CHAPTER 5

EMULSION LIQUID MEMBRANE PROCESS

This Chapter presents the results of the batch extraction of synthetic berberine and crude berberine (Khamin Khruea) in the absence of any carrier species: unfacilitated extraction. In the external phase, aqueous berberine solution must be in the free base form, so the pH of the external phase was basic. The emulsifying agent Span-80 and kerosene were the components in the organic membrane phase. The internal phase is hydrochloric acid solution. The berberine free base form partitions into the external/membrane phases interface, and diffuses through the membrane to the membrane/internal phases interface. Thus the berberine chloride solution is separated and concentrated in the internal phase as shown schematically in Figure 5.1

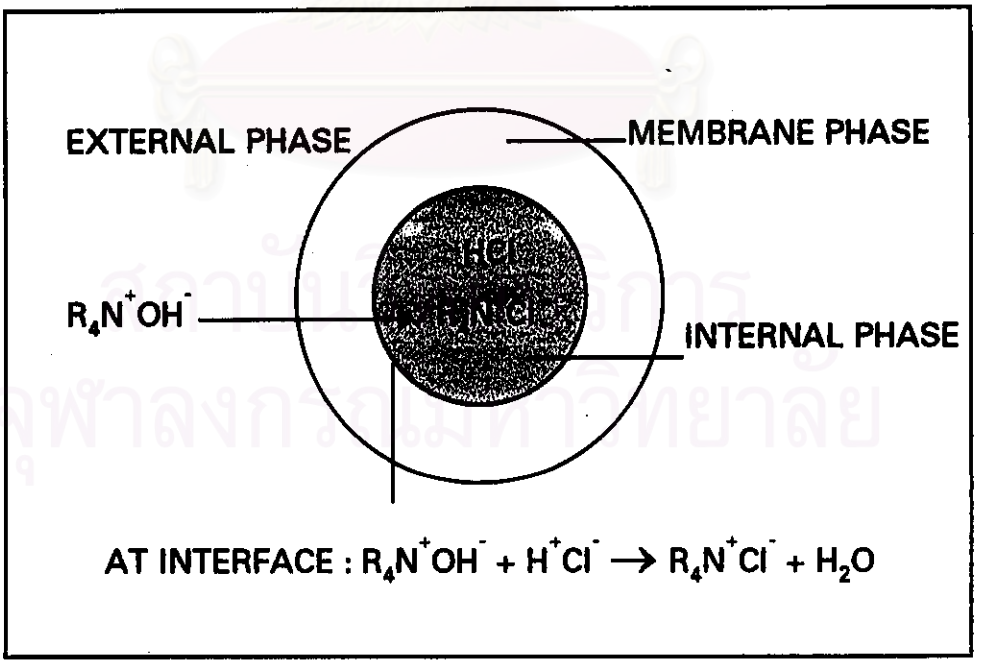

Figure 5.1 Schematic Diagram of the Transport Mechanism for Berberine

5.1 Experimental Materials and Methods

5.1.1 Experimental Materials

The reagents used in the experiment were previously described in Chapter 4. The emulsion liquid membrane process consisted of three parts as follows :

a. External phase

- 0.05 g/l synthetic berberine solution
- 0.11 g/l crude berberine solution from extracted Khamin Khruea

b. Membrane phase

organic solvent : kerosene

surfactant : Span-80

c. Internal phase

0.1, 0.01, 0.02 and 0.03 M hydrochloric acid solution

5.1.2 Experimental Methods

5.1.2.1 ELM for Synthetic Berberine Solution

At first, 100 ml of membrane phase was formed by blending 99 ml of kerosene as solvent and 1 ml of Span-80 as surfactant. This condition was 1% (v/v) of Span-80. The w/o emulsion was prepared by homogenizing 60 ml of 0.01 M HCl solution as internal phase and 60 ml of kerosene plus Span-80 as membrane phase with a high speed homogenizer (IKA laboratechnik model T25) at 8000 rpm for thirty minutes. The 100 ml of w/o emulsion, as mentiond above was poured into 300 ml of synthetic berberine solution (the external phase). By using the mechanical stirrer at 240 rpm, the w/o/w system was formed and the extraction was started. The

extraction time of this experiment was started from the time that emulsion was poured and 1 ml of samples of the external phase and membrane phase were carefully drawn out of the system with respect to time. After the end of extraction, the mixture was allowed to settle in a separatory funnel until two distinct phases appeared. The volume of each phase was measured and the synthetic berberine concentrations in the external phase were analyzed using HPLC (Water™ 600, 717, 996) on octyl column (15 cm). The mobile solution was prepared by mixing 70% acetonitrile in 0.1% H_3PO_4 which was adjusted to pH 6 with concentrated ammonia solution. This mobile solution was passed through the column at the flow rate of 1 ml/min. Berberine detection (UV detector) was achieved at 345 nm. The pH of the external phase solution was measured by using pH meter (Schott, CG 825). The internal droplets were analyzed by particle size analyzer (Coulter® LS SERIES). The experimental data are shown in Appendix B.

The experiments were carried out under the conditions shown in Table 5.1

Table 5.1 The Experimental Conditions of Synthetic Berberine Extraction on ELM at Room Temperature

Parameters	Conditions
Initial pH in the external phase	pH 8, 9, 10, 11 and 12
Initial synthetic berberine concentration in the external phase	0.05 g/l
Surfactant concentration	1, 3, 5 and 7% (v/v)
Internal HCl concentration	0.1, 0.01, 0.02 and 0.03 M

5.1.2.2 ELM for Crude Berberine Solution

The results of the experiment is Section 5.1.2.1 lead to do the experimental investigation in this Section. The membrane components and the method of emulsion liquid membrane extraction were the same as in Section 5.1.2.1 The external phase was crude berberine solution. The concentrations of crude berberine in the external phase were analyzed as same as described in Section 5.1.2.1 The experimental data of this particular section are shown in Appendix C.

The experiments were carried out under the conditions shown in Table 5.2

Table 5.2 The Experimental Conditions of Crude Berberine Extraction on ELM at Room Temperature

Parameters	Conditions
Initial pH in the external phase	pH 8, 9, 10, 11 and 12
Initial crude berberine concentration in the external phase	0.11 g/l
Surfactant concentration	1% (v/v)
Internal HCl concentration	0.01, 0.02 and 0.03 M.

5.1.2.3 Calculation of Crude Berberine Concentration in the Internal Phase

In ELM extraction, the concentrations of crude berberine in the external phase were measured and the concentrations of crude berberine in the

internal phase were calculated by mass balance based on the assumption that there was no accumulation of berberine in the membrane phase. The following is an example of how the concentration is calculated.

Experimental conditions

External phase	: 0.05 g/l of synthetic berberine solution at pH 11
Membrane phase	: 1% of Span-80 and 99% of kerosene
Internal phase	: 0.01 M HCl solution
Membrane preparation	: 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

The initial concentration of berberine in external phase was 0.056 g/l

The concentration of berberine in external phase after 1 minute extraction was 0.015 g/l

The amount of berberine that penetrated into the internal phase was 0.056 - 0.015 = 0.041 g/l

The volume of external phase was 300 ml

At 1 minute, the volume of internal phase was 50 ml

Therefore, the internal berberine concentration was $\frac{0.041 * 300}{50} = 0.246$ g/l

5.1.2.4 Calculation of %Swelling in the Internal Phase

The calculation of %swelling in the internal phase can be done by measuring the internal droplets size by particle size analyzer (COULTER® LS SERIES). The percentage of emulsion swelling can be calculated as the following:

Experimental conditions

External phase : 0.11 g/l of crude berberine solution at pH 9

Membrane phase : 1% of Span-80 and 99% of kerosene

Internal phase : 0.02 M HCl solution

Membrane preparation : 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

Data of internal droplet size :

Time (min)	Internal droplet size (μm)
0	2.548
1	2.551
2	2.585
3	2.611
4	2.619
5	2.627

at 0 minute, internal droplet volume $= \frac{4}{3}\pi r^3$

$= \frac{4}{3}\pi(1274 * 10^{-3})^3$

$= 8.6*10^{-9}$ mm^3

at 1 minute, internal droplet volume $= \frac{4}{3}\pi r^3$

$= \frac{4}{3}\pi(1305 * 10^{-3})^3$

$= 9.3*10^{-9}$ mm^3

$$\% \text{ swelling} = \frac{(9.3 - 8.6) * 10^{-9}}{8.6 * 10^{-9}} * 100$$

$$= 8.6\%$$

5.2 Results and Discussion

In this experiment, the chemical structure of berberine changes significantly with changes in pH, as shown in Chapter 3. Generally, berberine is used as medicinal in the forms of salt such as berberine chloride and berberine sulphate etc., as separated and concentrated by ELM process at the same time. Thus, berberine in the external phase must exist in basic form and the reagents in the internal phase must exist in acidic form. When berberine diffuses through the internal phase will get berberine in salt form. In order to separated and concentrated more berberine in salt form, a large difference of pH between the external and internal phases must be established

5.2.1 ELM for Synthetic Berberine Solution

5.2.1.1 The Effect of Surfactant Concentration

The relationship between the concentrations of the surfactant Span-80 and berberine extraction are depicted in Figure 5.2. The experimental data are shown in Table B.2 (Appendix B). The experimental conditions used at room temperature were 0.05 g/l of initial synthetic berberine solution at pH 9 with 1%, 3%, 5% and 7% (v/v) of surfactant concentrations; kerosene at various volume (% kerosene due to % Span-80) as membrane phase, and 0.1 M HCl solution as the internal phase. From this Figure, X-direction is time (min) and the Y-direction is C/Co.

An increase of Span-80 concentration in membrane phase from basis 1% (v/v) will result in lowering in the amount of synthetic berberine extracted. After 20 minutes, the amount of synthetic berberine extracted will go toward constant value at each of surfactant concentration. In this system, Span-80 concentration of 1 % (v/v) seems optimal.

Figure 5.3 shows the initial rate of synthetic berberine extraction by ELM at various surfactant concentration. The experimental data are taken from Table B.3 (Appendix B). From this curve, X-direction is surfactant concentration (% v/v) and Y-direction is initial rate (g/l min). It shows that the initial rate of synthetic berberine extraction decreases with the increasing of Span-80 concentration (at first 1 minute). This can be explained that when the concentration of Span-80 was increased, the layer of surfactant film in the organic phase becomes thick which reflected to high mass transfer resistance resulting in lower berberine diffusion rate. In this experiment, the 1% (v/v) of Span-80 concentration was found to be the optimum value, gave rise to high initial extraction rate and gave 80% of extraction within 20 minutes.

Figure 5.4 illustrates the internal berberine concentration on ELM extraction of synthetic berberine solution at various surfactant concentrations (at first 1 minute). This Figure is derived from Table B.4 (Appendix B). From this curve, the X-direction is surfactant concentration (% v/v) and the Y-direction is internal berberine concentration (g/l). Apparently that, the internal berberine concentration decreases with the increasing of Span-80. At 1 % (v/v) of Span-80 concentration, the extraction process can be maximum with synthetic berberine concentrated for 3 times in the external phase.

