CHAPTER IV

RESULTS AND DISCUSSION

1. Recrystallization of asiaticoside in various solvents

The recrystallized product from methyl alcohol gave the highest yield. Products recrystallized in acetonitrile, butyl acetate, chloroform, ethyl acetate and methylene chloride could not be found because asiaticoside was not sufficiently dissolved in these solvents. The recrystallized products from acetone, 1-butyl alcohol and 2-butyl alcohol gave a very low yield due to the very slow evaporation rates of 1-butyl alcohol and 2-butyl alcohol were very slow because of their high boiling points. Thus, products recrystallized from acetone, 1-butyl alcohol and 2-butyl alcohol were not sufficiently collected for the future studies. All products were primarily identified and chracterized by thin layer chromatography and X-ray powder diffraction, respectively.

2. Identification of asiaticoside

2.1 Thin layer chromatography

Compound identification by TLC is based initially on R_f values compared to the reference standards. R_f values are generally not exactly reproducible from laboratory to laboratory or even in different runs in the same laboratory because many factors affect R_f values such as dimension and type of the chamber, nature and size of the layer, direction of mobile phase flow, the volume and composition of the mobile phase, equilibration conditions, humidity, and sample preparation methods preceeding chromatography. Further characterization of separated substances can be obtained by scraping the layer and elution of the analyte followed by infrared spectroscopy, Ultraviolet spectroscopy, nuclear magnetic resonance or mass spectrometry (Sherma,1991).

The TLC patterns of asiaticoside and recrystallized products were obtained using silica gel 60 F_{254} and ethyl acetate : methyl alcohol : water (8:2:1) as a mobile phase. The purple spot of asiaticoside was found after spraying with 10% H_2SO_4 in methyl alcohol and heating with a blow dryer. The R_f value of standard asiaticoside, madecassic acid and asiatic acid were 0.41, 0.74 and 0.80, respectively.The difference in R_f value indicated that this TLC condition was suitable for the separation of the above stated three commonly found components of *Centella asiatica* extract. All of recystallized asiaticoside showed the spots at the same R_f value as the asiaticoside standard. The R_f value is calculated by the following equation(Sherma, 1991).

$$R_f \text{ value} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by mobile-phase front}}$$

2.2 Fourier Transformed Infrared Spectroscopy

Infrared spectroscopy have been widely used for identification of pharmaceutical compounds by comparing FTIR pattern of compound of interest with standard substance or international references such as pharmacopiea. For solid state study, Fourier transformed infrared spectroscopy may be use for the qualitative and quantitative characterization of polymorphic and pseudopolymorphic compounds of pharmaceutical of interest.

The evaluation of solid state FTIR pattern by KBr method between raw material asiaticoside, recrystallized asiaticoside by methyl alcohol and asiaticoside synthesized by Arunya (Arunya, 1997) were similar. The FTIR spectrum shows broaded peak at 3400 cm^{-1} representing OH stretching, the peak at 2920 cm^{-1} representing CH stretching, the sharp peak at 1740 cm^{-1} representing C=O stretching, the peak at 1640 cm^{-1} representing CH bending, and the peak at 1060 for CO stretching.

However, Some wave frequencies of raw material asiaticoside (called asiaticoside I) and recrystallized asiaticoside by methyl alcohol (called asiaticoside II)

were different such as CH stretching (2926.03 versus 2921.91) , CH bending (1637.00 versus 1640.29) , CO stretching (1064.95 versus 1063.78) (Figures 10 and 11). This may be due to some differences internal structure of asiaticoside I and II which will be confirmed by other experiments later in the discussion.

