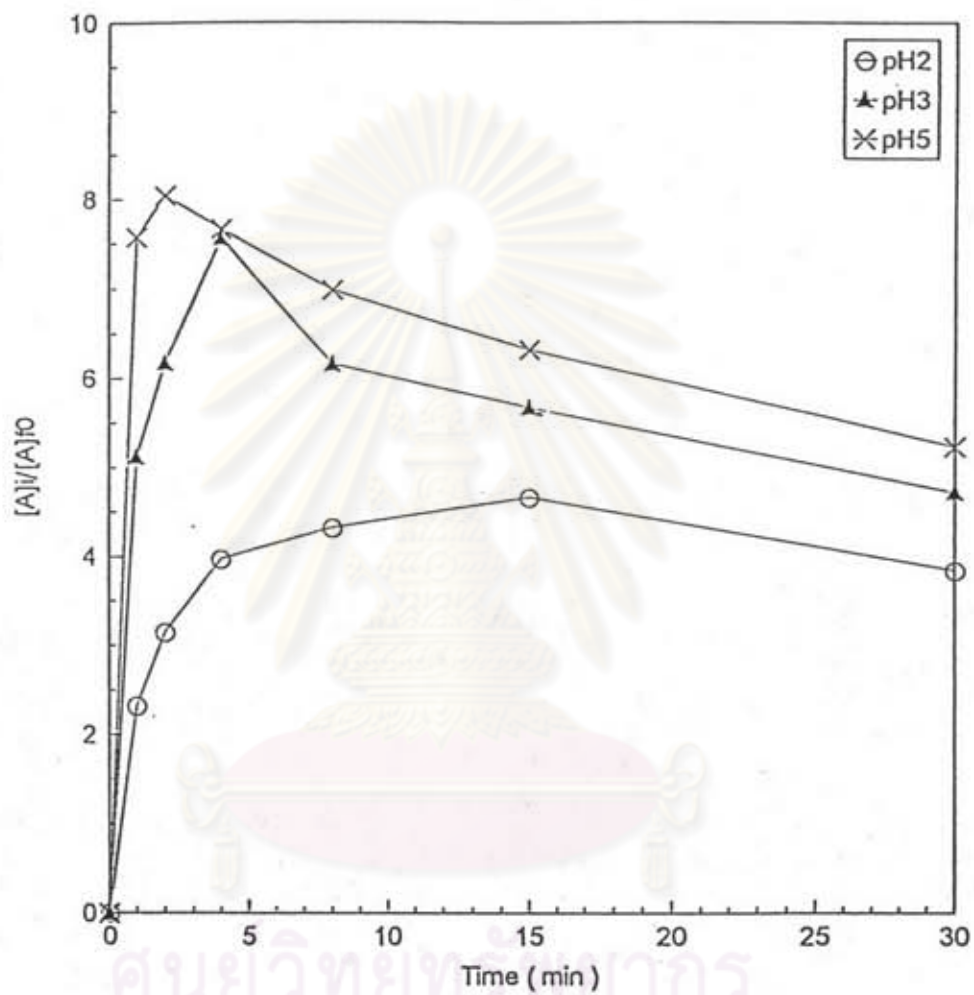[A]f0 = Initial amino acid concentration in the feed phase.

Figure 5.3 Concentration of 0.006M phenylalanine in the internal phase.

$[\text{Á}]i$ = Amino acid concentration in the internal phase.

$[A]f0$ = Initial amino acid concentration in the feed phase.

