CHAPTER 4
EXTRACTION EQUILIBRIUM

This Chapter presents the study of the extraction equilibrium berberine from synthetic berberine solution and crude berberine from crude berberine solution (Khamin Khruea) by using n-hexane, kerosene and paraffin as organic solvents.

4.1 Experimental Materials and Methods

4.1.1 Experimental Materials

All chemical used in this study are listed below. All of these reagents are of analytical grade unless otherwise stated.

a. n-Hexane (Mallickrodt Specialty Chemical Co., Ltd.) was used as organic solvent for extraction equilibrium from Nirin Supply Company.
b. Paraffin (Mallickrodt Specialty Chemical Co., Ltd.) was used as organic solvent for extraction equilibrium from Nirin Supply Company.
c. Kerosene was used as organic solvent for extraction equilibrium and was kindly supplied by Petroleum Authority of Thailand.
d. Synthetic berberine chloride (Sigma Chemical Co., Ltd.) was used as a solute in the external phase from Chatcharee Limited Part.
e. Khamin Khruea was used as a solute in the external phase from Boun-Zae (folkloric medicine shop).

f. Span-80: sorbitan monooleate, a non-ionic surfactant, (Sigma Chemical Co., Ltd.) was used to stabilize the emulsions from Chatcharee Limited Part.

g. Acetonitrite HPLC grade (Mallickrodt Specialty Chemical Co., Ltd.) was supplied from Nirin Supply Company.

h. Water HPLC grade (Mallickrodt Specialty Chemical Co., Ltd.) was supplied from Nirin Supply Company.

4.1.2 Experimental Equipments

The equipments in this experiment were

a. HPLC: High Performance Liquid Chromotography, (Water^{TM} 600, 717, 996) used to analyze berberine from Chemical Engineering Equipment Center, Chulalongkorn University.

b. Particle size analyzer (Coulter) used to analyze the particle sizes of internal droplet from Chemical Engineering Department, Chulalongkorn University.

c. Homogenizer (IKA Laboratechnik) used to prepare emulsion from Chemical Engineering Department, Chulalongkorn University.

d. pH meter (Schott CG825) used to measure pH of solution from Chemical Engineering Department, Rangsit University.

e. Mechanical Stirrer (IKA Laboratechnik) was from Chemical Engineering Department, Chulalongkorn University and Rangsit University.

4.1.3 Experimental Methods

4.1.3.1 Extraction Equilibrium of Synthetic Berberine Solution

At first 300 ml of synthetic berberine solution was poured into a 1000 ml of beaker. To this beaker, 300 ml of n-hexane was also added to give an aqueous mixture solution to organic ratio of 1:1 by volume. By using a mechanical stirrer (IKA, model Euroatar Digi-vis), the aqueous/organic solution in the beaker was agitated at 240 rpm at 25°C for a period of 96 hours to ensure equilibrium condition was reached. The aqueous phase was 0.05 g/l of synthetic berberine solution and the pH was adjusted to pH 8, 9, 10, 11, and 12, respectively with concentrated sodium hydroxide solution. The phases were allowed to settle under gravity. The sample was collected with respect to time until 96 hours. The aqueous solution was then assayed for berberine and the pH were then measured. The concentrations of berberine in the organic solution were determined by the difference of berberine concentrations between the initial and final stages. The berberine concentrations in the aqueous 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 aqueous phase solution was measured by using pH meter (Schott, CG825).

The experiments were repeated at the same experimental conditions. Exception of the organic solvents were changed to kerosene and paraffin, respectively.

The experiments were carried out under the conditions shown in Table 4.1

Table 4.1 The Experimental Conditions of Synthetic Berberine Solution at 25°C for Extraction Equilibrium

Organic Phase	Aqueous Phase	rpm	Time
n-Hexane or Kerosene or Paraffin	0.05 g/l of synthetic berberine solution (adjusted to pH 8, 9, 10, 11 and 12)	240	96 h

4.1.3.2 Extraction Equilibrium of Crude Berberine Solution (Khamin Khruea)

At the start, the infusion of Khamin Khruea was prepared according to the following procedure. 1000 g Dry small pieces of Khamin Khruea were soaked in 2000 ml distilled water for 72 hours (repeated four times). The solution was filtered and the filtrate was diluted to 8000 ml with distilled water to form a mother liquor for the succeeding tests.

Experiments on the extraction equilibrium of crude berberine were carried out as mentioned in Section 4.1.3.1 . But the solute in aqueous was changed from synthetic berberine solution to mother liquor of crude berberine.