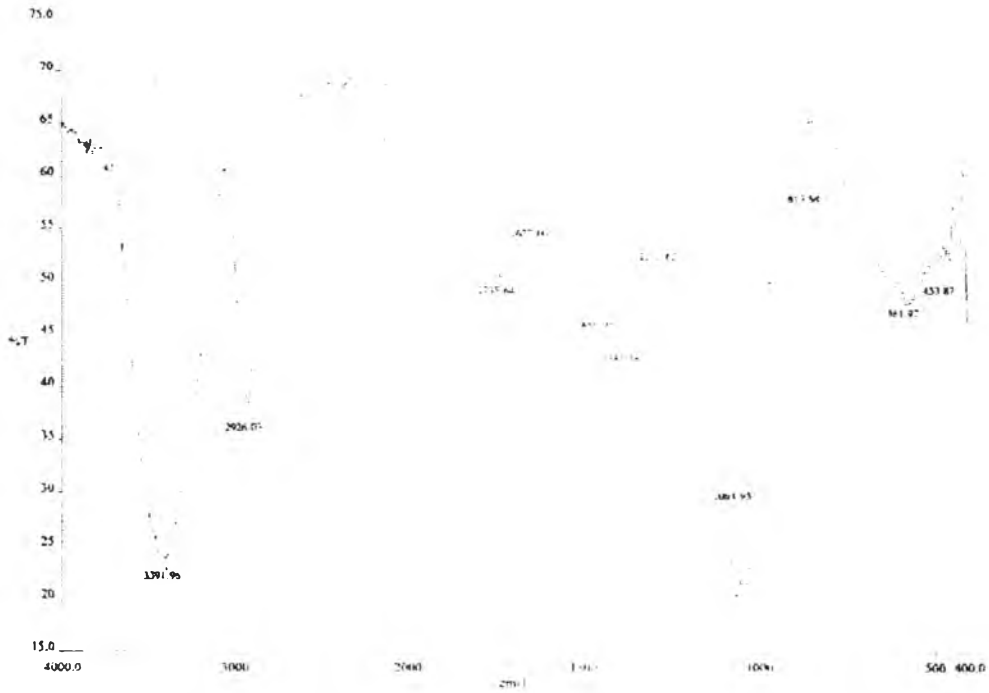

Figure 10: FTIR pattern of raw material asiaticoside (asiaticoside I)

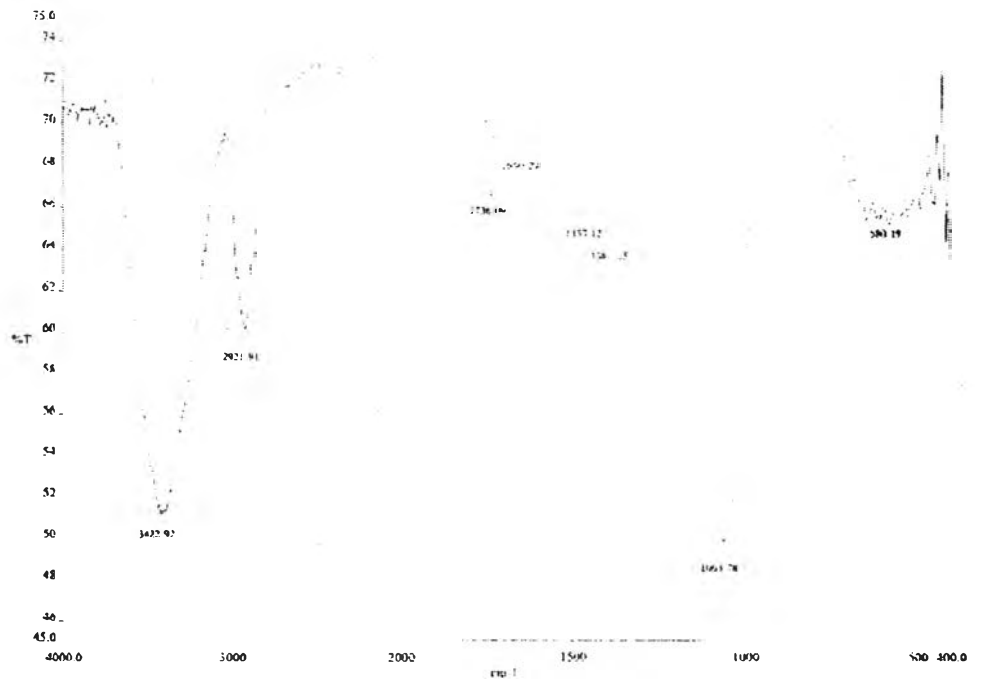

Figure 11: FTIR pattern of recrystallized asiaticoside from methyl alcohol (asiaticoside II)

2.3 Nuclear Magnetic Resonance

Proton and carbon nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) is widely used for the study of drugs and is employed for structure elucidation or identification. In polymorphic studies, the most useful nuclear magnetic resonance spectroscopy is solid state NMR. This technique has been applied to study polymorphism at the qualitative and quantitative levels. The solid state NMR has more advantages than solution NMR because the magnetic interactions are much larger in magnitude in the solid state than in solution phase and this method is a nondestructive method.

The solid state NMR require advanced hardware and software instruments and this is the limitation of this study. Thus, nuclear magnetic resonance spectroscopy study in this work was done with solution NMR only for chemical identification and comparision of the 2 forms of asiaticoside. The solid state structure are not possible to elucidate at this point.

The solution NMR can compare the spectrum of unknown with the standard substance or the reference data based on the same condition (same frequencies and same solvent). The ^{13}C NMR spectrum of asiaticoside I and asiaticoside II in Dimethyl sulfoxide were found to have similar pattern (Figures 12 and 13) indicating that the two samples are the same chemical entity, asiaticoside.

From TLC results, FTIR patterns and ^{13}C NMR spectrums, it may be concluded that asiaticoside I and asiaticoside II are the same in chemical structure but the molecular arrangement in solid state may be different as seen from the shift in wave number from FTIR experiments. Thus, a solid state investigation must be done.