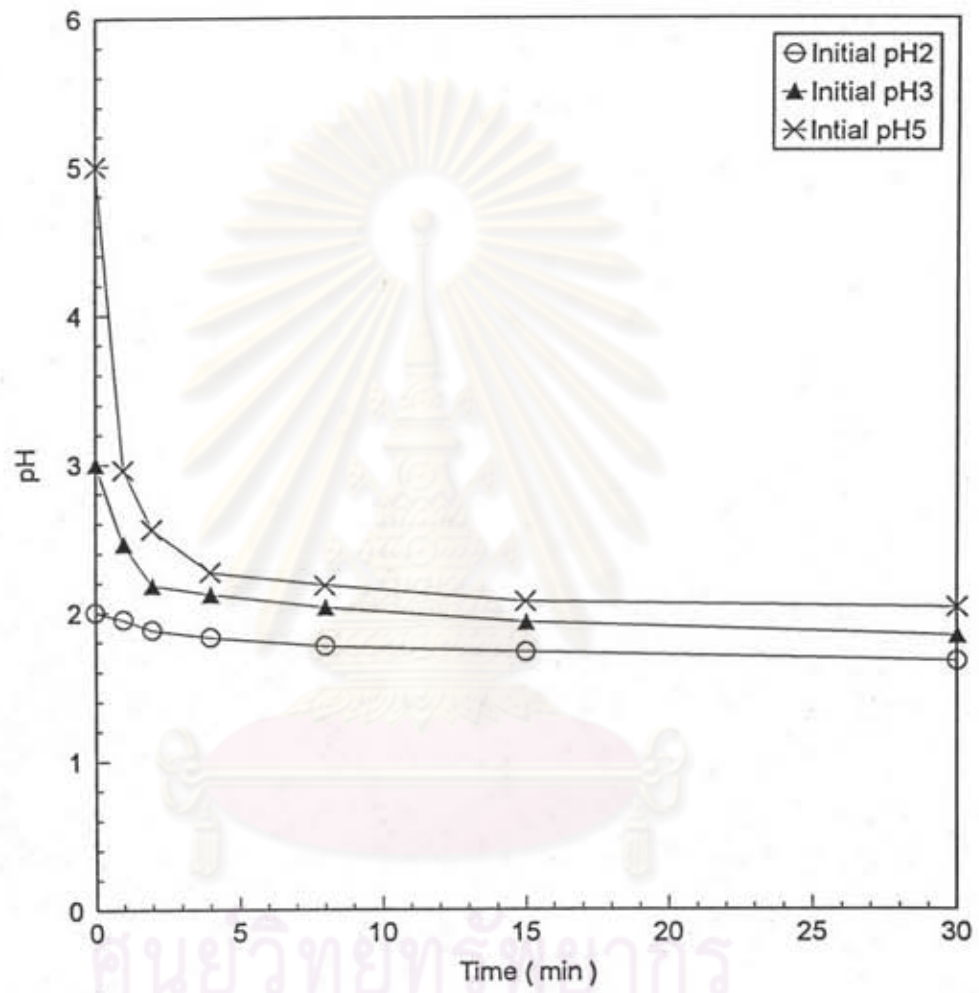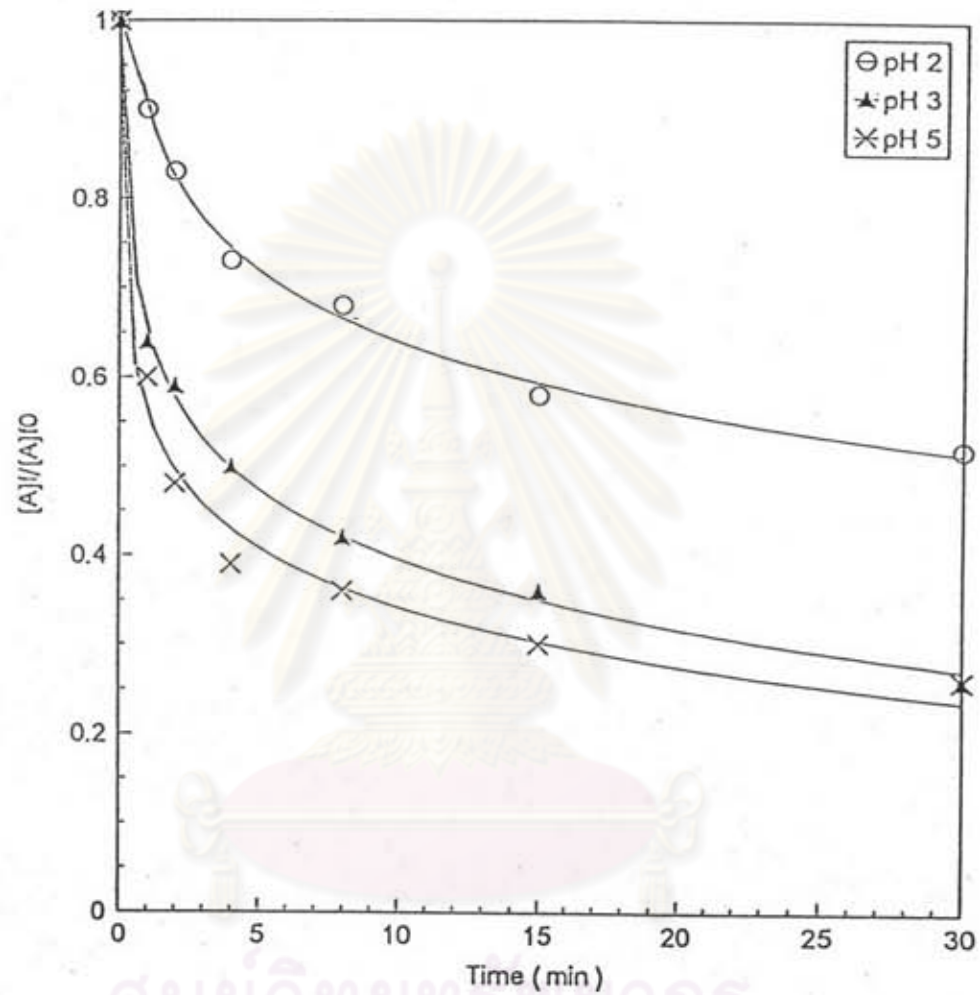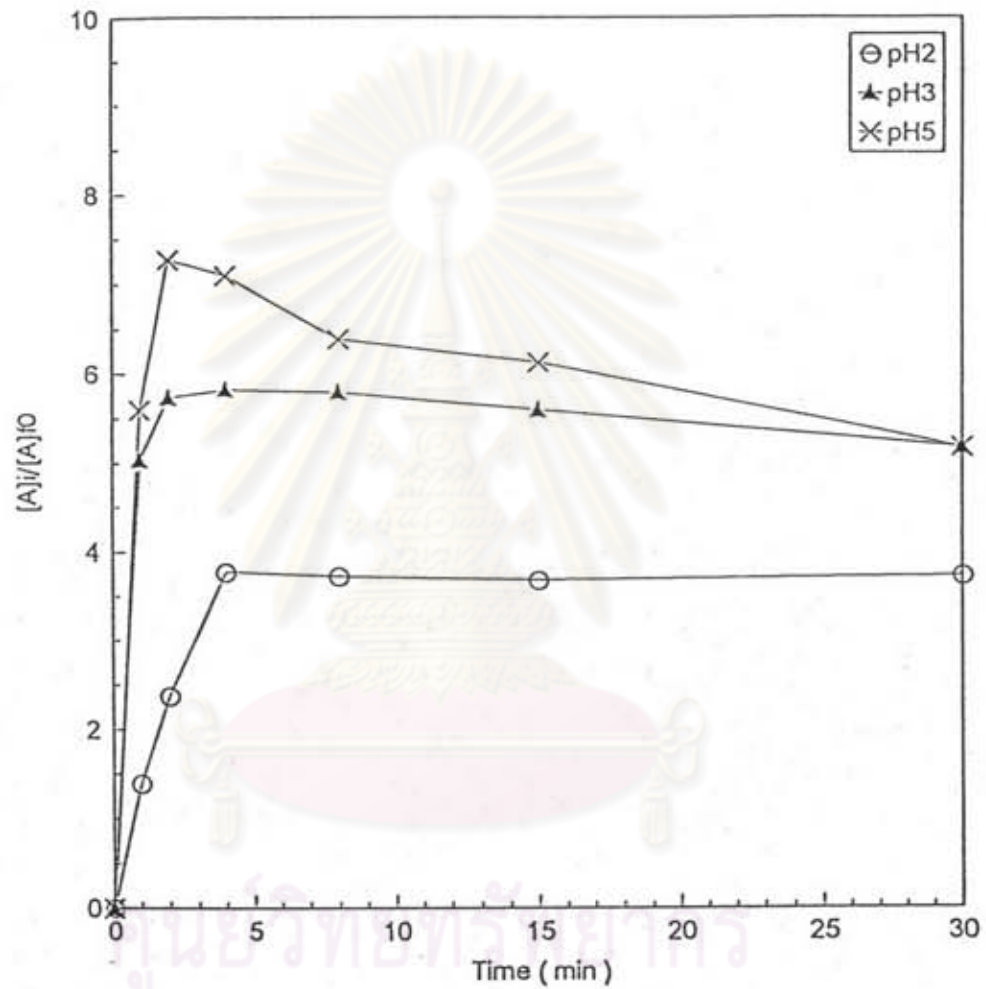Figure 5.4 Effect of pH on swelling in emulsion liquid membrane extraction of 