The experiments were carried out under the conditions shown in Table 4.2

Table 4.2 The Experimental Conditions of Crude Berberine Solution at 25°C for Extraction Equilibrium

Organic Phase	Aqueous Phase	rpm	Time
n-Hexane or Kerosene or Paraffin	0.11g/l of crude berberine solution (adjusted to pH 8, 9, 10, 11 and 12)	240	96 h

4.1.3.3 Calculation of Distribution (Partition) Coefficient

After the experiments in Section 4.1.3.1 and 4.1.3.2, the concentrations of berberine in synthetic berberine solution and the concentrations of crude berberine in crude berberine solution were measured. The berberine concentrations in the organic phase were calculated by mass balance of berberine concentrations in the external phase between initial and final stages. The example of calculation is shown in the following:

Experimental Conditions:

Aqueous phase : 0.05 g/l of synthetic berberine solution at pH 12

Organic phase : kerosene at volume 1:1 (aqueous : organic)

Agitation speed : 240 rpm

Temperature : room temperature

At o hours, the concentration of berberine in the external phase was 0.045 g/l

At 96 hours, the concentration of berberine in the external phase was

0.04 g/l

The amount of berberine that partition into the organic phase (kerosene) was

0.045-0.04 g/l

Therefore, the distribution (partition) coefficient becomes,

$$K_D = (0.045-0.04)/0.04 = 0.1250$$

4.2 Results and Discussion

4.2.1 Extraction Equilibium of Synthetic Berberine Solution

The ELM extraction of synthetic berberine solution follows the Type 1 Unfacilitated Transport Mechanism. The external phase was synthetic berberine solution, the membrane phase was organic solvent and the internal phase was aqueous phase (HCl solution). Therefore this Chapter presents the effect of organic solvents which were used in ELM system. A comparison of these solvents was made by measuring the distribution (partition) of berberine solute between aqueous solution and organic solvents at equilibrium. The extraction equilibruim data are shown in Appendix A.

Figure 4.1 shows the concentration ratio of berberin in synthetic berberine solution; C_{BS} (aqueous phase) and n-hexane; C_{BH} (organic phase) at various pH of aqueous phase, where the X-direction is time (h) and the Y-direction is the concentration ratio of berberin in synthetic berberine solution and n-hexane (C_{BS}/C_{BH}). It shows that the increase of basicity in aqueous phase was increased from pH 8 to pH 12, the concentration ratio of berberin in synthetic berberine solution and n-hexane was also increased. But for each pH value, the concentration ratio of berberine in synthetic berberine solution and n-hexane increases fast at the initial time and becomes slightly constant when the time passed.

Figure 4.2 describes the concentration ratio of berberin in synthetic berberine solution; C_{BS} (aqueous phase) and kerosene; C_{BK} (organic phase) at various pH of aqueous phase, while Figure 4.3 depicts the ratio of concentration of berberin in synthetic berberine solution; C_{BS} (aqueous phase) and paraffin; C_{BP} (organic phase) at various pH of aqueous phase,. Apparently these results are the same as in Figure 4.1, but at different concentration ratio.

Figure 4.4 shows the comparison of the concentration ratio of berberin in synthetic berberine solution; C_{BS} (aqueous phase) and organic solvents; C_{BO} (n-hexane, kerosene and paraffin) at pH 12 of aqueous phase. it was shown that at equilibrium, the distribution (partition) efficient; K_D of berberine in n-hexane was about 0.152, the the distribution (partition) efficient; K_D of berberine in kerosene was about 0.1186 and the the distribution (partition) efficient; K_D of berberine in paraffin was about 0.0288. This means that berberine solute was soluble in n-hexane much better than kerosene and paraffin.

Generally, berberine solute is very poorly soluble in non-polar solvents, which are inert solvents. Inert solvents are usually characterized by very low (10 to 2) dielectric constants. Hydrocarbons are typical examples of inert solvents. But other aromatic hydrocarbons are known to be π-electron donors and can accept protons under drastic conditions. Similarly, some hydrocarbons lose their hydrogen in strongly basic media, forming carbanions. Still, even how acidic or basic hydrocarbons may posses are many orders of magnitude below those of typical protogenic or protophilic solvents (Orest, and Reginald, 1981).