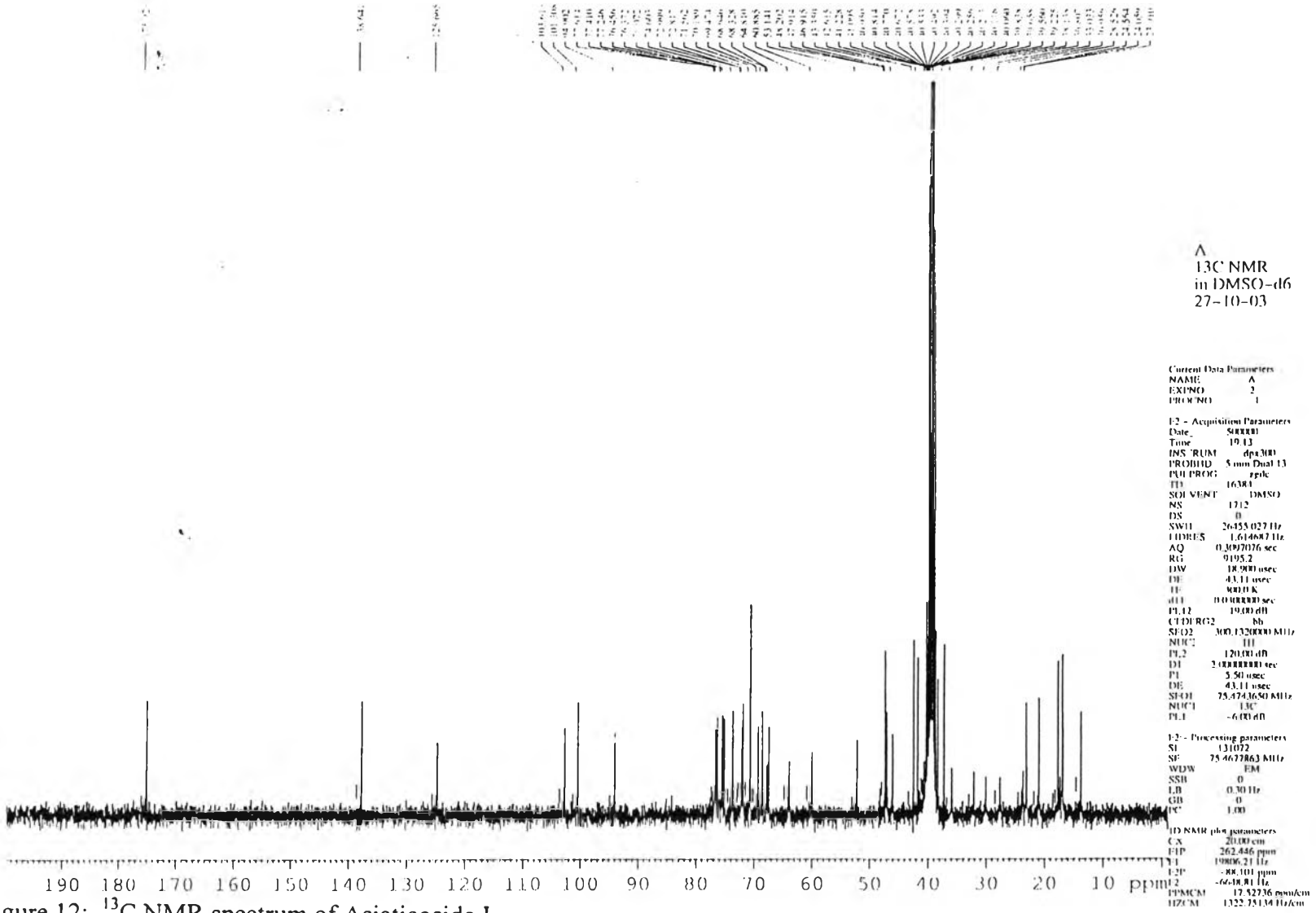

Figure 12: ^{13}C NMR spectrum of Asiaticoside I

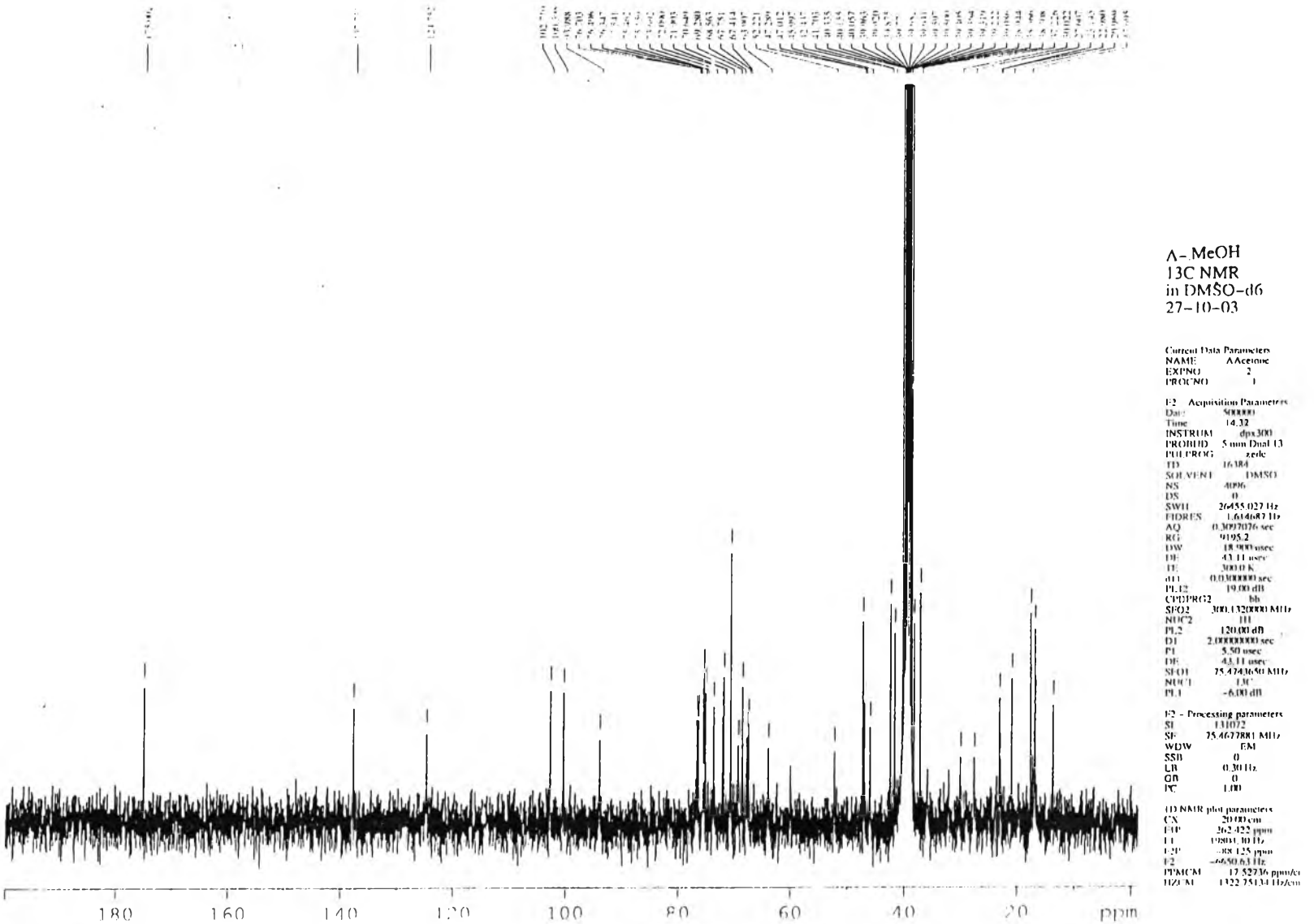

Figure 13 : ^{13}C NMR spectrum of Asiaticoside II

3 Solid State Characterization of asiaticoside

3.1 Microscopy

Morphology of asiaticoside I and recrystalized products from several solvents were observered by scanning electron microscopy and are shown in Figures 14 – 23.The morphology of asiaticoside I is needle-like (acicular form) and the recrystailized products obtained from methyl alcohol , ethyl alcohol in method I and methyl alcohol/water , methyl alcohol/acetonitrile in method II are also needle-like (acicular form) (Figures 14-16, 22-23). The products from n-propyl alcohol , isopropyl alcohol are acicular form but agglomerated (Figures 17-18). The recrystallized products from 1-butyl alcohol, 2- butyl alcohol and acetone are agglomerated small and thin needle-like (acicular form) crystals that may resulted from the amount of asiaticoside in recrystallization solution was low due to asiaticoside's low solubility in these solvents(Figures 19-21).

When asiaticoside I and asiaticoside II were observed under hot stage microscope. Gas bubbles appeared when the temperature increased to approximately 80°C. The small gas bubbles slowly appeared and became larger but not continuous indicating that the water or solvent in the crystals are released. Because the bubble was observed at the temperature about 80°C, it could be assumed that the bubbles were generated from water. Asiaticoside I and asiaticoside II both melted at approximately 230°C.

3.2 Qualitative X-ray Powder Diffraction analysis

The X-ray powder diffraction is a powerful method for the investigation of crystalline solids. The X-ray powder pattern of every crystalline forms of a compound is unique, so this technique is most suitable for the identification of different polymorphic forms and other molecular arrangements of a solid substance. The X-ray powder diffraction data obtained from different samples must be analysed on the same instrument and compared by overlaying and aligning the respective films or plots or an alternative method, it may involve comparison of 2θ values versus intensities of the various solid forms (Brittain, 1995).