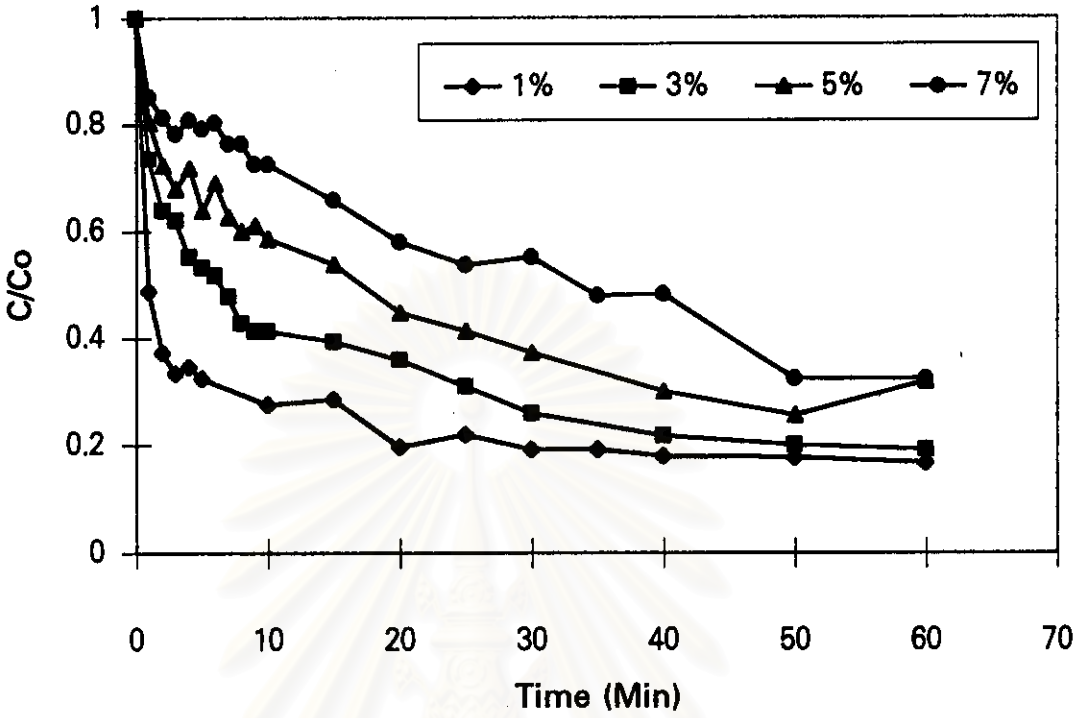

Figure 5.2 Effect of Surfactant Concentration on ELM Extraction of Synthetic Berberine Solution at Various Surfactant Concentrations

Experimental Conditions

External phase	:	0.05 g/l of synthetic berberine solution at pH 9
Membrane phase	:	Span-80 at various concentration and kerosene at various volume (% kerosene due to % Span-80)
Internal phase	:	0.1 M HCl solution
Membrane preparation	:	at 8000 rpm of homogenizing speed for 30 minutes
Agitatiion speed	:	240 rpm
Temperature	:	room temperature

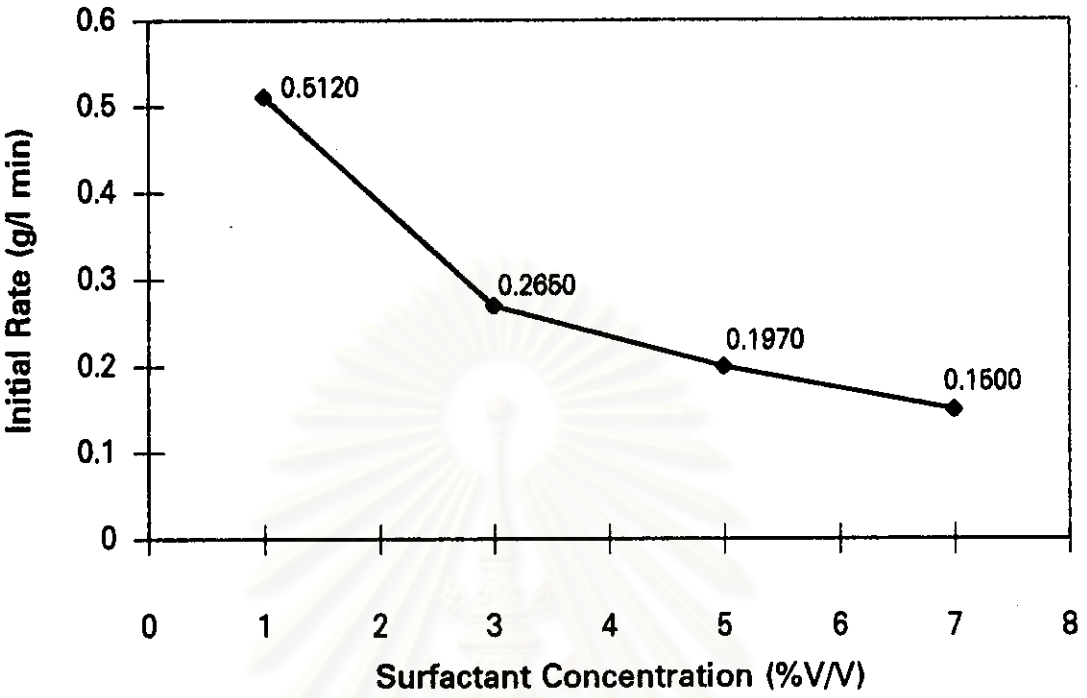

Figure 5.3 Initial Rate of Berberine Extraction by ELM at Various Surfactant Concentrations

Experimental Conditions

External phase	:	0.05 g/l of synthetic berberine solution at pH 9
Membrane phase	:	Span-80 at various concentration and kerosene at various volume (% kerosene due to % Span-80)
Internal phase	:	0.1M HCl solution
Membrane preparation	:	at 8000 rpm of homoginizing speed for 30 minutes
Agitation speed	:	240 rpm
Temperrature	:	room temperature

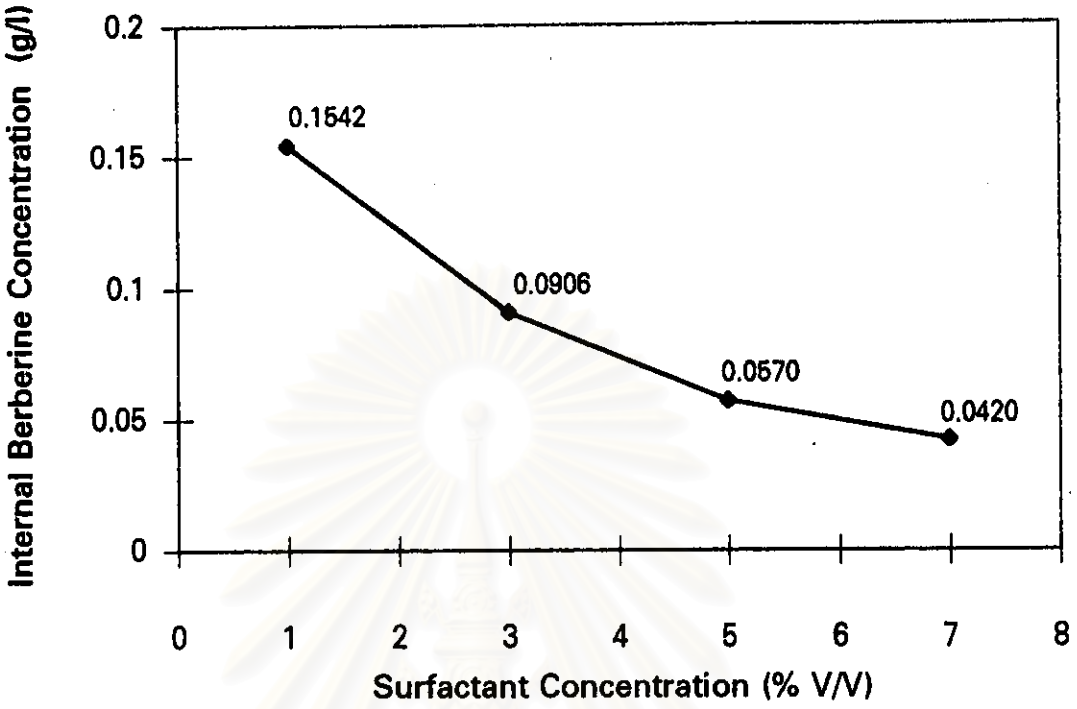

Figure 5.4 Internal Berberine Concentration on ELM Extraction of Synthetic Berberine Solution at Various Surfactant Concentrations (at first 1 minute)

Experimental Conditions

External phase	:	0.05 g/l of synthetic berberine solution at pH 9
Membrane phase	:	Span-80 at various concentration and kerosene at various volume (% kerosene due to % Span-80)
Internal phase	:	0.1 M HCl solution
Membrane preparation	:	at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	:	240 rpm
Temperature	:	room temperature

5.2.1.2 The Effect of Initial pH in the External Phase

As described earlier, the chemical structure of berberine changes significantly with changes in pH. The relationship between initial pH of the external phase and berberine extraction is shown in Figure 5.5. The data of this curve are shown in Table B.6 (Appendix B). This curve illustrates the effect of initial pH in external phase on ELM extraction of synthetic berberine solution which the X-direction is time (min) and the Y-direction is C/Co. The experimental conditions used at room temperature were 0.05 g/l of initial synthetic berberine solution at various pH from pH 8 to pH 12 , 1% (v/v) of Span-80 and 99% of kerosene as a membrane phase, and 0.01 M HCl solution as the internal solution. The amount of synthetic berberine extracted increased with the increasing of pH value of the external phase. At the same pH value, the amount of synthetic berberine extracted will increase with time until it becomes constant. Comparing pH 12 with pH 11 (at first 1 minute), the amount of synthetic berberine extracted decreased due to leakage which caused apparent of partial emulsion breakage or instability of the emulsion, although the amount of synthetic berberine transported through the membrane actually increased, the amount of synthetic berberine leakage due to the breakage of emulsion also increased and the resulting net amount of synthetic berberine transported did not much increase.

Figure 5.6 shows the initial synthetic berberine extraction rate on ELM extraction of synthetic berberine solution at various external phase pH (at first 1 minute) which data are obtained from Table B.7 (Appendix B). The X-direction is pH and the Y-direction is initial rate (g/l min). It was found that the increasing of pH value will increase in free base form of synthetic berberine in the external phase. Therefore, the pH value of the external phase increased with the increasing of the initial

synthetic berberine extraction rate. However, at pH 12 the initial berberine extraction rate decreased. The reason can be explained in the same as mentioned in Figure 5.5. In this experiment, the optimum pH value of the external phase was pH 11, which gave 0.7358 g/l min of initial extraction rate and produced nearly 100% of extraction within 5 minutes.

Figure 5.7 as also shows the internal berberine concentration on ELM extraction of synthetic berberine solution at various external phase pH (at first 1 minute). The data of this Figure are obtained from Table B.8 (Appendix B). The X-direction is pH and the Y-direction is the internal synthetic berberine concentration (g/l). It was shown that the internal synthetic berberine concentration at 1 minute of extraction increased with the increasing of initial pH value of the external phase, but it decreases at pH 12. It was also found that at pH 11 (0.01M HCl solution) the internal berberine concentration was 4.4 times the external berberine concentration.

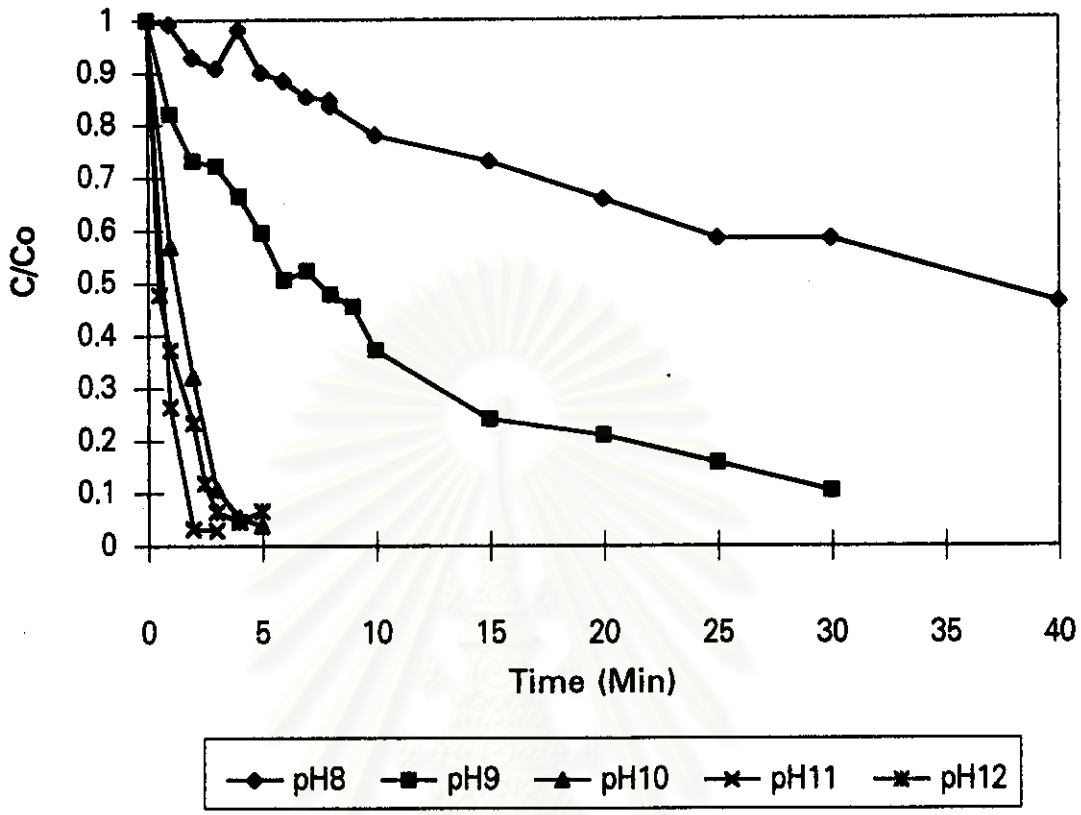

Figure 5.5 Effect of Initial pH in External Phase on ELM Extraction of Synthetic Berberine Solution at Various pH of External Phase (at first 1 minute)