0.006 M phenylalanine solution.
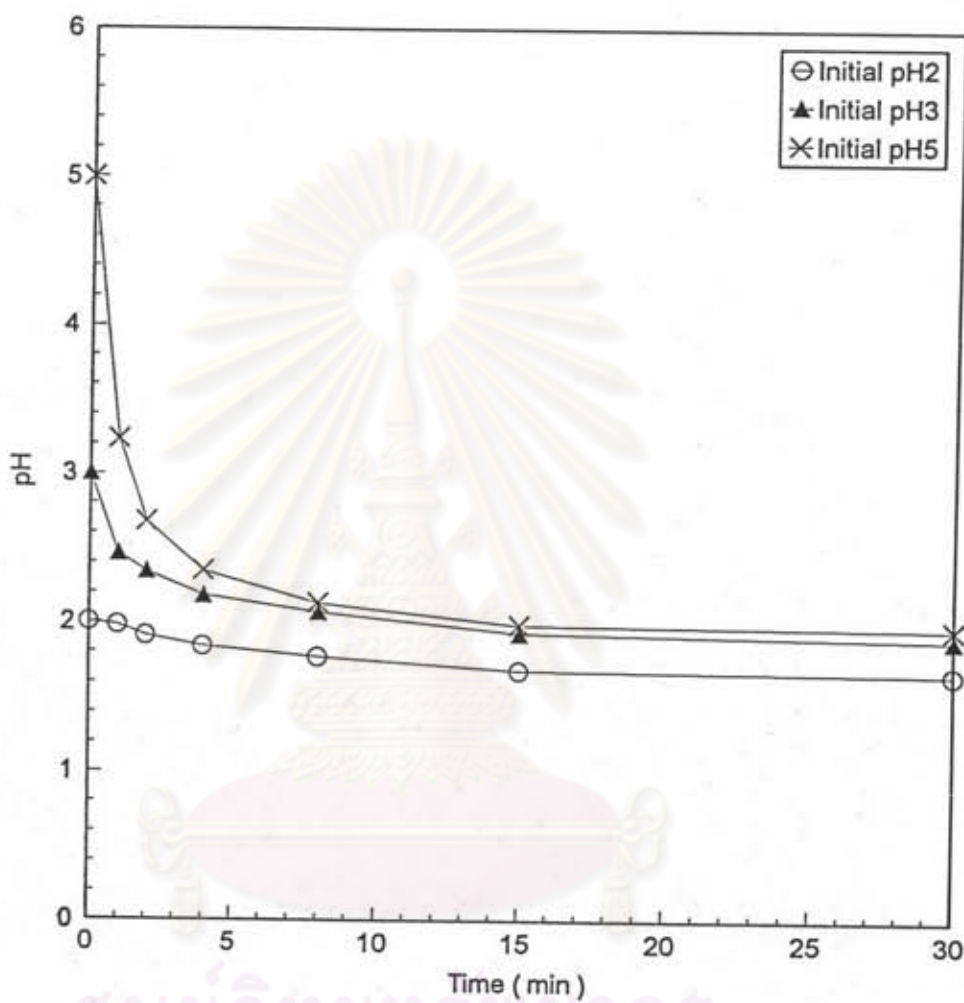
Figure 5.5   Change of pH in the feed phase during the emulsion liquid
membrane extraction of 0.006 M phenylalanine solution.