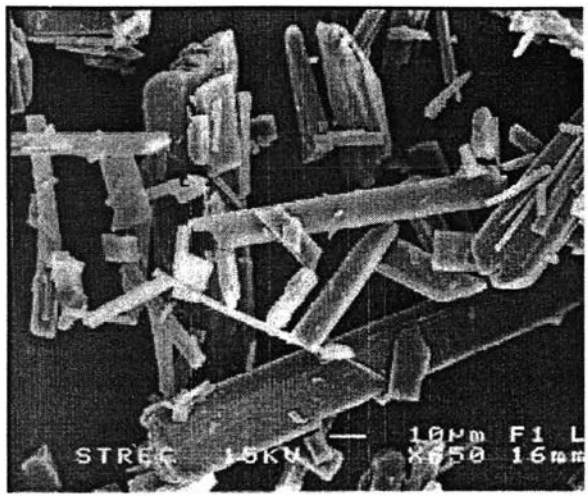

Figure 14 Morphology of raw material Asiaticoside (x 650)

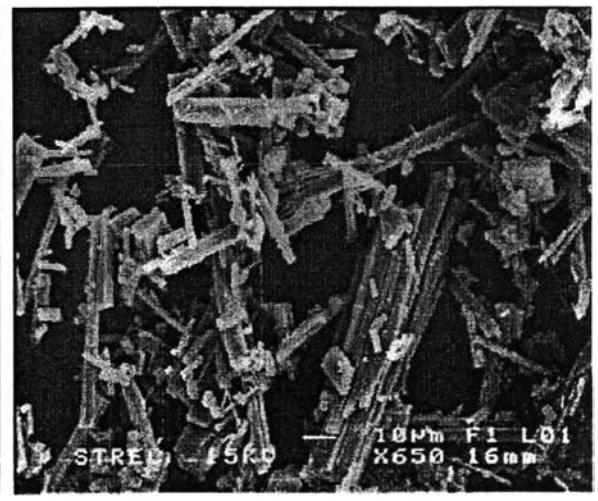

Figure 15 Morphology of recrystallized Product from Methyl alcohol (x 650)

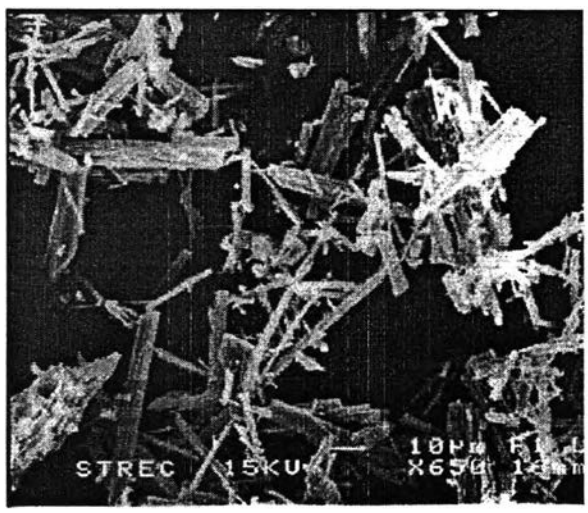

Figure 16 Morphology of recrystallized Product from Ethyl alcohol (x 650)

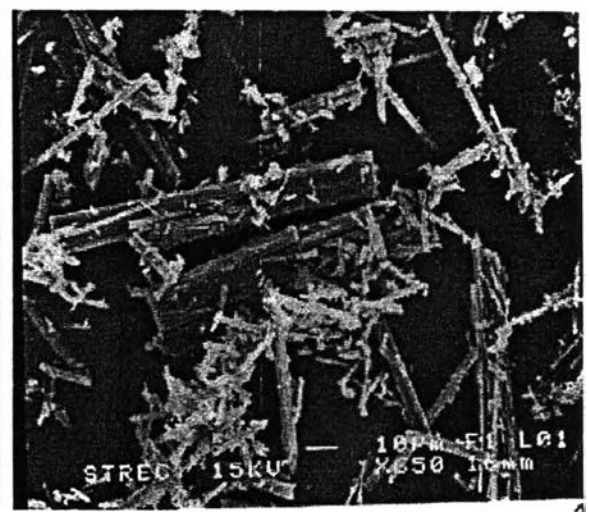

Figure 17 Morphology of recrystallized Product from n-propyl alcohol (x 650)

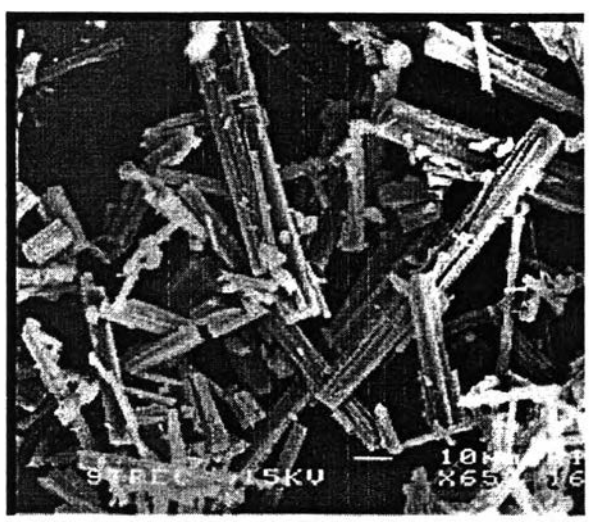

Figure 18 Morphology of recrystallized Product from Isopropyl alcohol (x 650)

Figure 19 Morphology of recrystallized Product from 1-buthyl alcohol (x 650)