Experimental Conditions

External phase : 0.06 g/l of synthetic berberine solution at various external pH

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

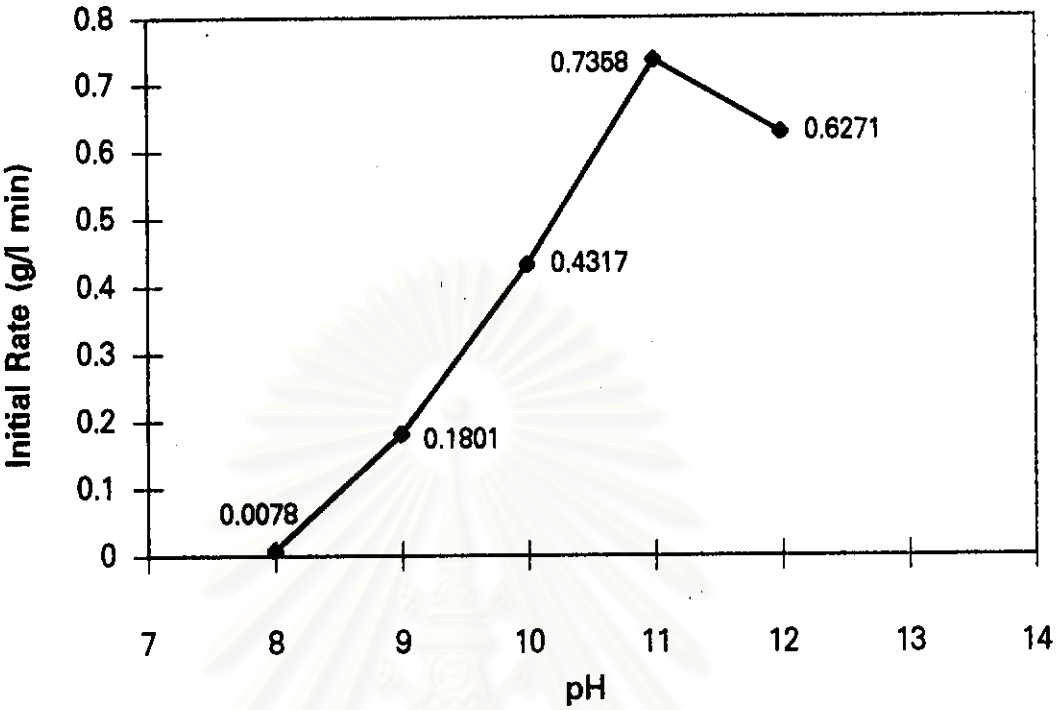

Figure 5.6 Initial Rate on ELM Extraction of Synthetic Berberine Solution at Various External Phase pH (at first 1 minute)

Experimental Conditions

External phase : 0.05 g/l of synthetic berberine solution at various external phase pH

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

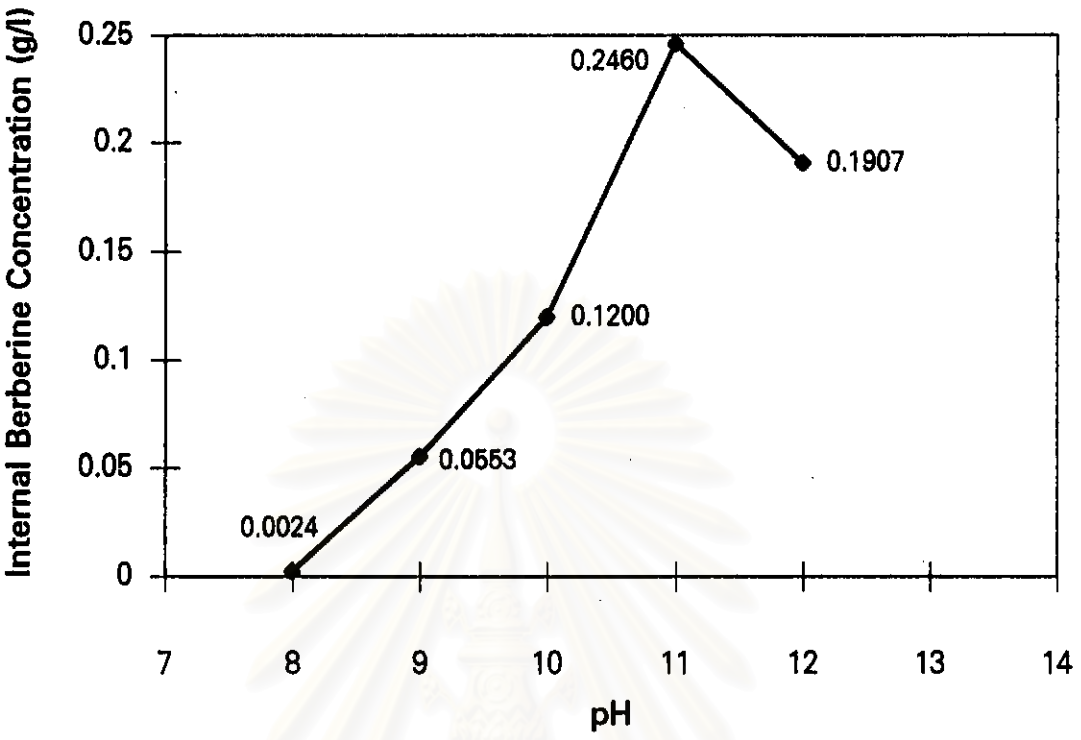

Figure 5.7 Internal Berberine Concentration on ELM Extraction of Synthetic Berberine Solution at Various pH of External Phase (at first 1 minute)

Experimental Conditions

External phase : 0.05 g/l of synthetic berberine solution at various external phase pH

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

5.2.1.3 The Effect of Hydrochloric Acid Concentration in the Internal Phase

Difference in hydrogen ion concentration between external and internal phase is the driving force in the emulsion liquid membrane extraction. The relationship between hydrochloric acid concentration in the internal phase and synthetic berberine extraction with time is shown in Figure 5.8. The effect of HCl concentration in the internal phase on ELM extraction of synthetic berberine solution is also depicted, which the X-direction is time (min) and the Y-direction is C/Co. The data of this curve are shown in Table B.10 (Appendix B). The experimental conditions used at room temperature were 0.05 g/l of initial synthetic berberine concentration at pH 11 , 1% (v/v) of Span-80 and 99% of kerosene as membrane. The internal phases were 0.01, 0.02 and 0.03 M HCl, respectively. The Figure also shows that the amount of synthetic berberine extracted increased with the increasing of HCl concentration in the internal phase. At this curve, it can be seen that the amount of synthetic berberine extracted at 0.02 and 0.03 M HCl solution at first 1 minute is found to be nearly the same.

Figure 5.9 describes the initial rate on ELM of synthetic berberine solution at internal phase concentration variables (at first 1 minute). Which data are listed Table B.11 (Appendix B). The X-direction is HCl concentration (M) and the Y-direction is the initial rate (g/l min). As mentioned earlier, a higher concentration of free base form berberine existed at higher basicity. Therefore, the increase of HCl concentration in the internal phase resulted in the increase of berberine extraction rate due to high degree of acid/base reaction. At 0.02 and 0.03 M HCl solution, the extraction rate for the first 1 minute is nearly the same, so that lower acid concentration should be utilize.

Figure 5.10 shows the internal berberine concentration on ELM extraction of synthetic berberine solution at internal phase concentration variables (at first 1 minute) (as seen in Table B.12, Appendix B). The X-direction is HCl concentration (M) and the Y-direction is the internal synthetic berberine concentration (g/l). It shows that at 0.02 M HCl solution in the internal phase can achieve 5 times higher concentration than the initial one.

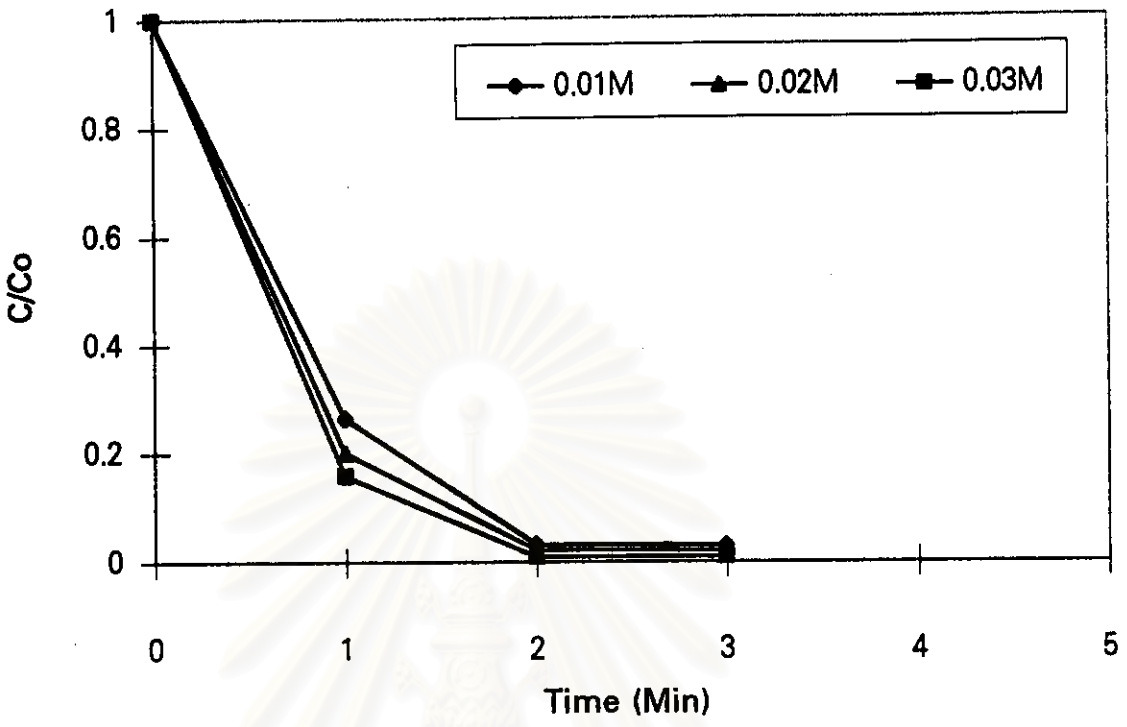

Figure 5.8 Effect of HCl Concentration in the Internal Phase on ELM Extraction of Synthetic Berberine Solution

Experimental Conditions

External phase : 0.05 g/l of synthetic berberine solution at pH 11

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : HCl solution at various concentrations