Figure5.6 Extraction of 0.006M tryptophan by emulsion liquid membrane
[A]f = Amino acid concentration in the feed phase.
[A]f0 = Initial amino acid concentration in the feed phase.

Figure 5.7 Concentration of 0.006M tryptophan in the internal phase.

[A]i = Amino acid concentration in the internal phase.

[A]f0 = Initial amino acid concentration in the feed phase.
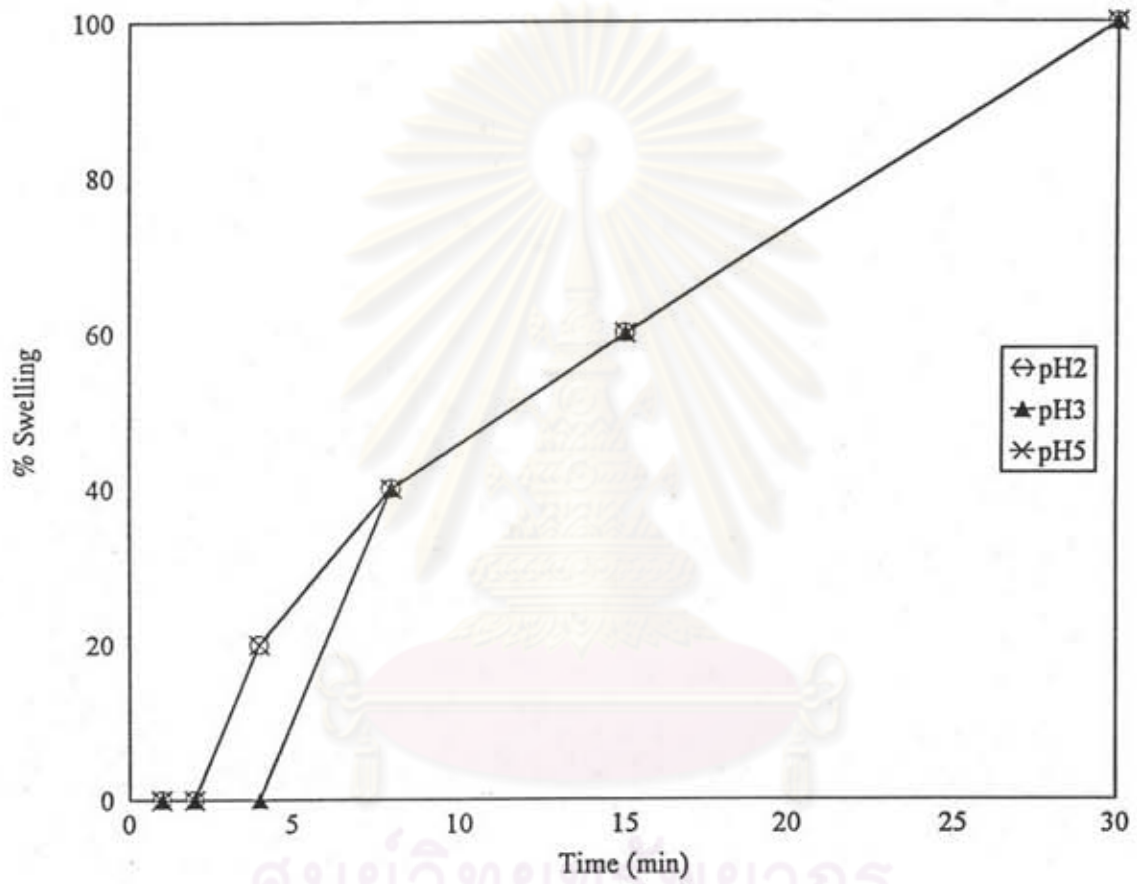
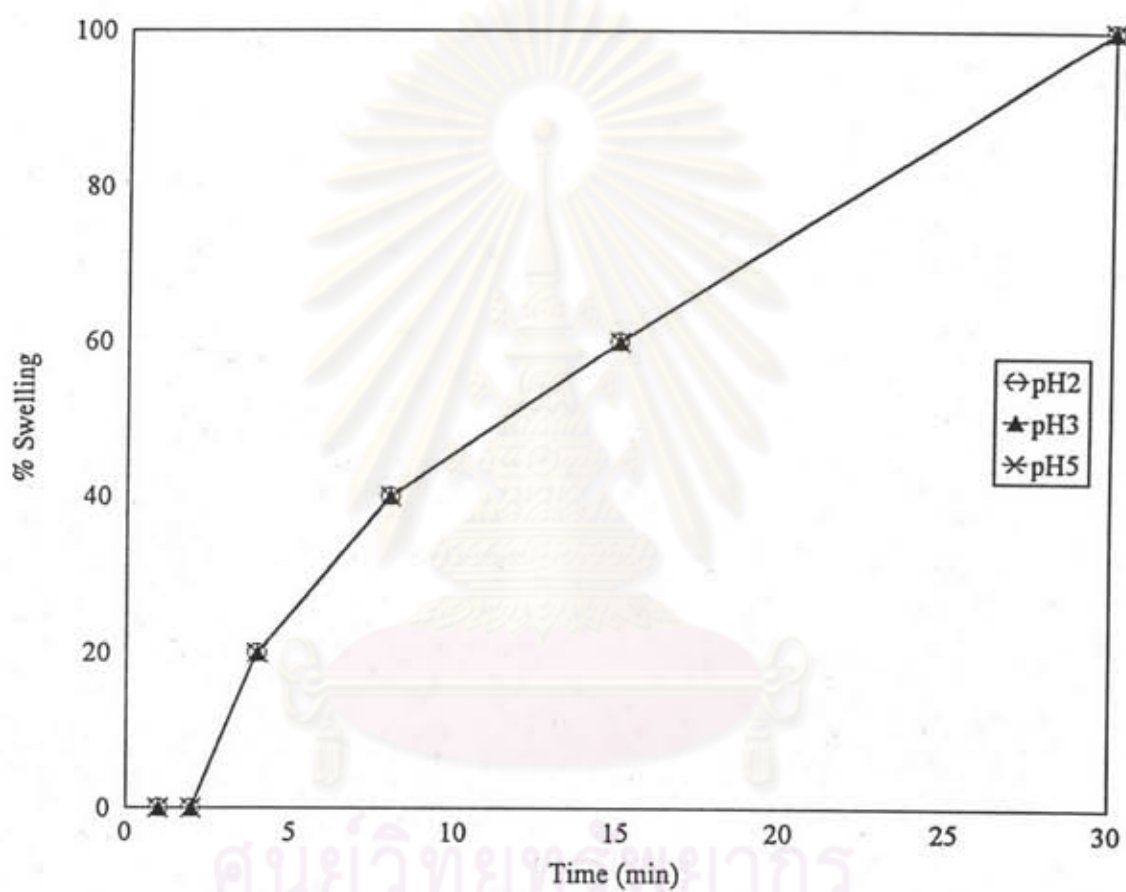Figure 5.8 Effect of pH on swelling in emulsion liquid membrane
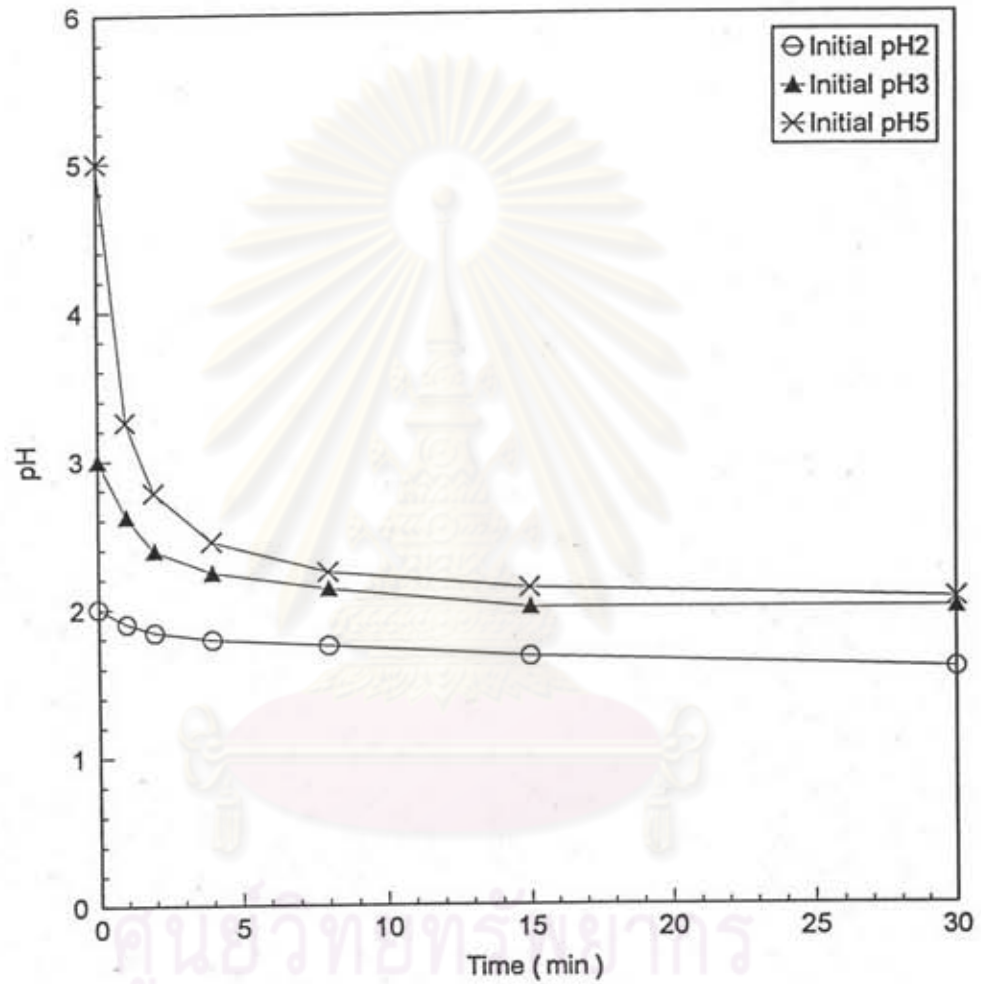extraction of 0.006 M tryptophan solution.