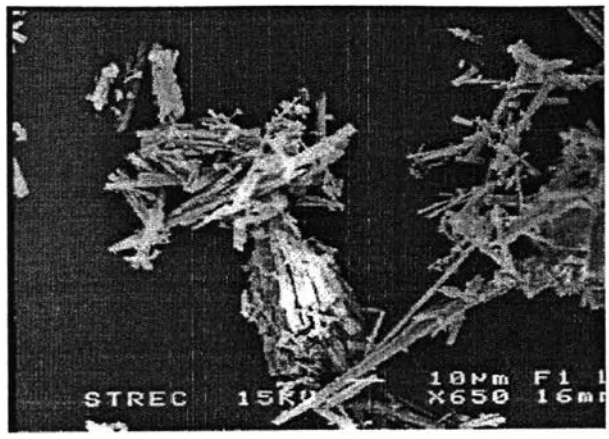

Figure 20 Morphology of recrystallized Product from 2-buthyl alcohol (x 650)

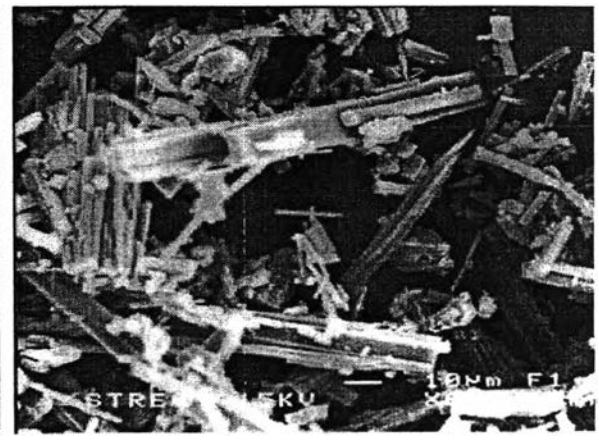

Figure 21 Morphology of recrystallized Product from Acetone (x 650)

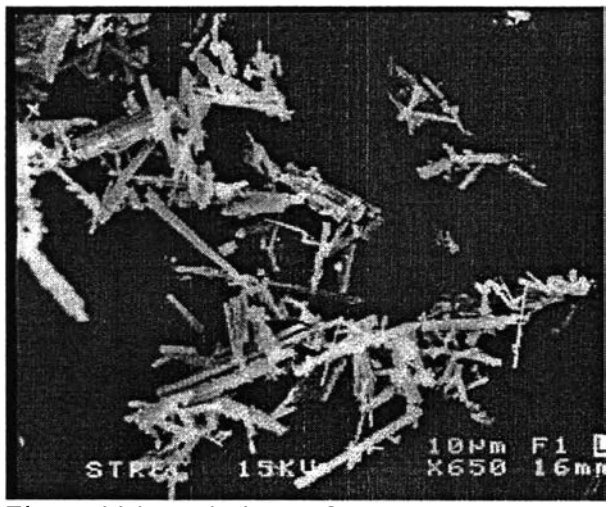

Figure 22 Morphology of recrystallized Product from methyl alcohol and acetonitrile (x 650)

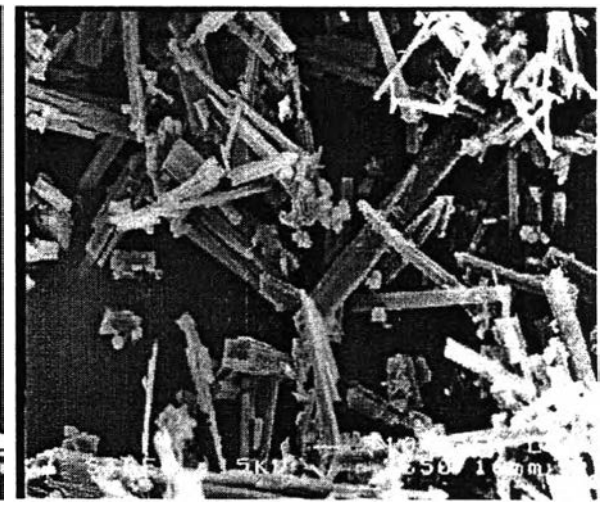

Figure 23 Morphology of recrystallized Product from methyl alcohol and water (x 650)

The XRPD patterns of asiaticoside I and its recrystallized products in various solvents were determined under the same condition. The patterns were interpreted by overlaying data pattern and comparing the 2θ values.

XRPD patterns of asiaticoside was classified into 2 groups as follows (Figures 24-26) ;

(a) Asiaticoside I (raw material asiaticoside , recrystallized product of methyl alcohol after treated under 105 °C for 3 hr) showed dominant XRPD peaks as seen in Figure 26;

- 3 separate peaks at 8.78, 9.66 and 10.34 °2θ
- tripplet peak at 28.54, 29.12 and 29.60 °2θ

(b) Asiaticoside II (recrystallized products in methyl alcohol, ethyl alcohol and other solvents) showed dominant peaks as seen in Figures 24-25;

- tripplet peak at 9.92 to 10.60 °2θ
- tripplet peak at 28.54, 29.12 and 29.60 °2θ were absent from the trace.