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

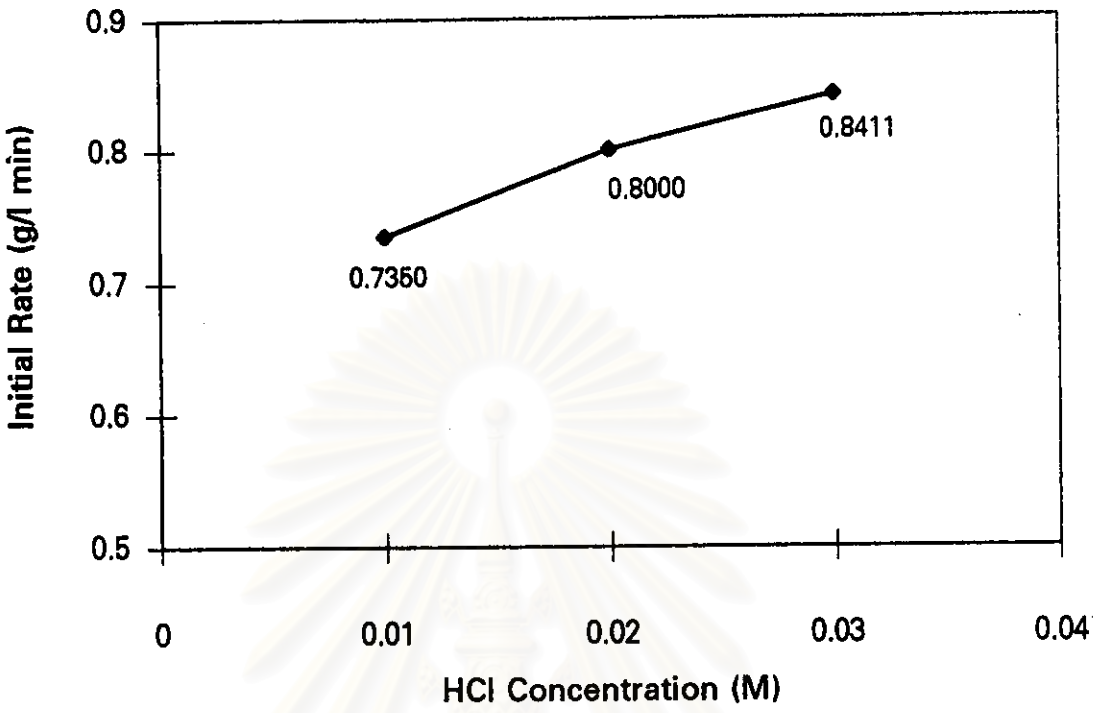

Figure 5.9 Initial Rate on ELM Extraction of Synthetic Berberine Solution at Intertnal Phase Concentration Variables (at first 1 minute)

Experimental Conditions

External phase : 0.05 g/l of synthetic berberine solution at pH 11

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : HCl solution at various concentrations

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

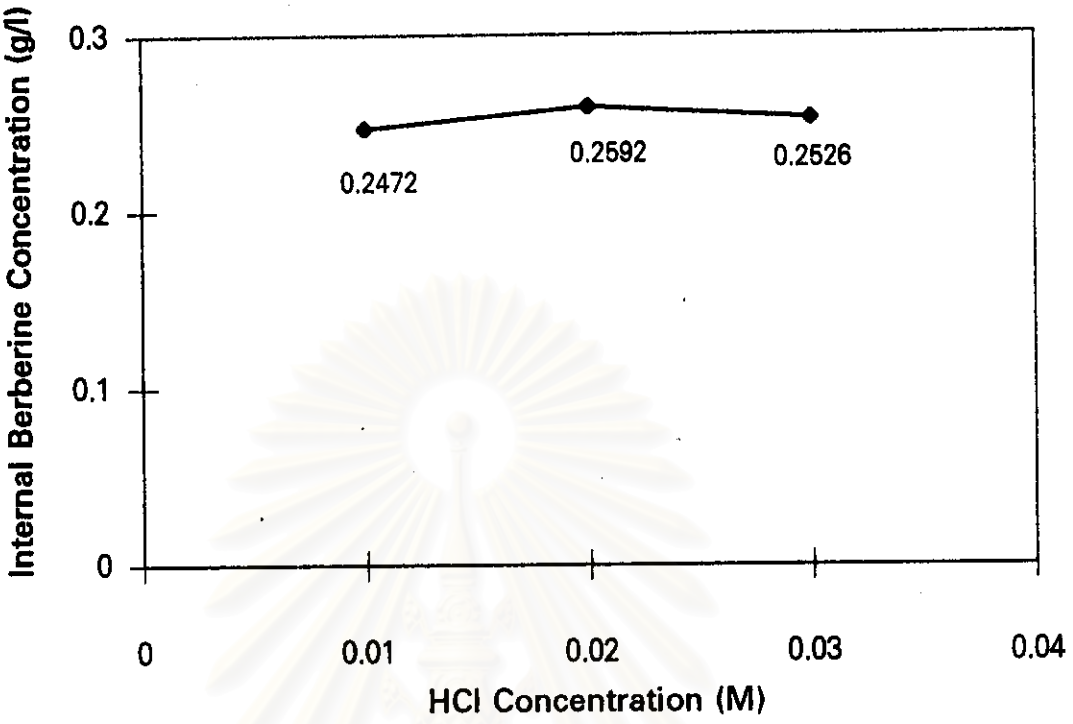

Figure 5.10 Internal Berberine Concentration on ELM Extraction of Synthetic Berberine Solution at Internal Phase Concentration Variables (at first 1 minute)

Experimental Conditions

External phase : 0.05 g/l of synthetic berberine solution at pH 11

Membrane phase : 1% Span-80 and 99% kerosene

Internal phase : HCl solution at various concentrations

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

5.2.2 ELM for Crude Berberine Solution

5.2.2.1 The Effect of Initial pH of the External Phase

From Section 5.2.1.2 in this Chapter, it was found that the increasing of pH value will increase the free base form of berberine. The relationship between initial pH of external phase and crude berberine extraction is shown in Figure 5.11, 5.12 and 5.13. The data are from Table C.2, C.6, and C.10 (as seen in Appendix C). The experimental conditions used at room temperature were about 0.11 g/l of initial crude berberine solution at various external phase pH from pH 8 to pH 12; 1% v/v of Span-80 and 99% of kerosene, as membrane. The internal phase was 0.01, 0.02, and 0.03 M HCl solution, respectively.

Figure 5.11, 5.12, and 5.13 illustrate the plots between C/Co and time at various initial pH of the external phase and 0.01 M, 0.02 M, and 0.03 M HCl solutions, respectively. The X-direction is time (min) and the Y-direction is C/Co. All these Figures have similar findings the amount of crude berberine extraction increased with the increasing of pH value of the external phase. At the same pH value, the amount of berberine extraction increased with time until it became constant. Comparison pH 11 with pH 12, due to leakage or breakage the amount of crude berberine extracted decrease. Actually the amount of crude berberine transported through the membrane increased, the amount of crude berberine leakage also increased and the resulting net amount of crude berberine transported did not much increase.

Figure 5.14 shows the initial crude berberine extraction rate at various external phase pH (at first 1 min) which data are from Table C.3, C.7, and C.11 (as seen in Appendix C). The X-direction is pH and the Y-direction is the initial rate (g/l

min). These were found that the increasing of external phase pH value will increase the initial crude berberine extraction rate. The reason can be explained that the increasing of external phase pH was caused the increment of berberine free form. At the same external phase pH, the increasing of HCl concentration (internal phase concentration) will increase the initial berberine extraction rate, e.g. at the external phase pH 12, the initial crude berberine extraction rate at 0.01, 0.02 and 0.03 M HCl were 0.5419, 0.6473 and 0.6985 g/l min, respectively.

Figure 5.15 shows the comparison of the initial rate of berberine from synthetic berberine solution with crude berberine solution at various pH of external phase. The experimental data are from Table B.7 and C.3 (as seen in Appendix B and C, respectively). The X-direction is pH and the Y-direction is initial rate of berberine (g/l min). The experimental conditions used the same conditions; excepted different concentrations and types of external phase as following : about 0.05 g/l of synthetic berberine solution and 0.11 g/l of crude berberine solution at various pH of external phase from pH 8 to pH 12 with 1% (v/v) of Span-80 and 99% of kerosene as membrane phase and 0.01 M HCl solution as internal phase. It was found that both initial rate of berberine extraction rate increases with the increasing of initial pH value of external phase as mentioned before. Exception at pH 12 of synthetic berberine solution, it was decreased due to leakage. Comparing the initial rate of synthetic berberine solution with crude berberine solution at the same concentration, the initial rate of synthetic berberine was higher than the initial rate of crude berberine. The reason can be explained like these. Owing to the crude berberine solution which is extracted from Khamin Khruea is not a pure berberine solution as the other one. They are comprised with many derivatives and many forms of alkaloid which were able to react with HCl solution in the internal phase. Therefore this result was decreasing of crude berberine initial rate in extraction system.

Figure 5.16 shows the internal berberine concentration on ELM extraction of crude berberine solution at various pH of external phase which data are shown in Table C.4, C.8, and C.12 (as seen in Appendix C). The X-direction is pH and the Y-direction is internal crude berberine concentration (g/l). It was found that the internal crude berberine concentration increase with the increasing of pH value of the external phase. At the same external phase pH, the initial berberine concentration increase with the increasing of HCl concentration, e.g. at pH 12, the internal crude berberine concentration at 0.01, 0.02, 0.03 M HC l were 0.3768, 0.4152 and 0.4986 g/l, respectively. It was also found that at the same pH 12, the internal concentration at 0.01, 0.02, and 0.03 M HCl were 3.25, 3.88, and 4.10 times higher concentration than the initial berberine concentration in the external phase respectively.

Figure 5.17 shows the comparison of the internal berberine concentration between systhetic berberine solution and crude berberine solution at various external phase pH (at first 1 minute). The experimental data derived from Table B.8 and C.4 (Appendix B and C). The experiment data have been done at the same conditions, the initial concentration of crude berberine solution was about 0.11 g/l and 0.05 g/l, respectively. The concentration of synthetic berberine solution was about 0.05 g/l. The X-direction is pH and the Y-direction is internal berberine concentration (g/l). It was shown that both internal berberine concentration increases with the increasing of initial pH value on the external phase. Comparing 0.05 g/l of synthetic berberine solution and 0.05 g/l of crude berberine solution, it was found that the internal berberine concentration of synthetic berberine solution was higher than the internal berberine concentration of crude berberine slution. The optimum internal berberine concentration of crude berberine and synthetic berberine solution were 3.25

times (pH 12) and 4.39 times (pH 11) higher concentration than the initial concentration in external phase.

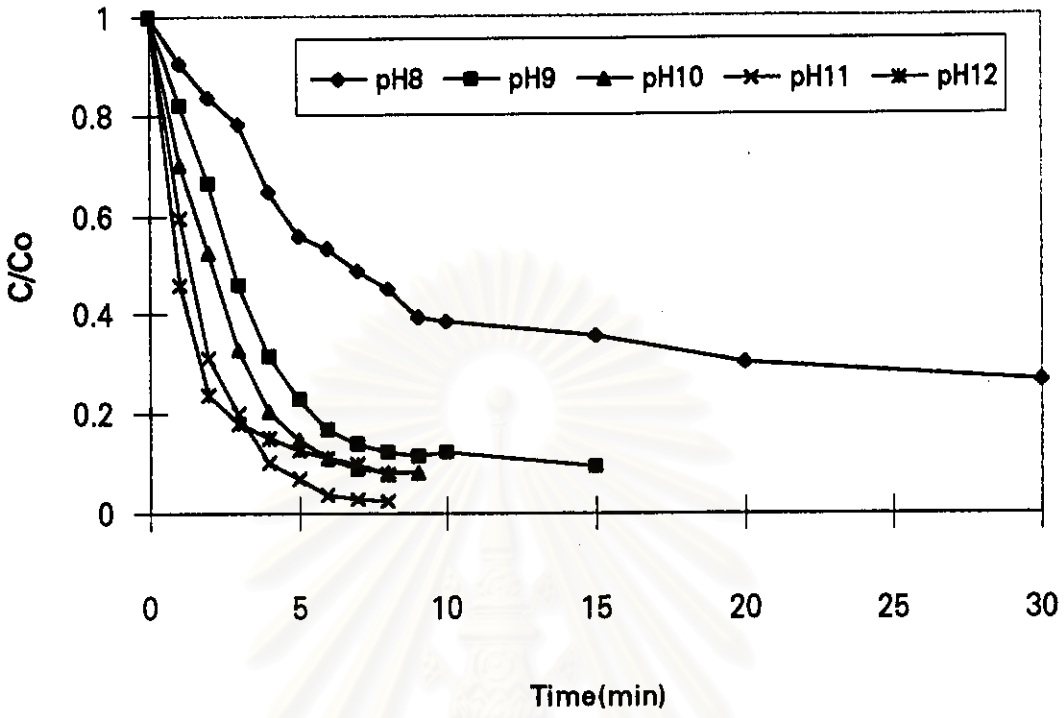

Figure 5.11 Effect of Initial pH in External Phase on ELM Extraction of Crude Berberine Solution at Various pH of External Phase (0.01 M HCl)