Therefore, from the reason as mentioned above and the physical properties of n-hexane and kerosene (Appendix D), it was found that n-hexane is slightly soluble in water, compare to kerosene and paraffin. The molecular weight of n-hexane is the lowest while that of paraffin is the highest. n-Hexane is more volatile when compared with kerosene and paraffin. Although the concentration ratio of berberine/n-hexane was the highest but n-Hexane was not suitable for used as organic solvent on ELM.

Comparison between kerosene and paraffin, the concentration ratio of berberine/kerosene was higher than the concentration ratio of berberine/paraffin. So kerosene was selected as organic solvent for ELM system. The paraffin was not chosen in this experiment because of its high viscosity.

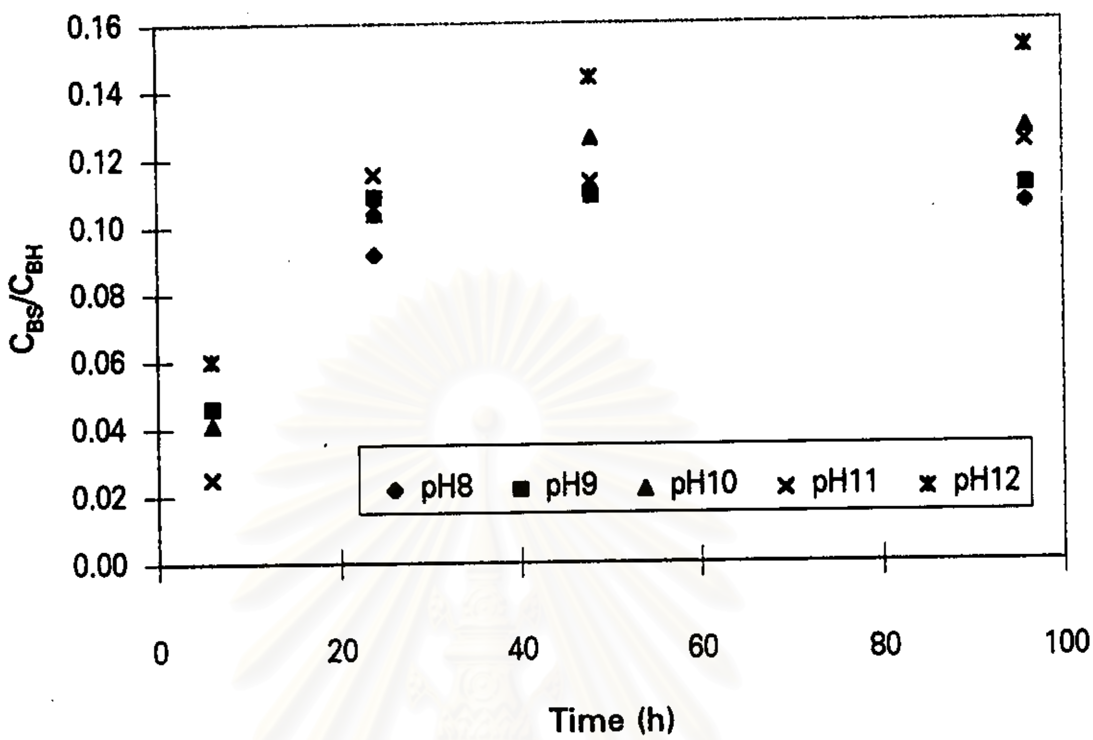

Figure 4.1 Concentration Ratio of Berberine in Synthetic Berberine Solution and n-Hexane at Various pH of Synthetic Berberine Solution