Figure 5.9   Change of pH in the feed phase during the emulsion liquid
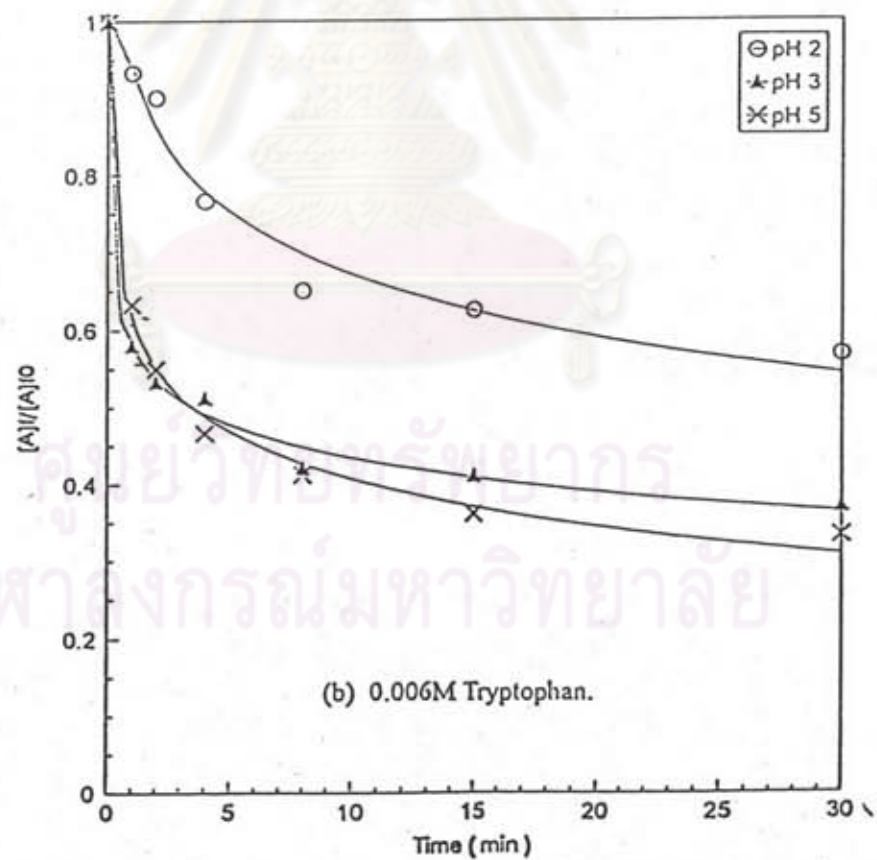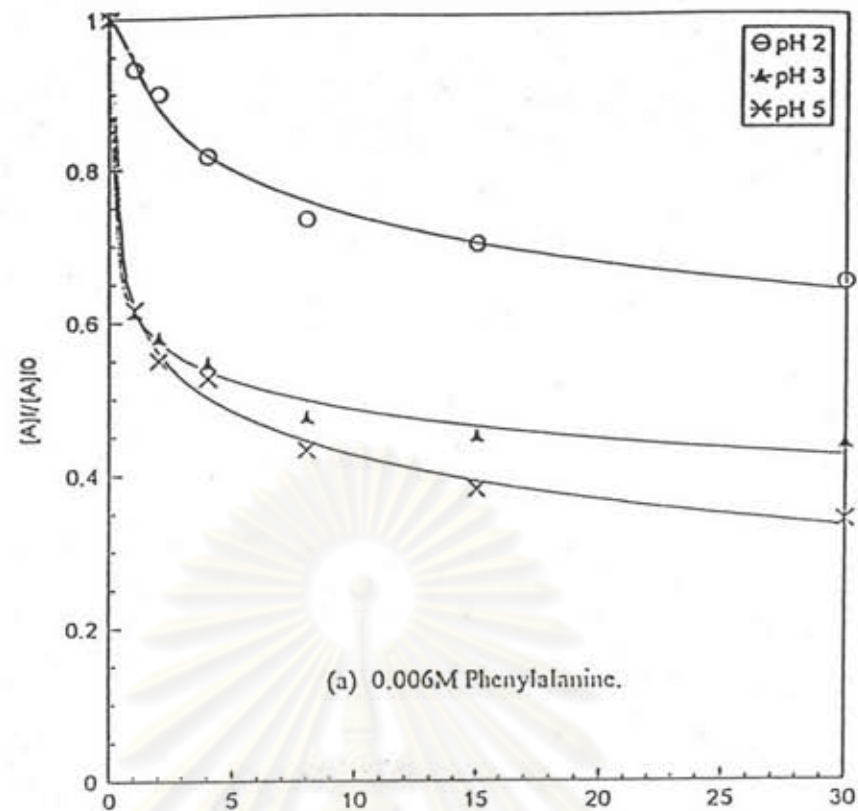membrane extraction of 0.006 M tryptophan solution.

Figure 5.10 Extraction of 0.001 M tryptophan by emulsion liquid membrane.