These data differences showed that solid state transformation of asiaticoside exist when recrystallized in various solvents. XRPD patterns of crystals obtained from 1-butyl alcohol, 2-butyl alcohol and acetone are low in crystallinity and appeared more amorphous. This may in fact be the result of its small particle size which lowers the X-ray scattering intensities seen in diffractograms Figure 24. The XRPD pattern of recrystallized product from methyl alcohol after heated under 105°C for 3 hours was different from untreated crystals and the pattern comformed to asiaticoside I. This result indicated that solid state interconversion of asiaticoside II to asiaticoside I existed after thermal treatment as seen in Figure 26.

On the otherhand, heated asiaticoside I retained its structure and showed the same pattern as asiaticoside I. It could be concluded that asiaticoside I was anhydrous and was more temperature stable than the recrystallized products of asiaticoside II.

From the data in these studies, asiaticoside had 2 polymorphs and showed that the solid state transformation could occur. Asiaticoside raw material and recrystallized product from methyl alcohol were used as a representatives of asiaticoside I and asiaticoside II, respectively, because it's ease of preparation and good yield.

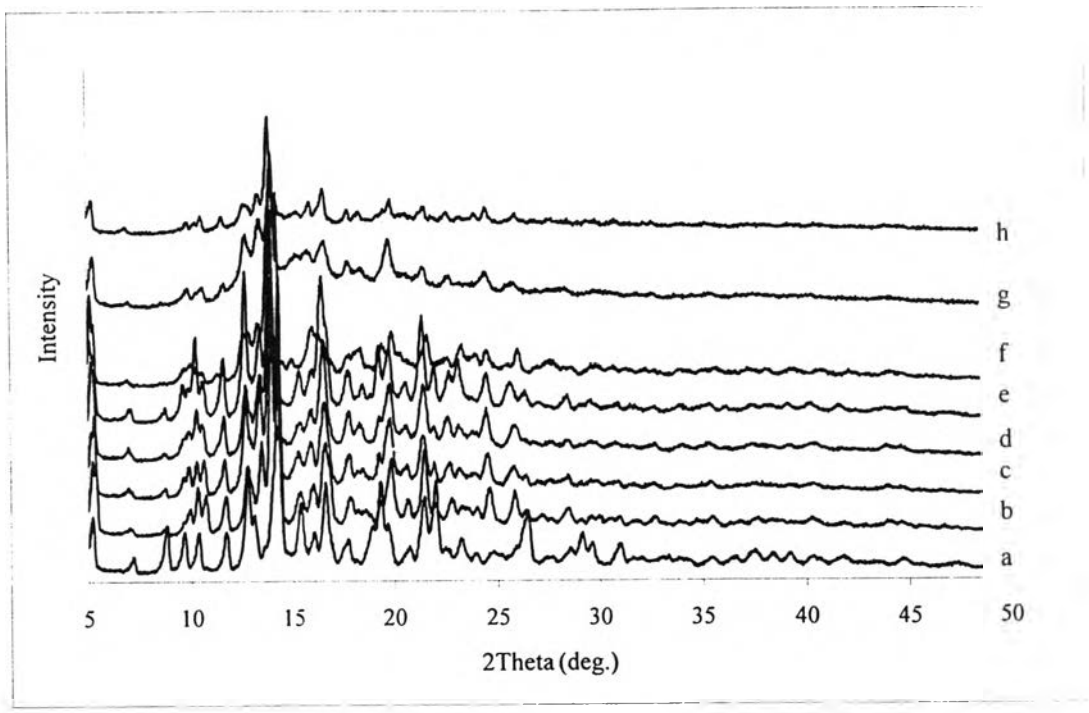

Figure 24 XPRD pattern of asiaticoside raw material and its recrystallized products from various solvents (a = asiaticoside raw material , b = product from methyl alcohol, c = product from ethyl alcohol, d = product from n-propyl alcohol, e = product from Isopropyl alcohol, f = product from 1 – butyl alcohol, g = product from 2- butyl alcohol, h = product from acetone)