Experimental Conditons

External phase : 0.11 g/l of crude berberine solution at various external phase pH

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

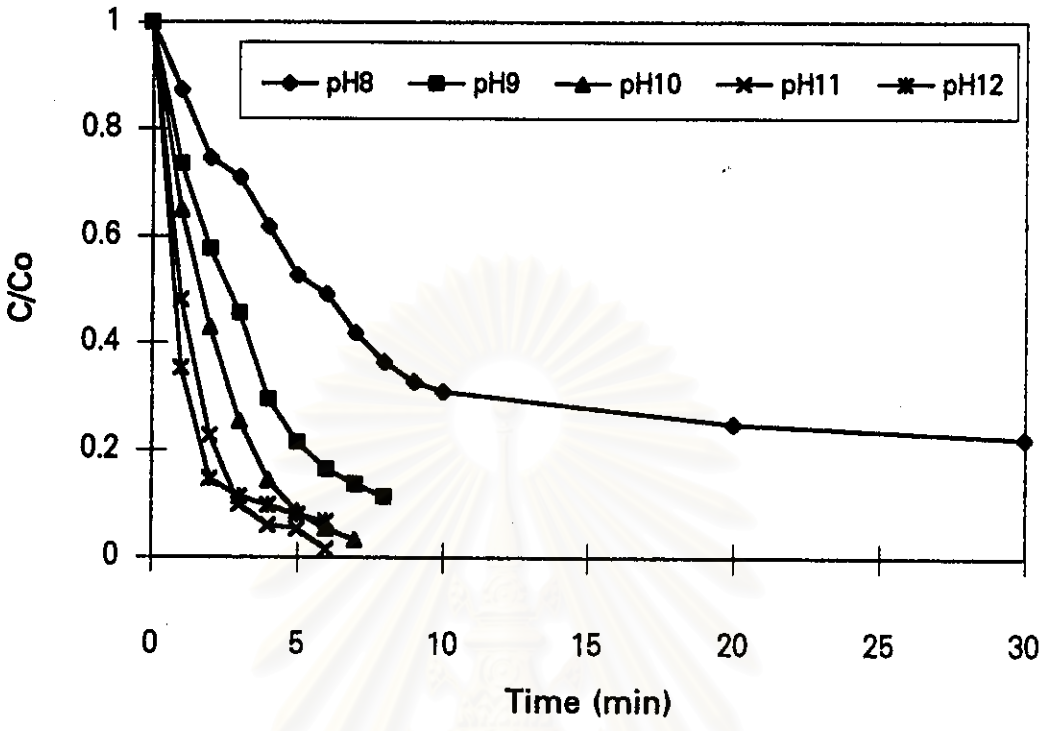

Figure 5.12 Effect of Initial pH in External Phase on ELM Extraction of Crude Berberine Solution at Various pH of External Phase (0.02 M HCl)

Experimental Conditons

External phase	:	0.11 g/l of crude berberine solution at various external phase pH
Membrane phase	:	1 % Span-80 and 99% kerosene
Internal phase	:	0.02 M HCl solution
Membrane preparation	:	at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	:	240 rpm
Temperature	:	room temperature

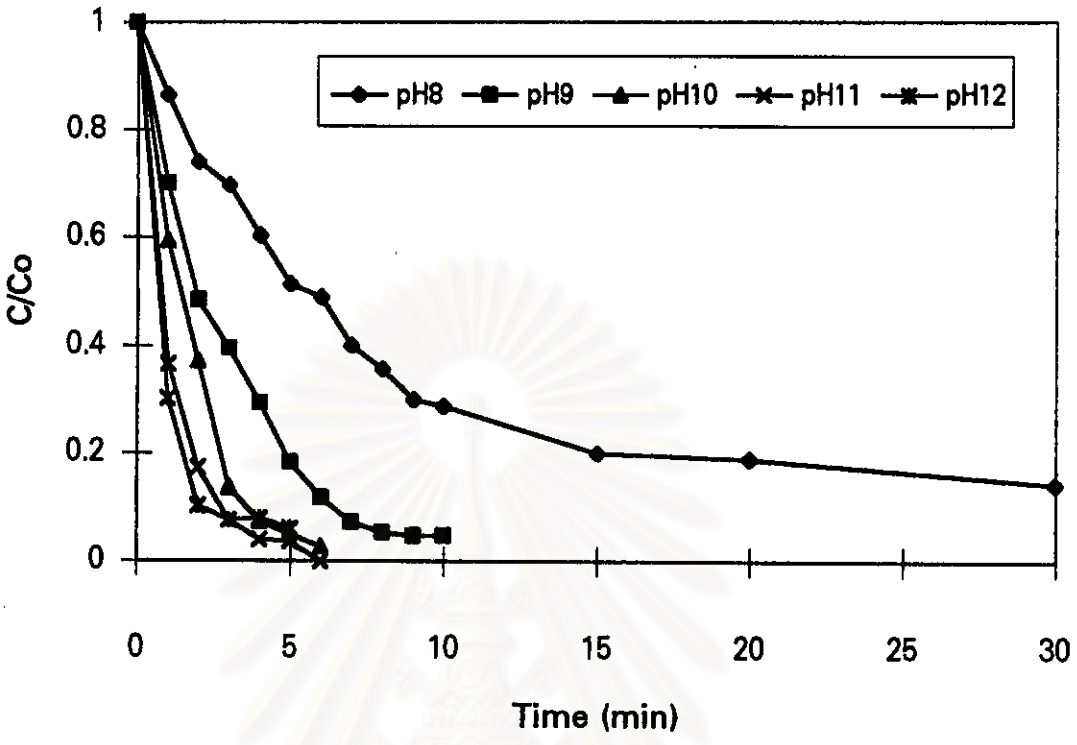

Figure 5.13 Effect of Initial pH in External Phase on ELM Extraction of Crude Berberine Solution at Various pH of External Phase (0.03 M HCl)

Experimental Conditons

External phase : 0.11 g/l of crude berberine solution at various external phase pH

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : 0.03 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

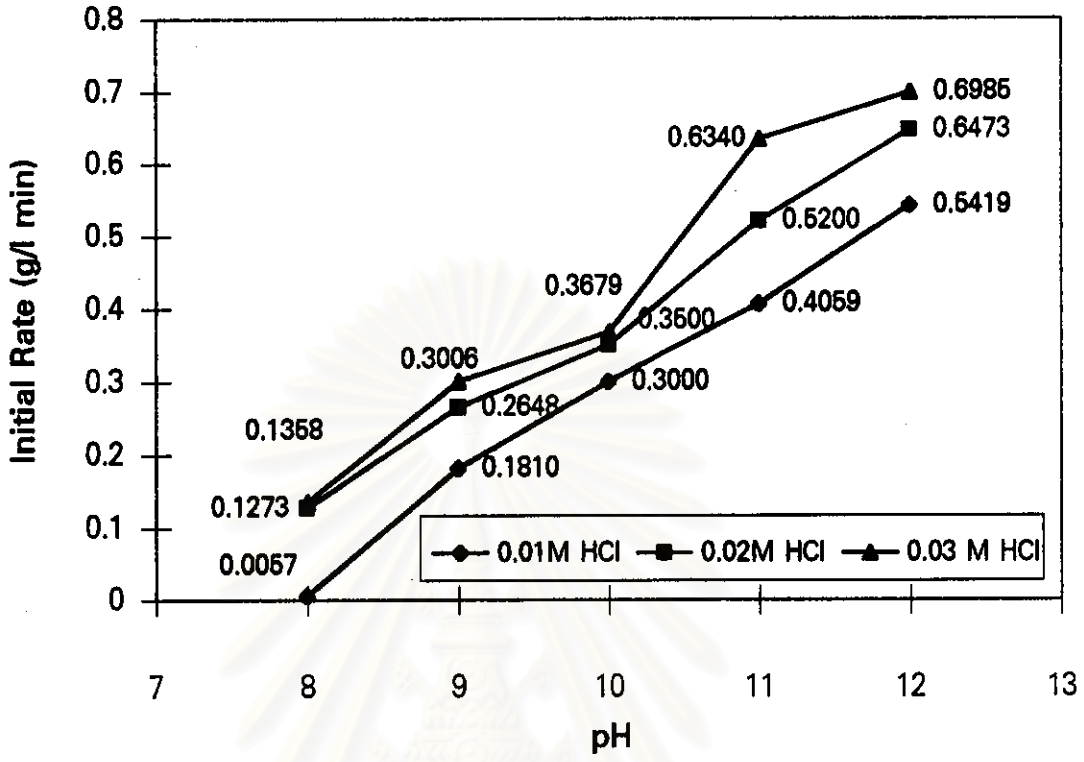

Figure 5.14 Initial Rate on ELM Extraction of Crude Berberine Solution at Various pH of External Phase (at first 1 minute)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at various pH of external phase
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: 0.01, 0.02, and 0.03 M HCl solution
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

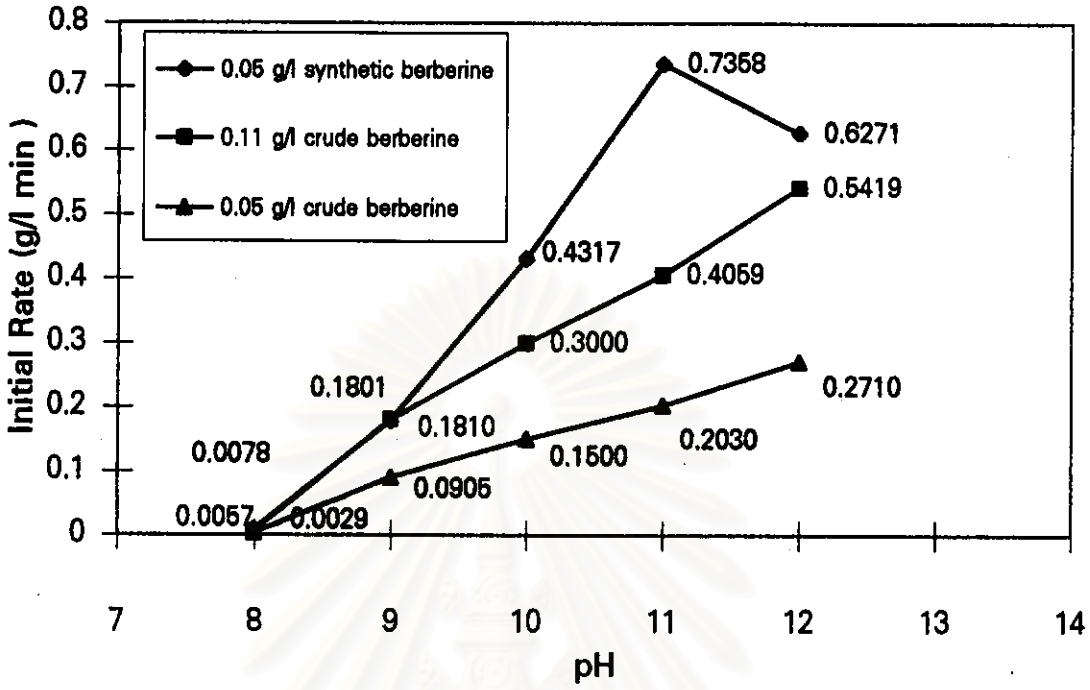

Figure 5.15 Comparison of the Initial Rate of Berberine Extraction between Synthetic Berberine Solution and Crude Berberine Solution at Various pH of External Phase

Experimental Conditons

External phase : a. 0.05 g/l of synthetic berberine solution
b. 0.11 g/l of crude berberine solution
c. 0.05 g/l of crude berberine solution