<u>Experimental Conditions</u>

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

Organic phase : n-hexane

Agitation speed : 240 rpm

Temperature : at 25°C

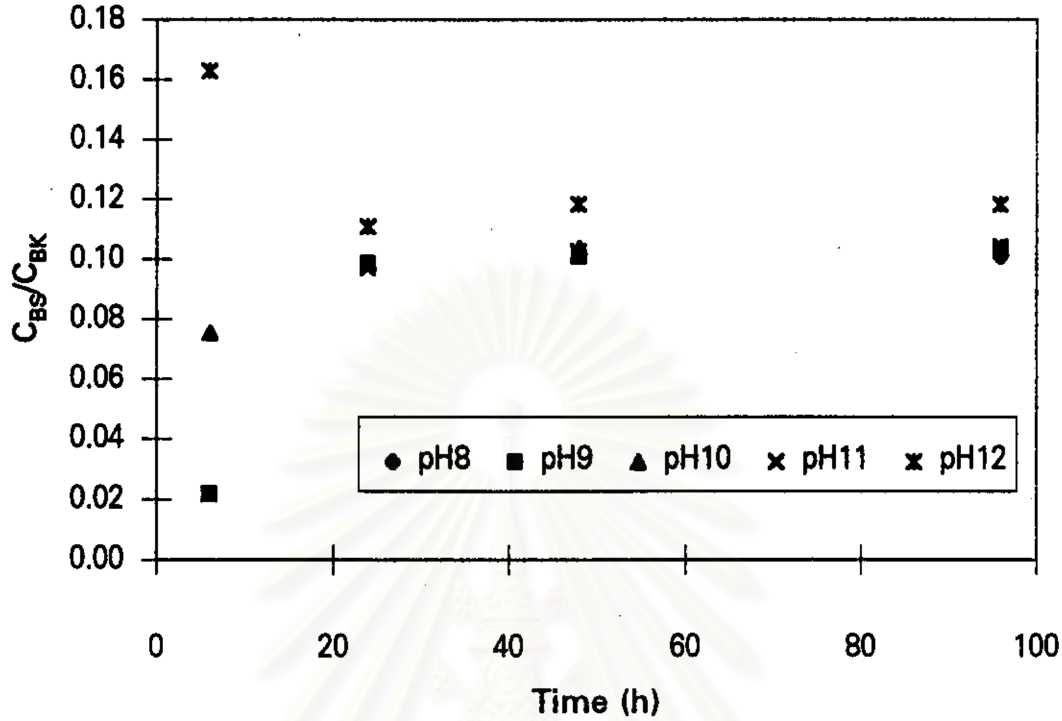

Figure 4.2 Concentration Ratio of Berberine in Synthetic Berberine Solution and Kerosene at Various pH of Synthetic Berberine Solution

Experimental Conditions

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

Organic phase : kerosene

Agitation speed : 240 rpm

Temperature : at 25°C

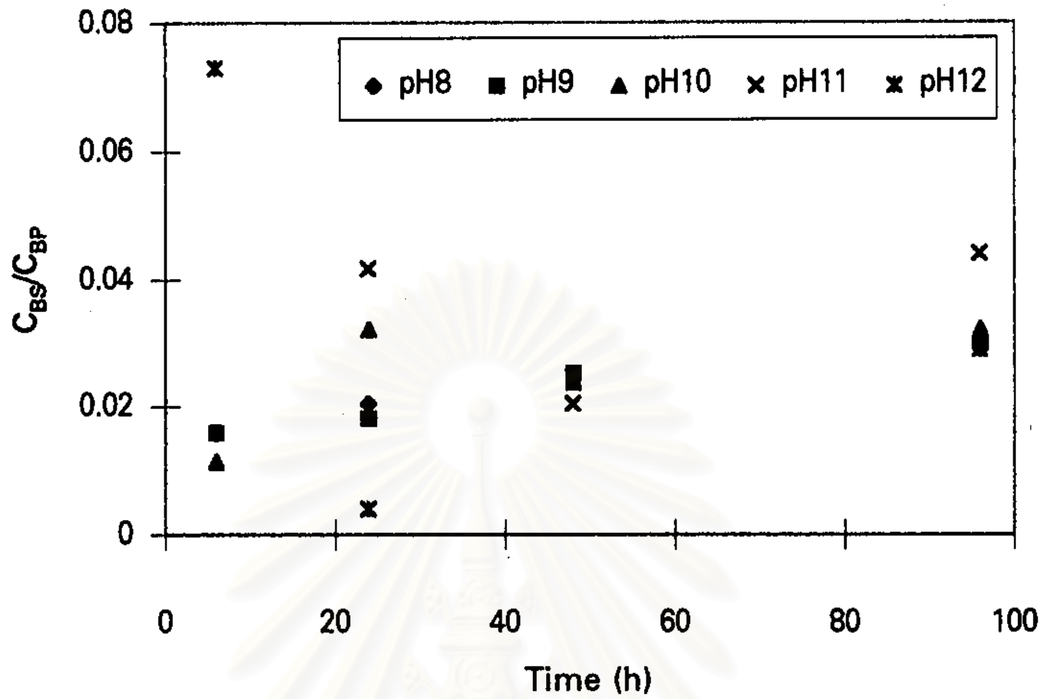

Figure 4.3 Concentration Ratio of Berberine in Synthetic Berberine Solution and Paraffin at Various pH of Synthetic Berberine Solution