$[A]f$ = Amino acid concentration in the feed phase.

$[A]f0$ = Initial amino acid concentration in the feed phase.

Figure 5.11 Concentration of 0.001M tryptophan in the internal phase.

$[A]i$ = Amino acid concentration in the internal phase.

$[A]f0$ = Initial amino acid concentration in the feed phase.

Figure 5.12 Effect of pH on swelling in emulsion liquid membrane
extraction of 0.001 M tryptophan solution.

Figure 5.13 Change of pH in the feed phase during the emulsion liquid membrane extraction of 0.001 M tryptophan solution.

Figure 5.14 Extraction of binary mixture of 0.006 M phenylalanine and
0.006 M tryptophan solution. (a) 0.006 M Phenylalanine.
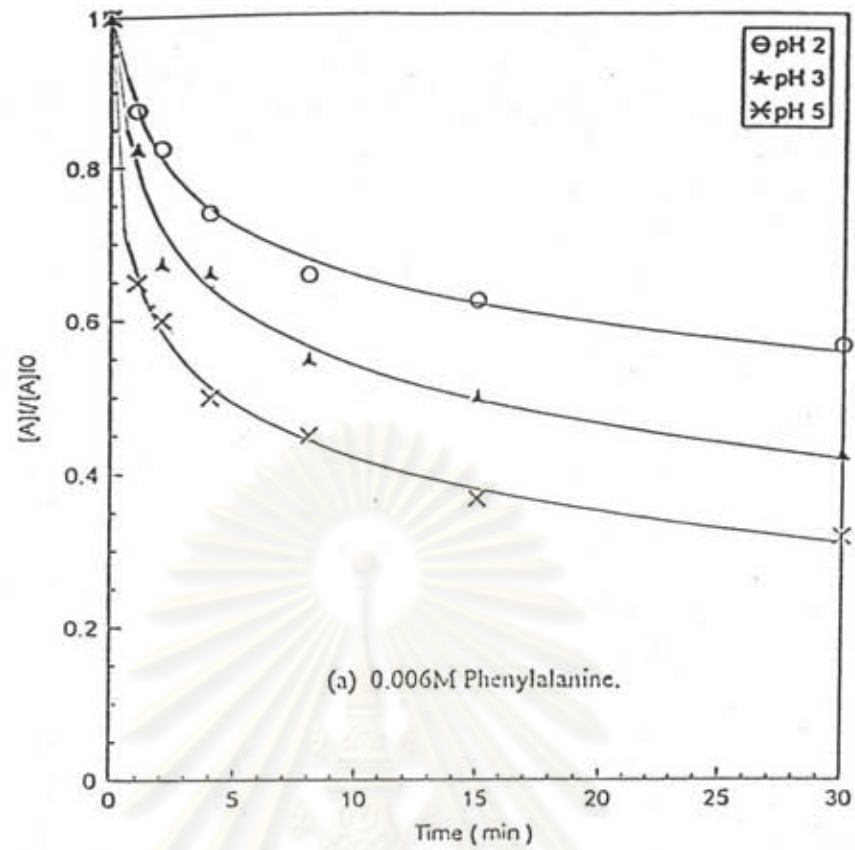(b) 0.006 M Tryptophan.
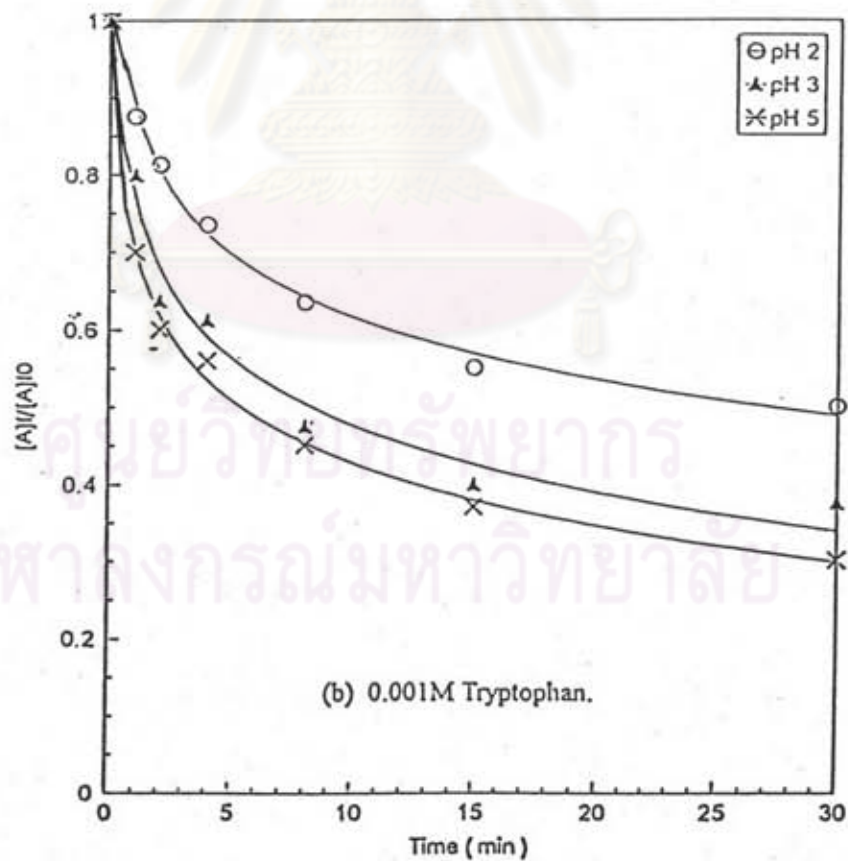
Figure 5.15 Extraction of binary mixture of 0.006 M phenylalanine and
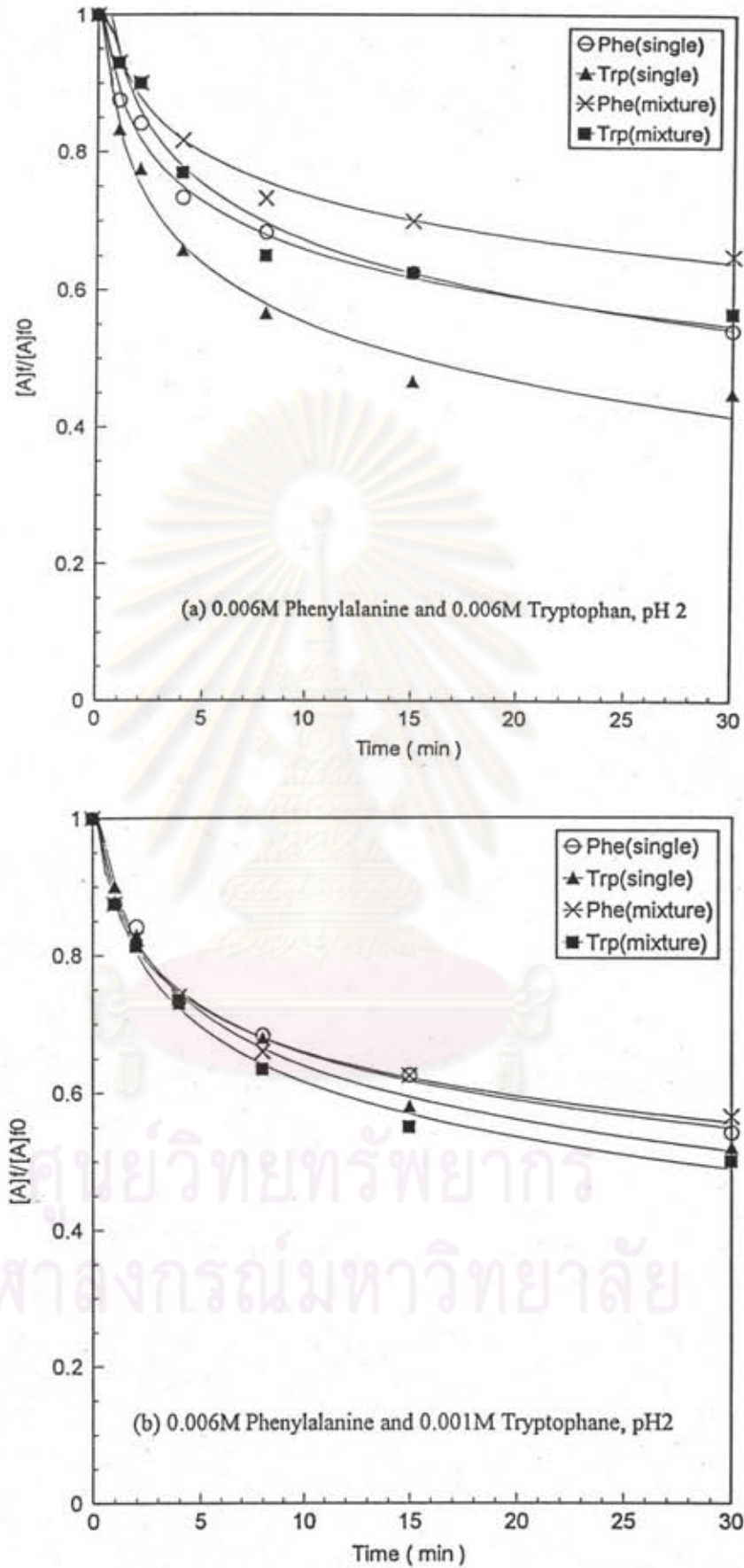0.001 M tryptophan solution. (a) 0.006 M Phenylalanine.
(b) 0.001 M Tryptophan.