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

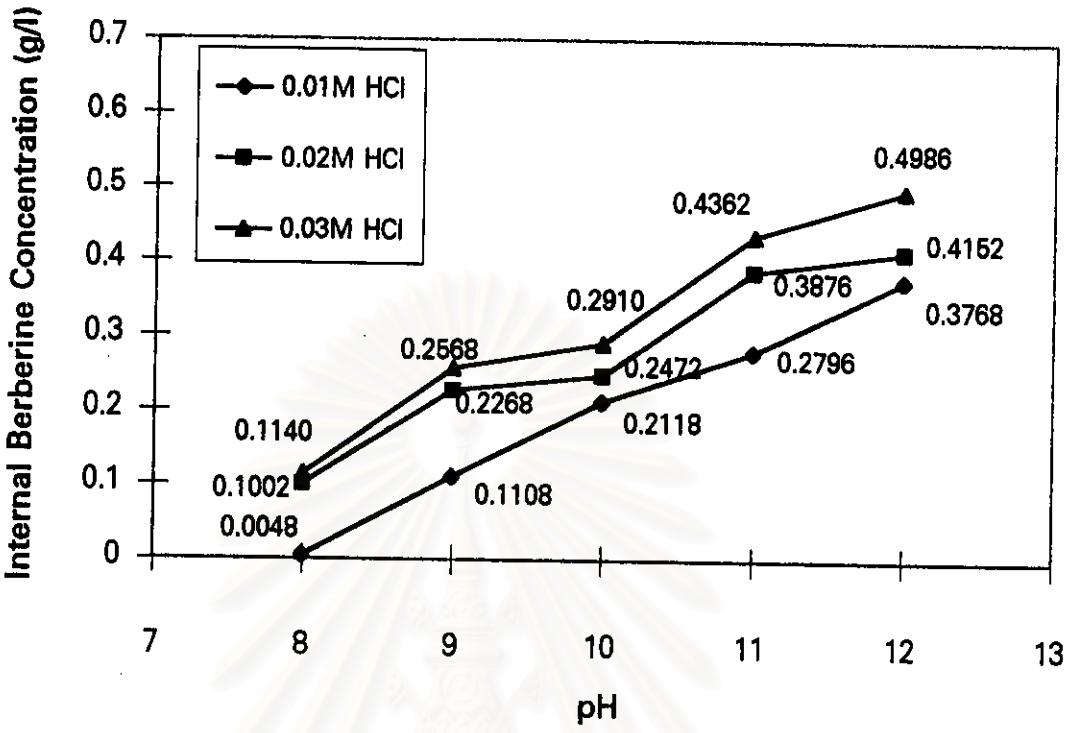

Figure 5.16 Internal Berberine Concentration on ELM Extraction of Crude Berberine Solution at Various pH of External Phase (at first 1 minute)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at various external phase pH
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: 0.01, 0.02, and 0.03 M HCl solution
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

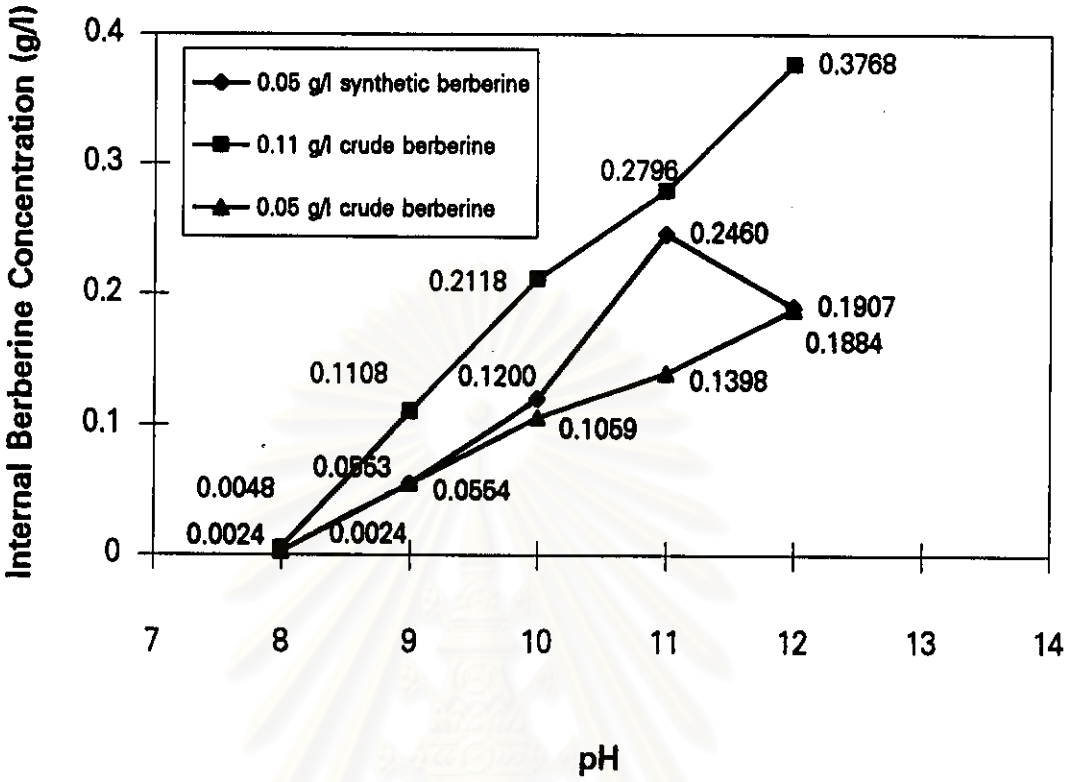

Figure 5.17 Comparison of the Internal Berberine Concentration between Synthetic Berberine Solution and Crude Berberine Solution at Various pH of External Phase (at first 1 minute)

Experimental Conditons

External phase : a. 0.05 g/l of synthetic berberine solution
b. 0.11 g/l of crude berberine solution
c. 0.05 g/l of crude berberine solution

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : 0.01 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

5.2.2.2 The Effect of Hydrochloric Acid Concentration in the Internal Phase

This relationship between hydrochloric acid concentration in the internal phase and crude berberine extraction with time is depicted in Figure 5.18 and Figure 5.19. The experiment data are shown in Table C.14 and C.18 (Appendix C). The experimental conditions used at room temperature were about 0.11 g/l of crude berberine solution at pH 11 and pH 12, 1% (v/v) Span-80 and 99% kerosene as the membrane phase. The internal solution was 0.01, 0.02, and 0.03 M HCl solution. The figure is ploted as time (min) in X-direction and C/Co in Y-direction. The results of these experiment were in the same as described in Section 5.2.1.3 (Chapter 5).

Figure 5.20 illustrates the initial berberine rate on ELM extraction of crude berberine alkaloid solution at internal phase concentration variables (at first 1 minute). This figure is derived from Table C.15 and C.19 (Appendix C). The X-direction is HCl concentration (M) and the Y-direction is initial rate (g/l min). It was shown that at the same pH value the internal rate concentration was increased which was effected to increase initial rate too, and also in different pH, comparing pH 12 with pH 11. It was found that at pH 12 the initial rate of crude berberine extraction (0.6985 g/l min at 0.03 M HCl) was higher than pH 11 (0.6340 g/l min at 0.03 M HCl).

Figure 5.21 shows the internal crude berberine at first 1 minute of 0.01 and 0.02 M HCl solution. The figure is derived from Table C.16 and C. 20 (Appendix C). The X-direction is HCl concentration (M) and the Y-direction is internal crude berberine concentration (g/l). It was found that at pH 12 of external phase achieves of 3.25 (at 0.01 M HCl), 3.88 (at 0.02 M HCl), and 4.19 (at 0.03 M HCl)times

higher concentration than the initial concentration of external phase. The internal berberine concentration increases with the increasing of the pH value of external phase and internal concentration of HCl solution.

Figure 5.22 shows the comparison to internal berberine concentration of synthetic berberine solution and crude berberine solution at internal phase concentration variables. The data were shown in Table B.12 and C.16 (Appendix B and C). The X-direction is HCl concentration (M) and the Y-direction is internal berberine concentration (g/l). It was shown that at the same pH (pH 11) the internal synthetic berberine concentration was higher than the internal crude berberine concentration e.g. at 0.03 M HCl the internal synthetic berberine concentration was 0.2526 g/l and the internal crude berberine concentration was 0.2181 g/l.

On this work, the berberine extrction from Khamin Khruea by ELM was studied. The suitable conditions at room temperature are the crude berberine solution at pH 12 as the external phase, 1% (v/v) Span-80 dissolved in kerosene as the membrane phase and 0.03 M HCl as the internal phase. These conditions could give 4.19 times higher concentration than the initial berberine concentration in the external phase(0.1189 g/l) within 1 minute. The initial rate of berberine at 1 minute is 0.6985 g/l min.

For Tang, Ma, Lui 's work, studied the berberine extraction from Rhizoma Coptidis (of Sichuan Origin). The suitable conditions are pH 11, 1% Span-80 dissolved and 0.03 M HCl. All details of condition were not specify in this journal. The alkaloid berberine contents are given in percent (7-9%). So that this cannot compare the recovery concentration after using ELM to this dissertation and the experiment was not done with the interval times, then it could not get the initial rate at 1-minute.

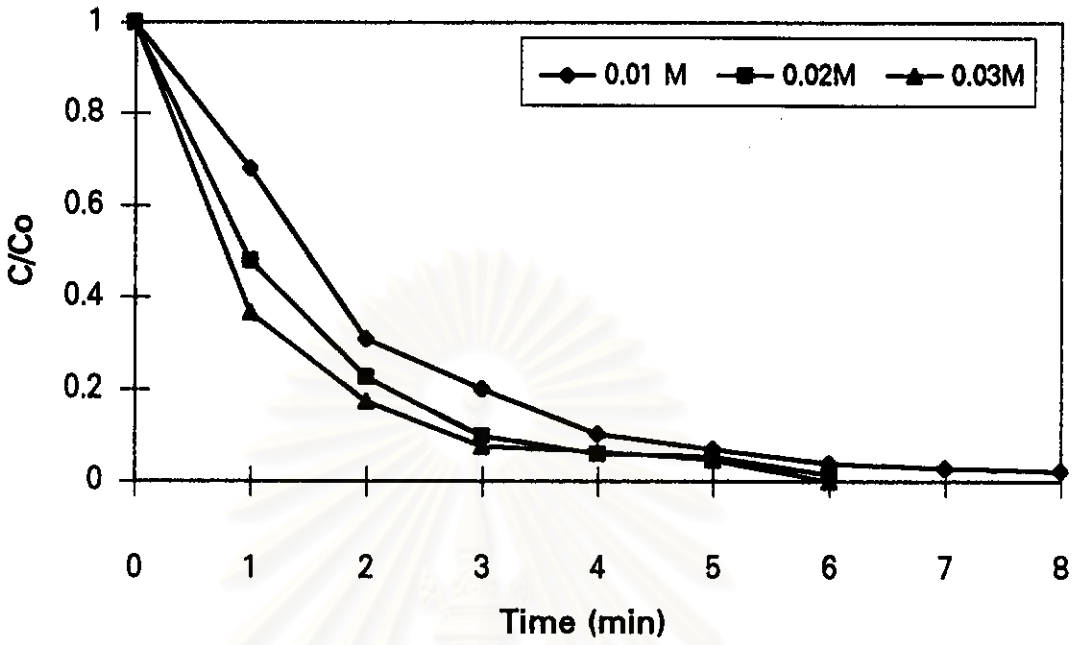

Figure 5.18 Effect of HCl Concentration in the Internal Phase on ELM Extraction of Crude Berberine Solution (pH 11)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at pH 11
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solution at various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