<u>Experimental Conditions</u>

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

Organic phase : paraffin

Agitation speed : 240 rpm

Temperature : at 25°C

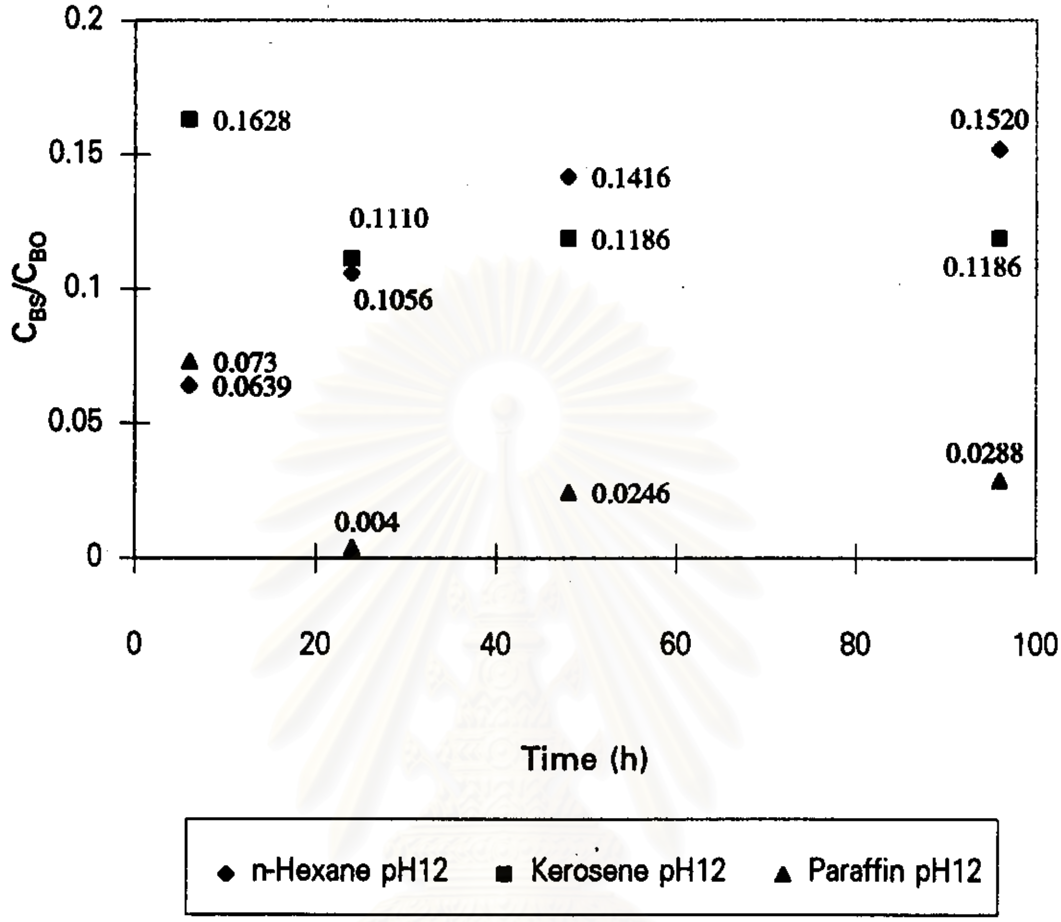

Figure 4.4 Concentration Ratio of Berberine in Synthetic Berberine Solution and Different Organic Solvents At pH 12 of Synthetic Berberine Solution

<u>Experimental Conditions</u>

Aqueous phase : 0.05 g/l of synthetic berberine solution at pH 12

Organic phase : n-hexane, kerosene, and paraffin

Agitation speed : 240 rpm

Temperature : at 25°C

4.2.2 Extraction Equilibrium of Crude Berberine Solution

In this study, the external phase of ELM system has been changed from synthetic berberine solution to mother liquor of extracted Khamin Khruea. The experimental methods have been done in the same way in order to see the effect of organic solvents on crude berberine. The extraction equilibrium data are seen in Appendix A.

Figure 4.5, 4.6, and 4.7 show the concentration ratio of berberine in crude berberine solution; C_{BC} (aqueous phase) and n-hexane; C_{BH} (organic phase), the concentration ratio of berberine in crude berberine solution; C_{BC} (aqueous phase) and kerosene; C_{BK} (organic phase), and the concentration ratio of berberine in crude berberine solution; C_{BC} (aqueous phase) and paraffin C_{BP} (organic phase) at various pH of aqueous phase respectively. The X-direction is time (h) and the Y-direction is the concentration ratio. The results of these experiments are the same as mentioned in Section 4.2.1 (Chapter 4), that when pH in aqueous phase was increased from pH 8 to pH 12, the concentration ratio was also increased.