Figure 5.16 Comparison of phenylalanine and tryptophan transport in emulsion liquid membrane extraction at pH 2.
(a) 0.006M Phenylalanine and 0.006M Tryptophan, pH 2.
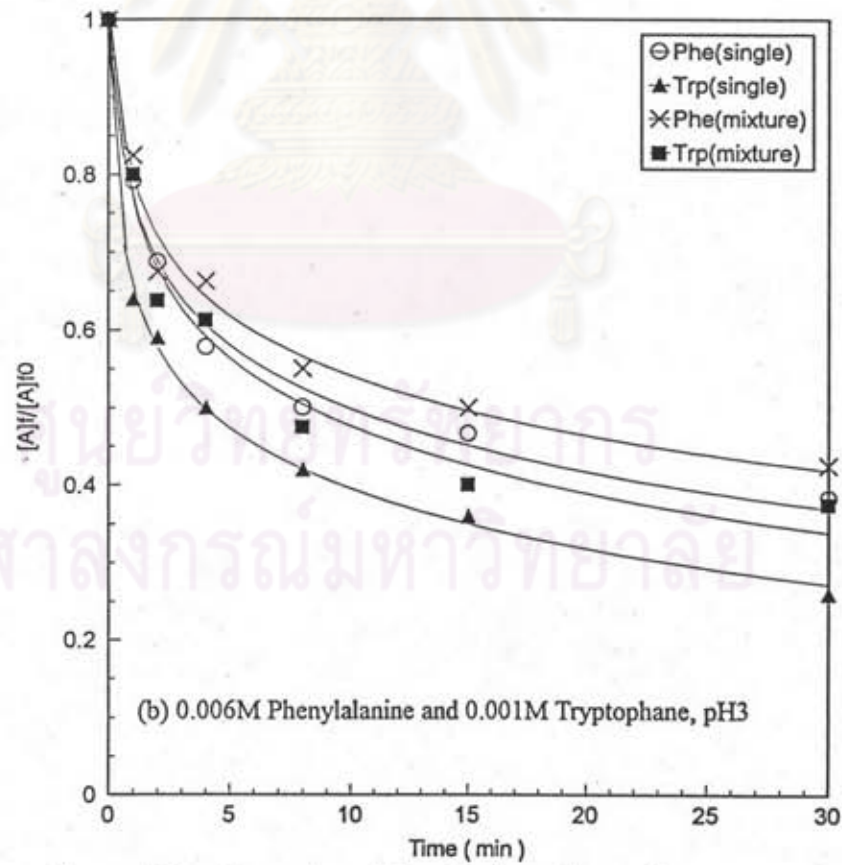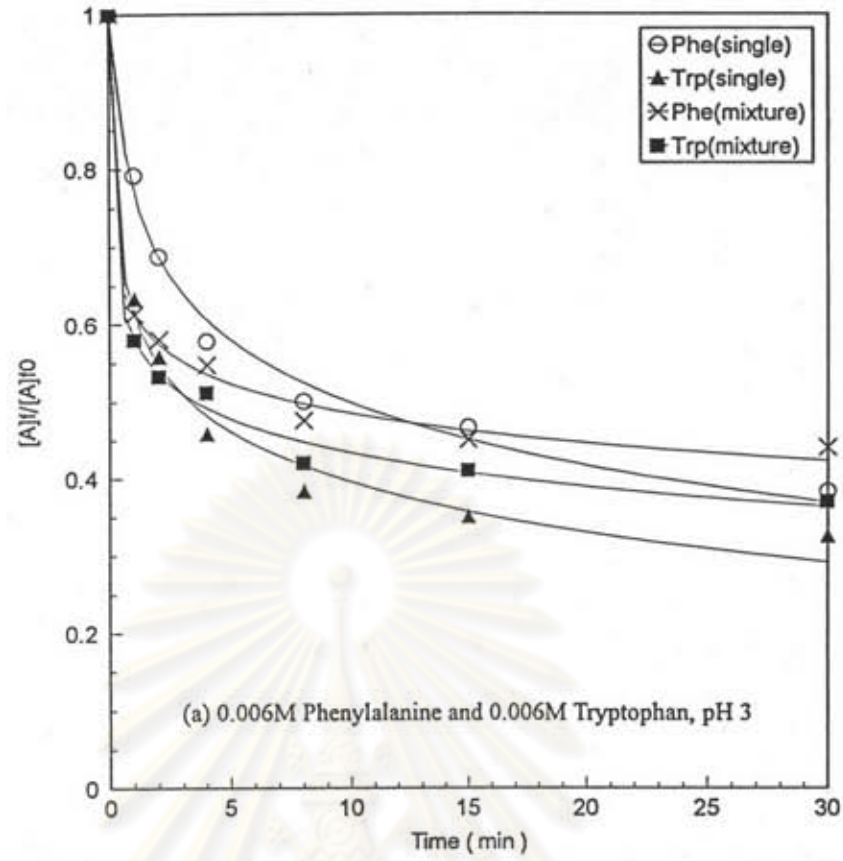(b) 0.006M Phenylalanine and 0.001M Tryptophan, pH 2.