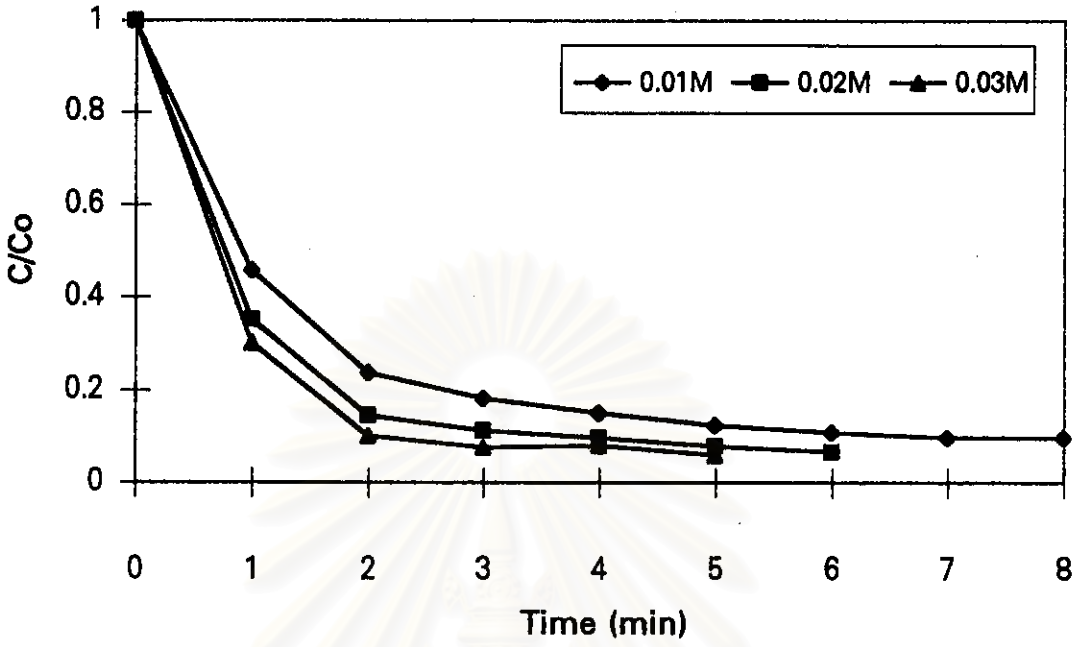

Figure 5.19 Effect of HCl Concentration in the Internal Phase on ELM Extraction of Crude Berberine Solution (pH 12)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at pH 12
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solution at various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

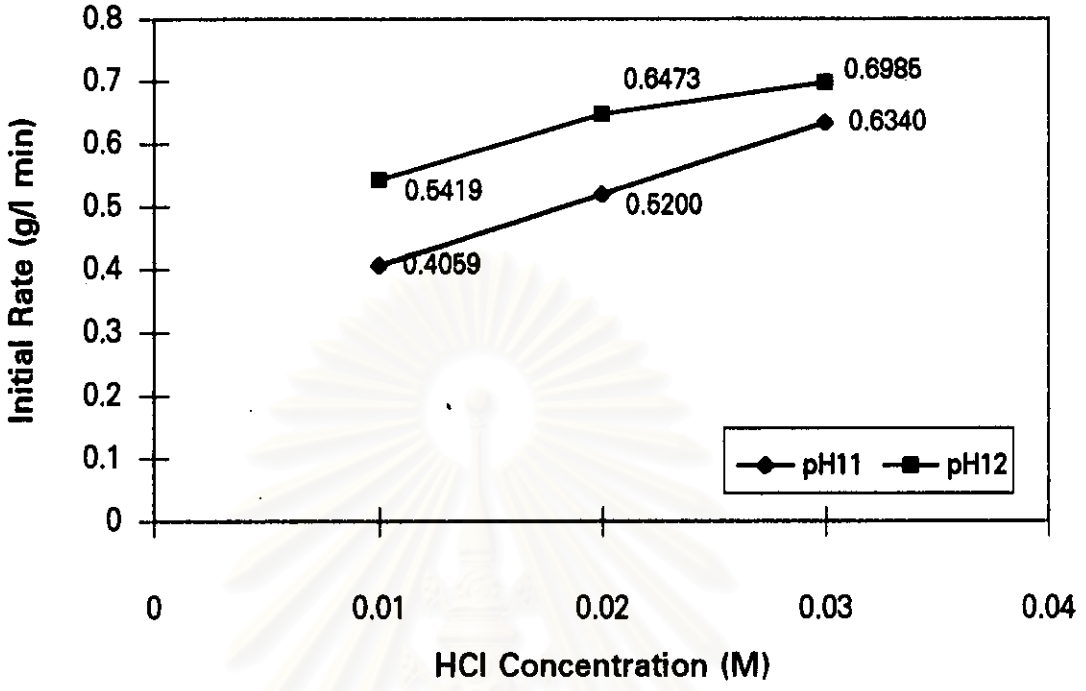

Figure 5.20 Comparison of Initial Rate at pH 11 and 12 of Crude Berberine Extraction on ELM at Internal Phase Concentration Variables (at first 1 minute)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at pH 11, pH 12
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solution at various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

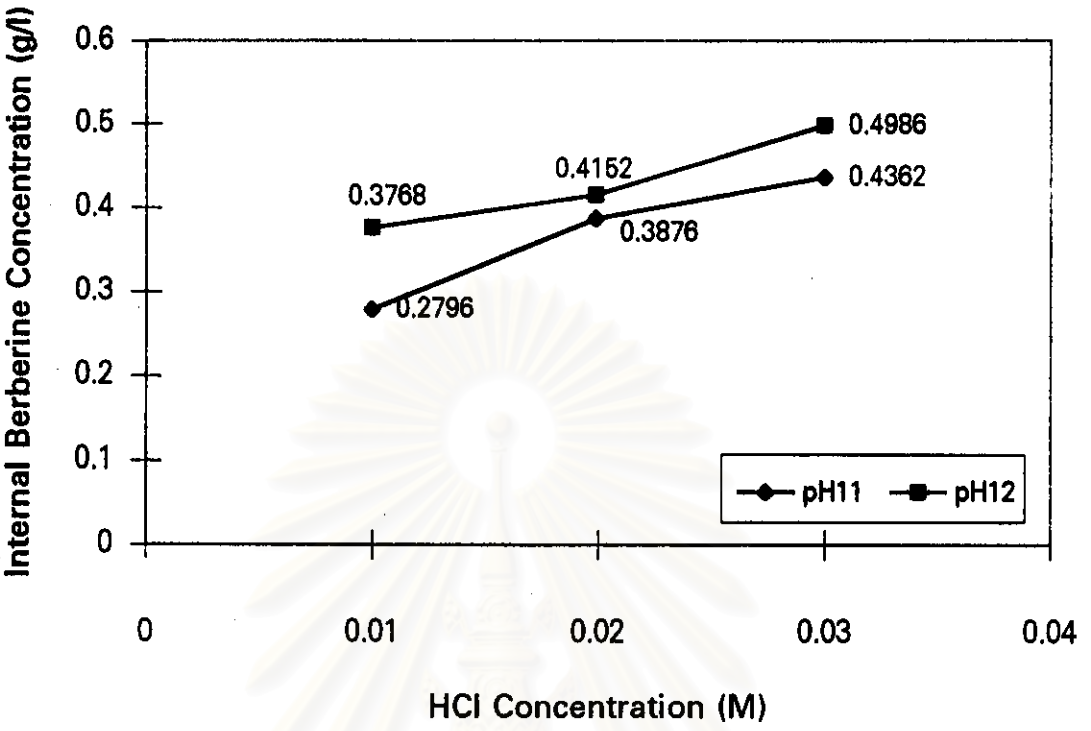

Figure 5.21 Comparison of Internal Berberine at pH 11 and 12 Concentration of Crude Berberine Solution at Internal Phase Concentration Variables (at first 1 minute)

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at pH 11, pH 12
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solution at various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

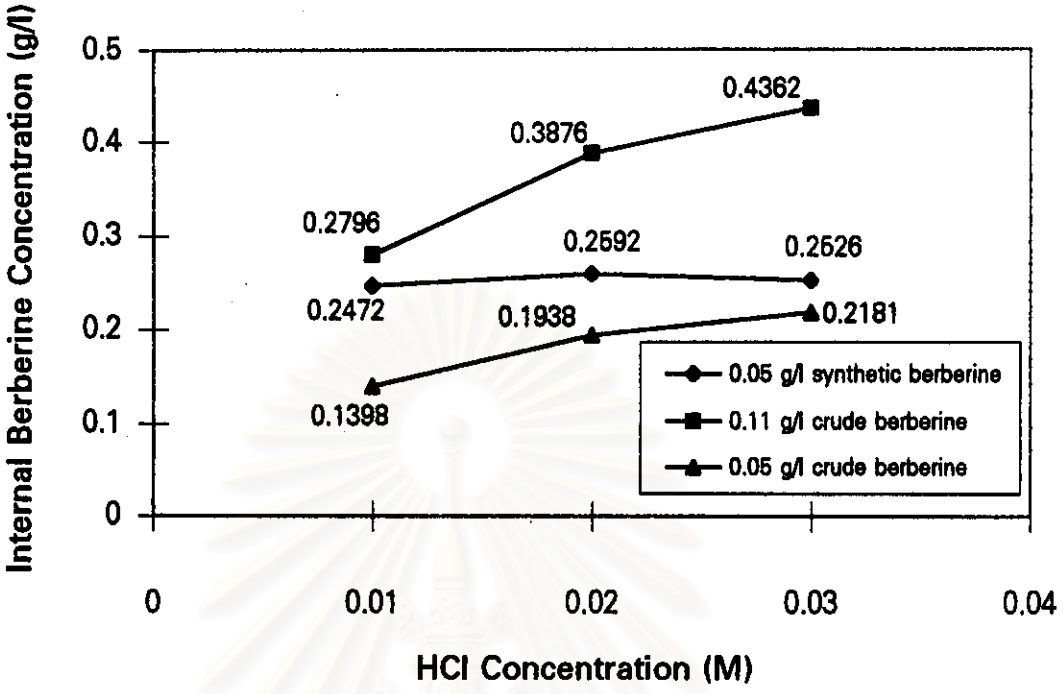

Figure 5.22 Comparison of Internal Synthetic Berberine Concentration and Internal Crude Berberine Concentration at Internal Phase Concentration Variables (at first 1 minute)

Experimental Conditons

External phase : 0.11 g/l of synthetic berberine solution at pH 11
0.05 g/l of synthetic berberine solution at pH 11
0.05 g/l ofcrude berberine solution at pH 11

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : HCl solution at various concentrations

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

5.2.2.3 Effect of % Swelling on the Extraction of Crude Berberine Solution

The effects of external pH on swelling are shown in Figure 5.23, 5.24, and 5.25 at different HCl concentration. The experimental data are shown in Table C.23, C.24, and C.25 (Appendix C). The X-direction is time (min) and the Y-direction is % swelling. When the extraction by ELM started, the swelling started also but at a very slow rate. The extraction rate at the first time interval was faster than the mediation of water. After that at higher pH, the extraction rate was higher than the mediation of water. On the other hand, at lower pH the extraction rate was lower than the mediation of water, e.g. at pH 8, 0.01 M HCl; the effect of swelling was 48.73% at 30 minutes. At pH 12 the % swelling of emulsion has no significantly difference when compared with the lower pH. Because of fast extraction rate of berberine on ELM at high pH value of external phase due to reducing of swelling.

Figure 5.26 and 5.27 show the effect of internal HCl concentration on % swelling in ELM at pH 8 and pH 12 respectively. At pH 8, swelling was increased while decreased the internal concentration. The increasing of internal concentration will resulted the higher driving force. While the solute was transfered from the external phase the molecule of water can be transfered but slowly rate when compared to extraction rate and also the long time of extraction will cause transported water into the emulsion. At pH 12, % swelling was low because of the contact time of extraction between aqueous phase and organic phase was shorted.