Figure 4.8 shows the comparison of the concentration ratio of berberine in crude berberine solution; C_{BC} and organic solvents; C_{BO} (n-hexane, kerosene and paraffin) at pH 12 of aqueous phase. The X-direction is time (h) and the Y-direction is the concentration ratio It was shown that, at equilibrium the distribution (partition) efficient; K_D of berberine in n-hexane was the highest while the distribution (partition) efficient; K_D of berberine in paraffin was the lowest as follows :

K_D of berberine/n-hexane(0.1345) > berberine/kerosene(0.1040) > berberine/paraffin(0.0247)

The comparison of distribution coefficient of berberine in synthetic berberine solution and in crude berberine solution at different organic solvents at pH 12 as shown in Table 4.3. It was found that K_D of synthetic berberine at different organic solvents was higher than K_D of crude berberine at different organic solvents.

Owing to many derivatives in crude alkaloidal extract from Khamin Khruea, therefore all the derivatives and berberine could partition into organic solvents.

Table 4.3 Comparison of K_D of Synthetic Berberine to Crude Berberine at Different Organic Solvents

Organic Solvents	K_D of Synthetic Berberine	K_D of Crude Berberine
n-hexane	0.1520	0.1345
kerosene	0.1186	0.1040
paraffin	0.0288	0.0247

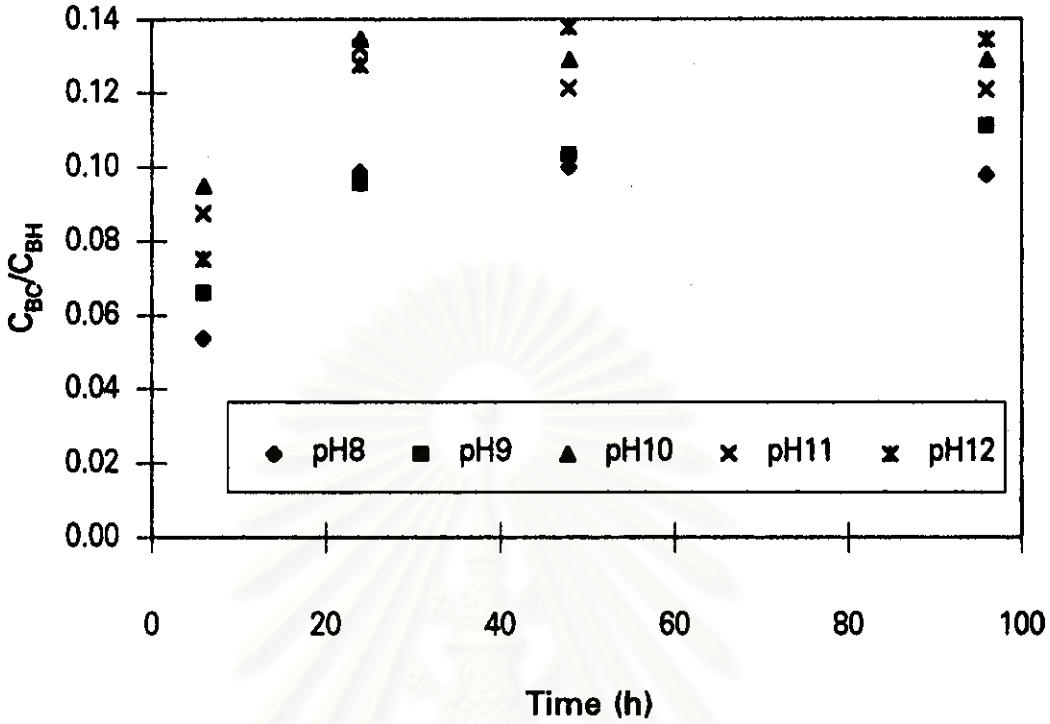

Figure 4.5 Concentration Ratio of Berberine in Crude Berberine Solution and n-Hexane at Various pH of Crude Berberine Solution