Figure 5.17   Comparison of phenylalanine and tryptophan transport in emulsion liquid membrane extraction at pH 3.

(a) 0.006M Phenylalanine and 0.006M Tryptophan , pH 3.
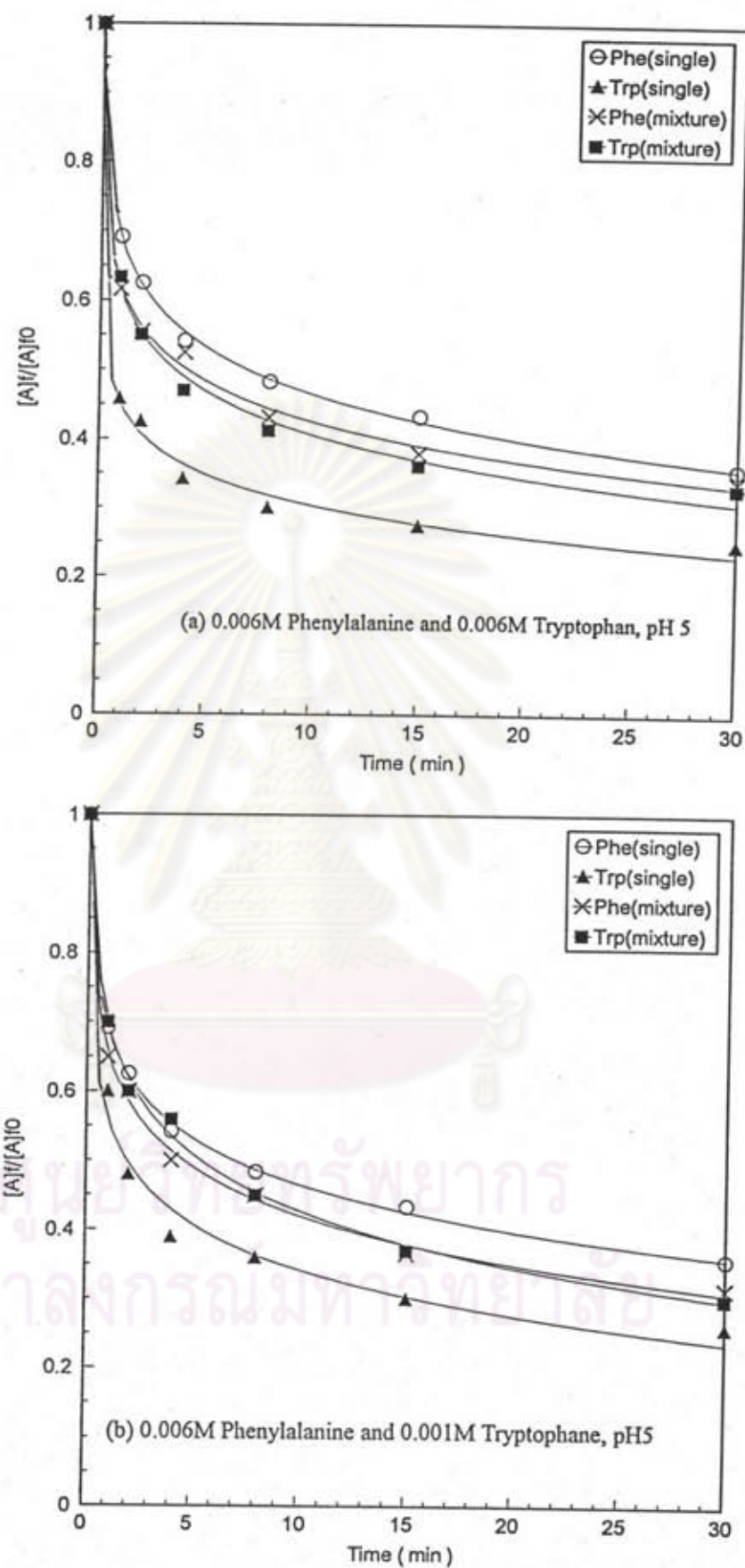
(b) 0.006M Phenylalanine and 0.001M Tryptophan , pH 3.

Figure 5.18 Comparison of phenylalanine and tryptophan transport in emulsion liquid membrane extraction at pH 5.
(a) 0.006M Phenylalanine and 0.006M Tryptophan, pH 5.
(b) 0.006M Phenylalanine and 0.001M Tryptophan, pH 5.