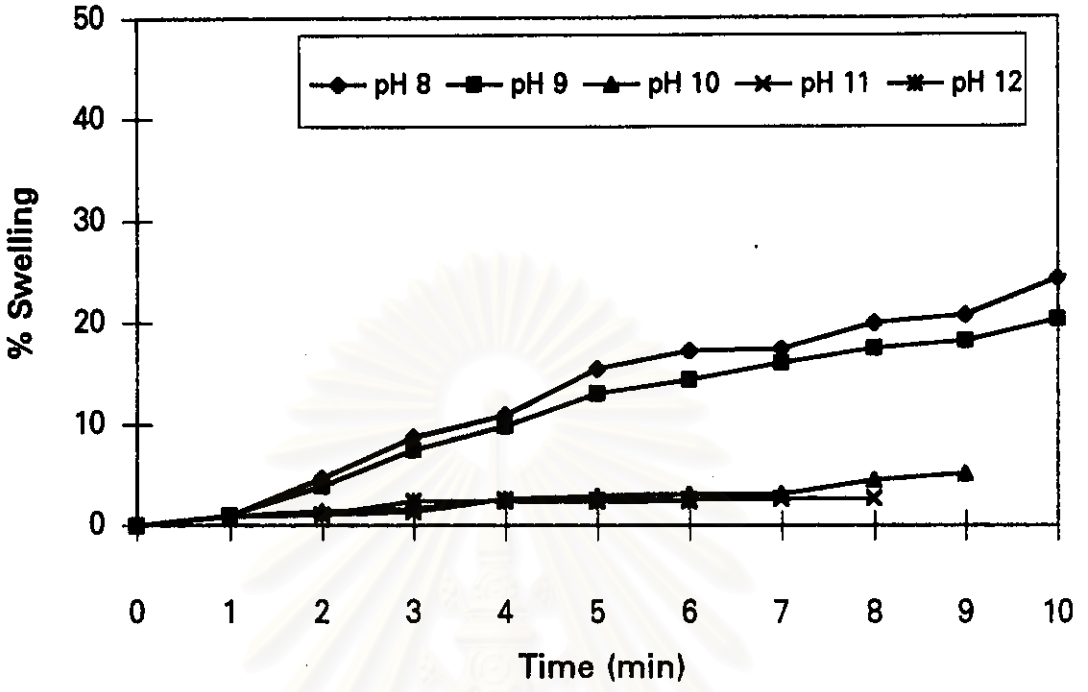

Figure 5.23 Effect of External Phase pH on % Swelling at 0.01 M HCl Solution

Experimental Conditons

External phase	:	0.11 g/l of crude berberine solution at various pH of external phase
Membrane phase	:	1 % Span-80 and 99% kerosene
Internal phase	:	0.01 M HCl solution
Membrane preparation	:	at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	:	240 rpm
Temperature	:	room temperature

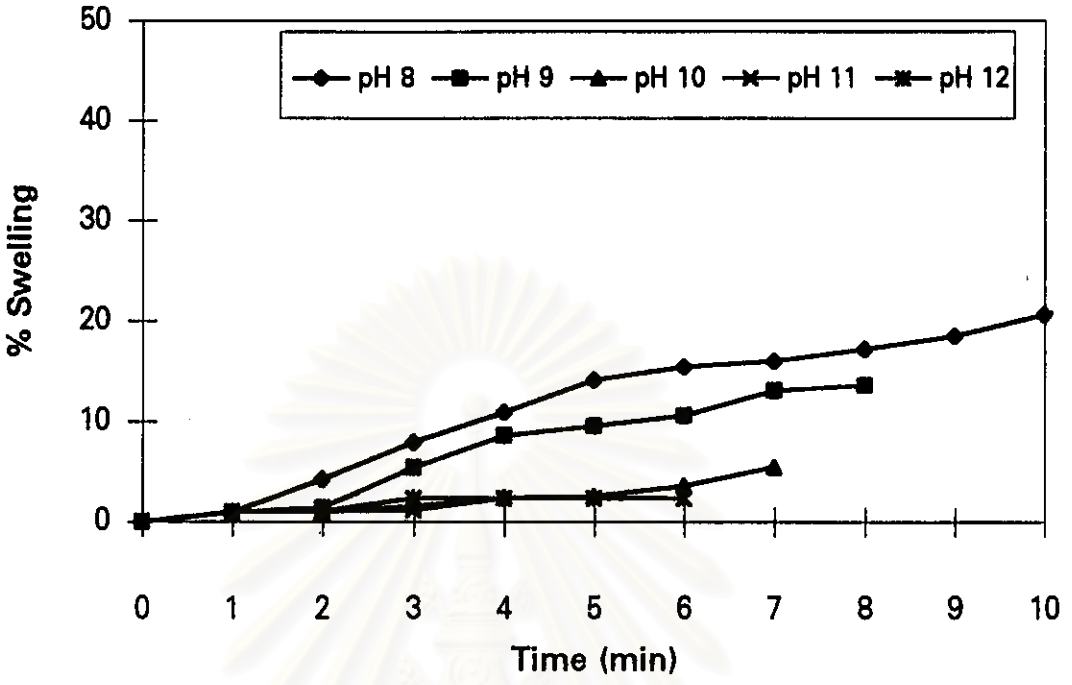

Figure 5.24 Effect of External Phase pH on % Swelling at 0.02 M HCl Solution

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at various pH of external phase
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: 0.02 M HCl solution
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

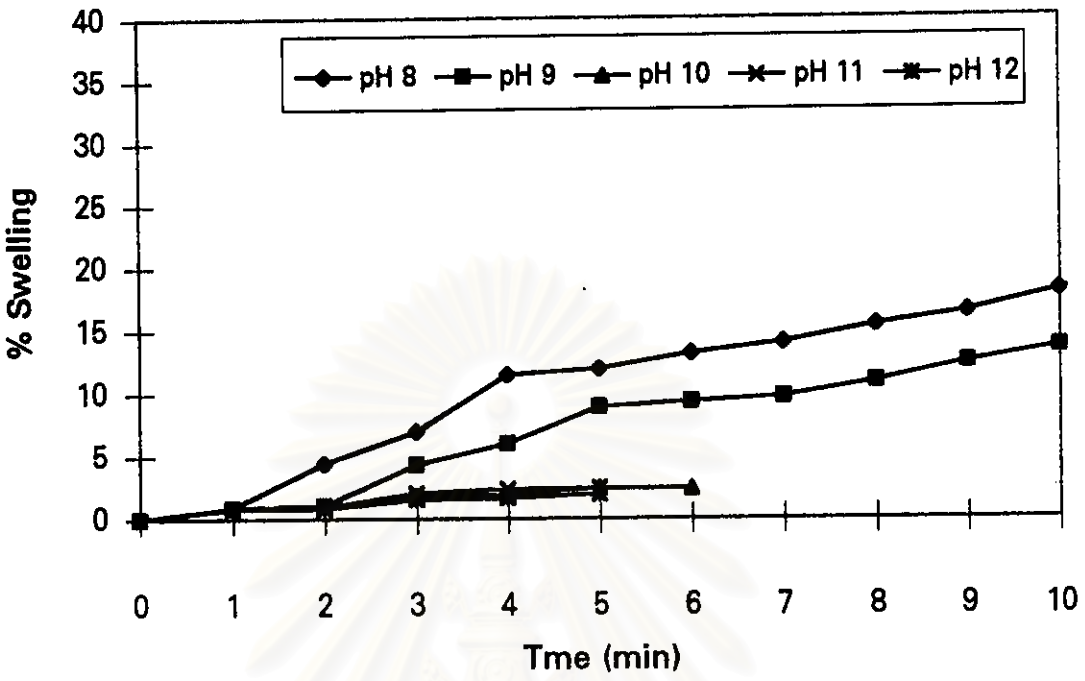

Figure 5.25 Effect of External Phase pH on % Swelling at 0.03 M HCl Solution

Experimental Conditons

External phase : 0.01 g/l ofcrude berberine solution at various pH of external phase

Membrane phase : 1 % Span-80 and 99% kerosene

Internal phase : 0.03 M HCl solution

Membrane preparation : at 8000 rpm of homogenizing speed for 30 minutes

Agitation speed : 240 rpm

Temperature : room temperature

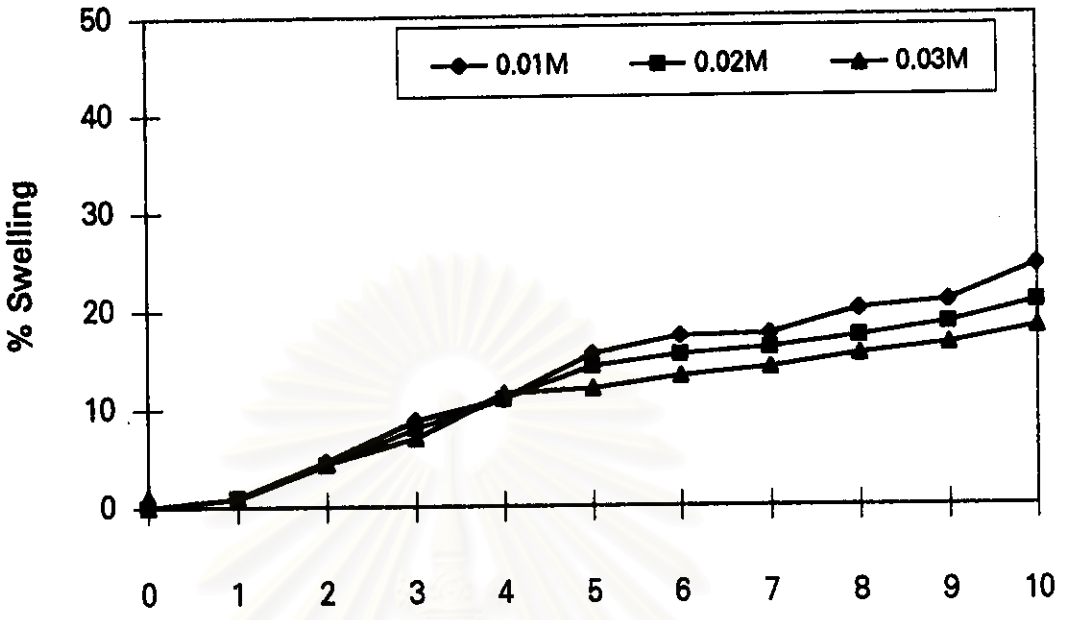

Figure 5.26 Effect of Internal HCl Concentration Phase on % Swelling in ELM at pH 8

Experimental Conditons

External phase	: 0.11 g/l of crude berberine solution at pH 8
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solution at various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature

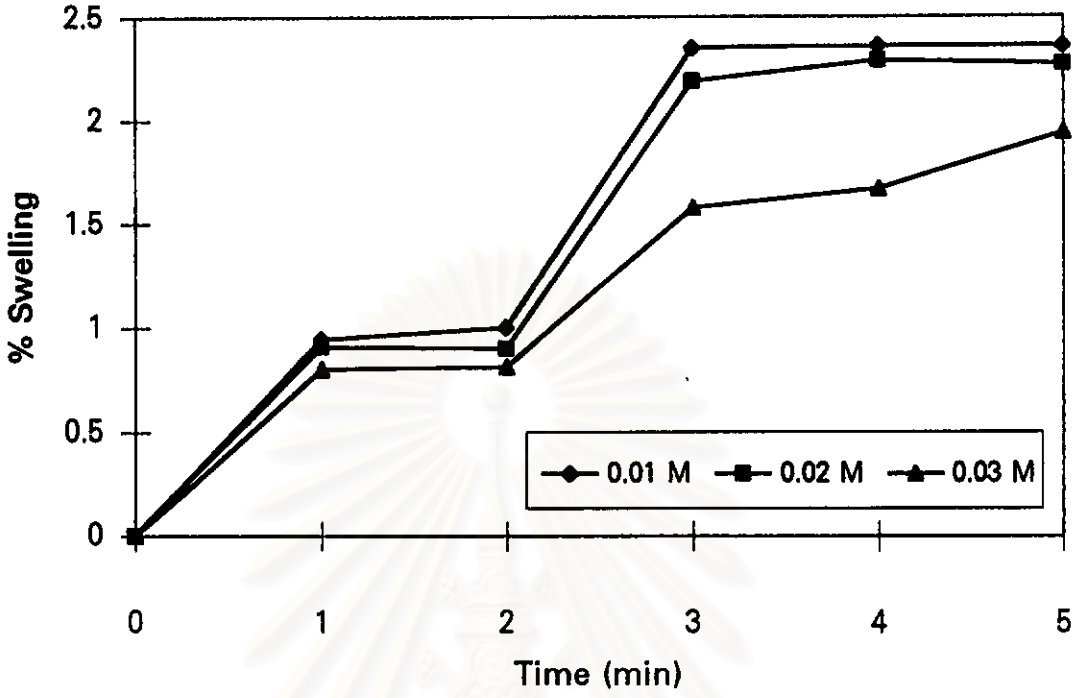

Figure 5.27 Effect of Internal HCl Concentration Phase on % Swelling in ELM at pH 12

Experimental Conditons

External phase	: 0.11 g/l ofcrude berberine solution at pH 12
Membrane phase	: 1 % Span-80 and 99% kerosene
Internal phase	: HCl solutionat various concentrations
Membrane preparation	: at 8000 rpm of homogenizing speed.. for 30 minutes
Agitation speed	: 240 rpm
Temperature	: room temperature