<u>Experimental Conditions</u>

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

Organic phase : n-hexane

Agitation speed : 240 rpm

Temperature : at 25°C

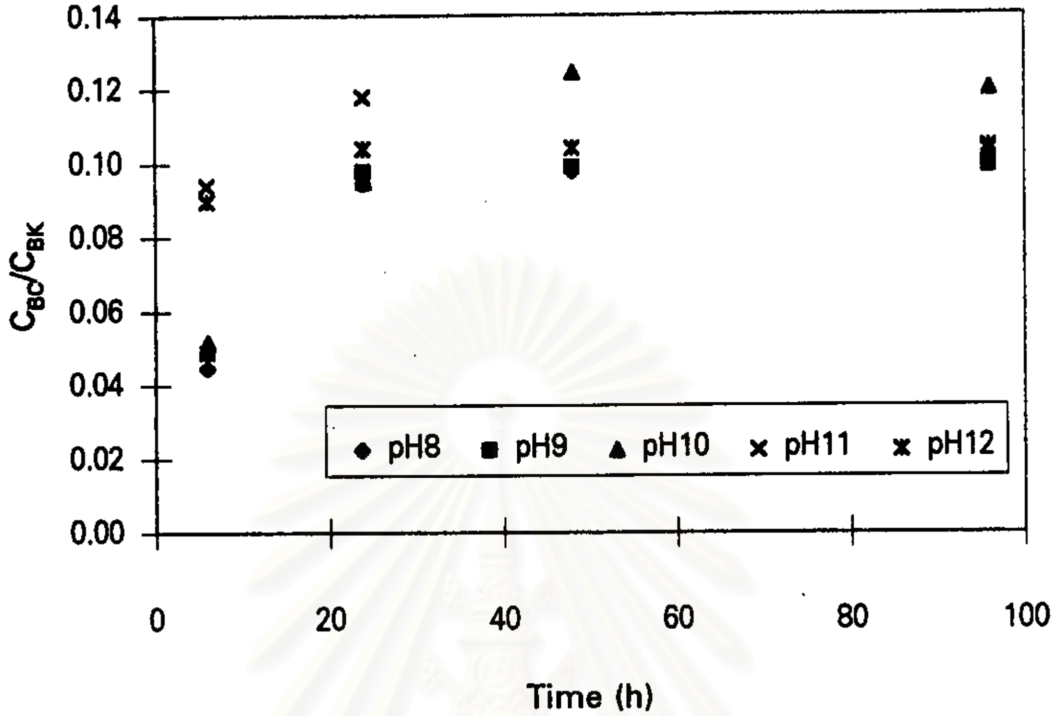

Figure 4.6 Concentration Ratio of Berberine in Crude Berberine Solution and Kerosene at Various pH of Crude Berberine Solution

<u>Experimental Conditions</u>

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

Organic phase : kerosene

Agitation speed : 240 rpm

Temperature : at 25°C

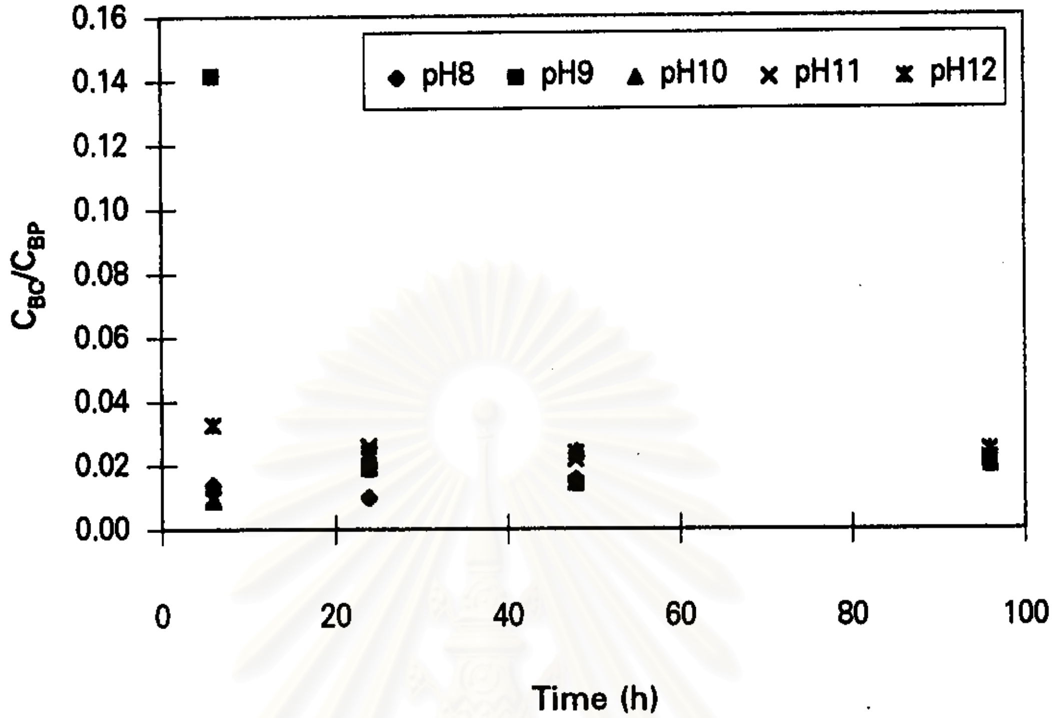

Figure 4.7 Concentration Ratio of Berberine in Crude Berberine Solution and Paraffin at Various pH of Crude Berberine Solution

Experimental Conditions

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

Organic phase : paraffin

Agitation speed : 240 rpm

Temperature : at 25°C

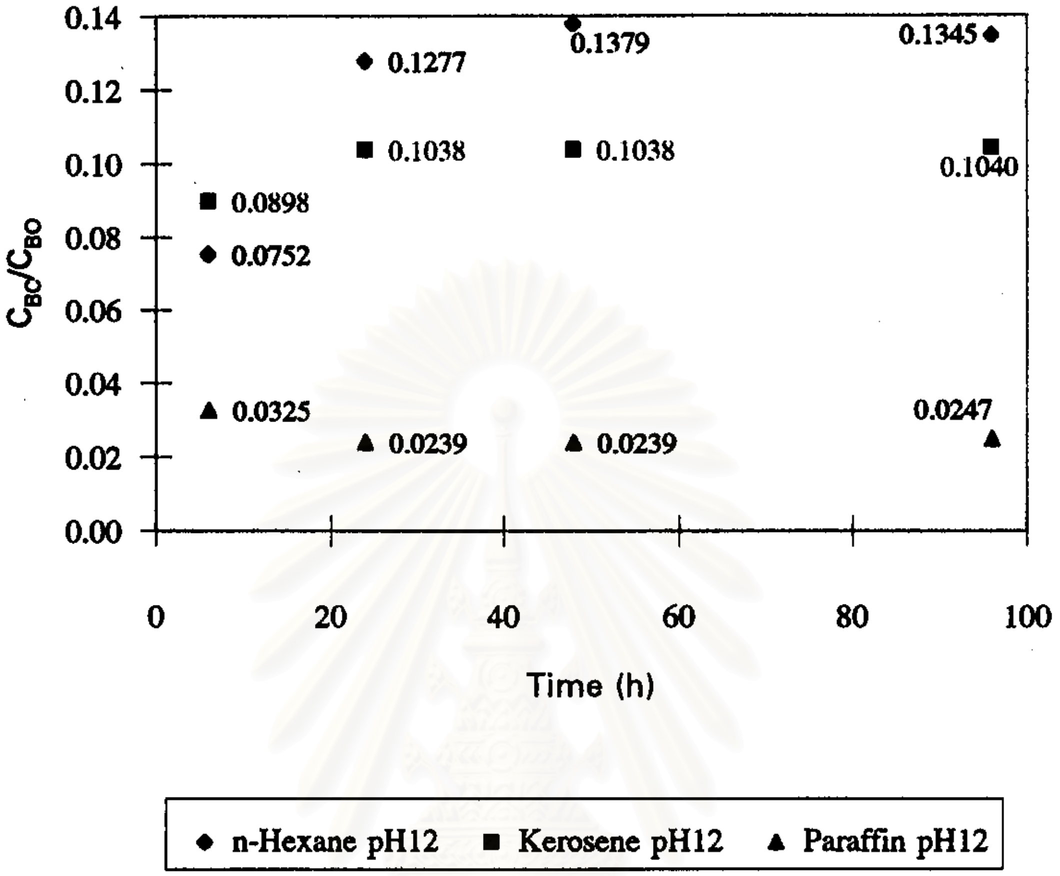

Figure 4.8 Concentration Ratio of Berberine in Crude Berberine Solution and Different Organic Solvents At pH 12 of Crude Berberine Solution

Experimental Conditions

Aqueous phase	:	0.11 g/l of crude berberine solution at pH 12
Organic phase	:	n-hexane, kerosene and paraffin
Agitation speed	:	240 rpm
Temperature	:	at 25°C