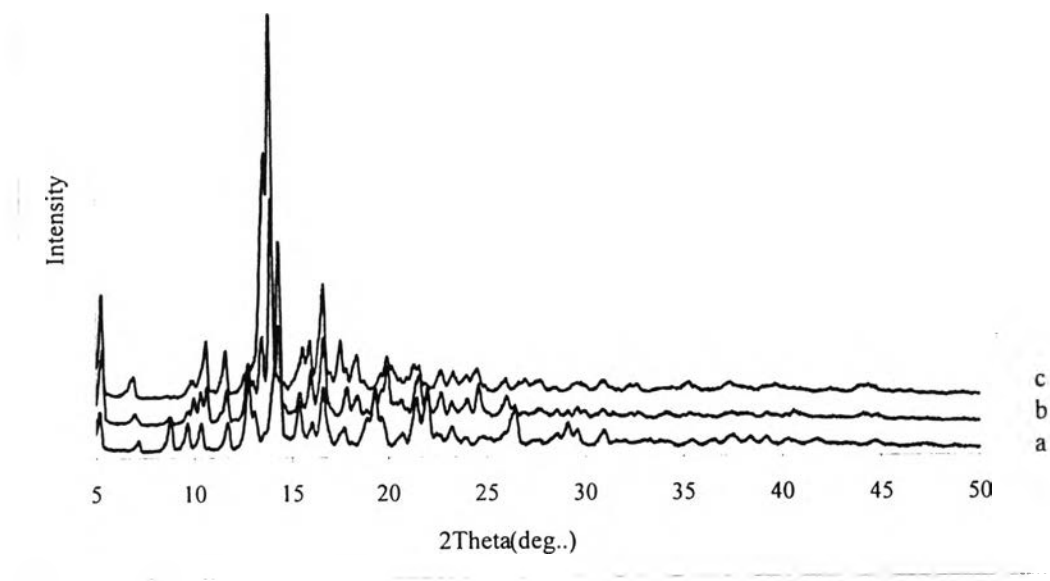

Figure 25 XPRD pattern of asiaticoside raw material and its recrystallized products from 2 solvent-systems (a = asiaticoside raw material, b = product from methyl alcohol/acetonitrile , c = product from methyl alcohol/water)

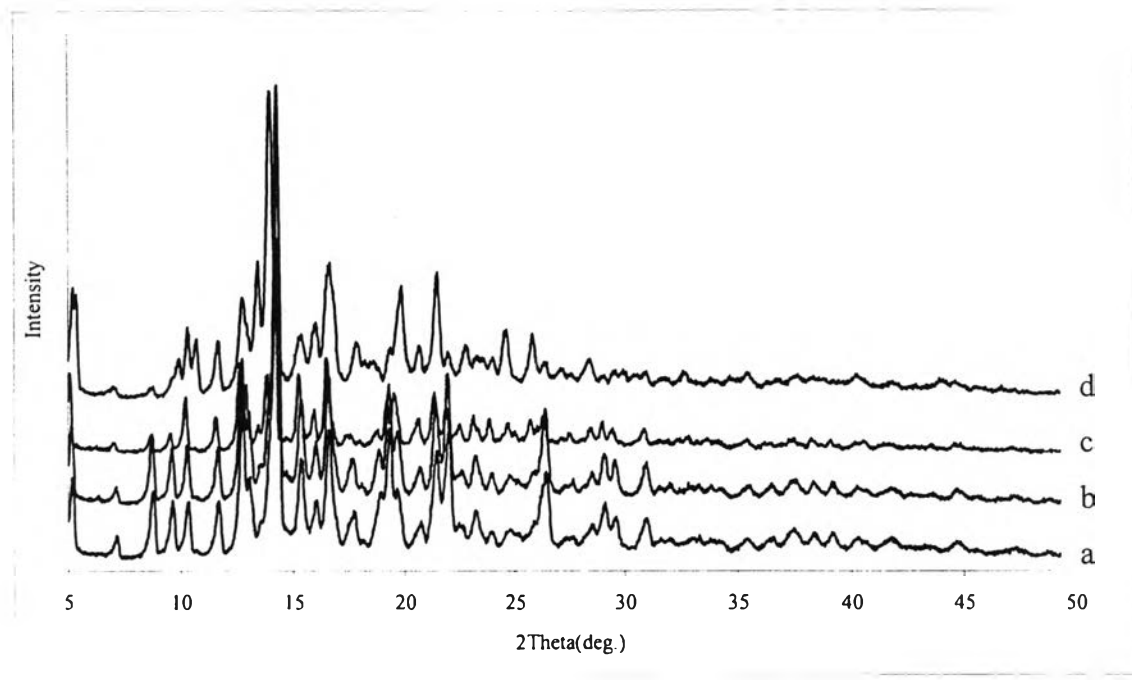

Figure 26 XPRD patterns of asiaticoside before and after heat treatment at 105°C for 3 hours (a = asiaticoside raw material , b = heated asiaticoside raw material , c = heated recrystallized product from methyl alcohol, d = recrystallized product from methyl alcohol)

3. Thermogravimetric Analysis

Thermogravimetric Analysis (TGA) is one of thermal methods in analysing solvates and polymorphs. TGA is a useful method for quantitative determination of solvents or volatile content in solids.

TGA thermogram of asiaticoside I and asiaticoside II present immediate weight lost at the starting temperature (Figures 27). Continuous weight loss occurred until a plateau was reached at approximately 90°C. Constant weight was observed until the substance melted with decomposition at around 240°C. From hot stage microscopy and TGA studies indicated that the initial weight lost is water or solvent deposited on the surface and was evaporated when heating started. The TGA thermogram of solvate or hydrate, which is considered as pseudopolymorph, usually show different patterns where desolvation occur at the later stage of heating. In

solvates or hydrates, the mass are usually constant during starting temperatures until boiling points of solvents which are bound in the solid molecular structure are reached. For example, Piretanide has 6 solvate forms which TGA thermograms showed weight loss at six various temperatures (Chikaraishi,1994).

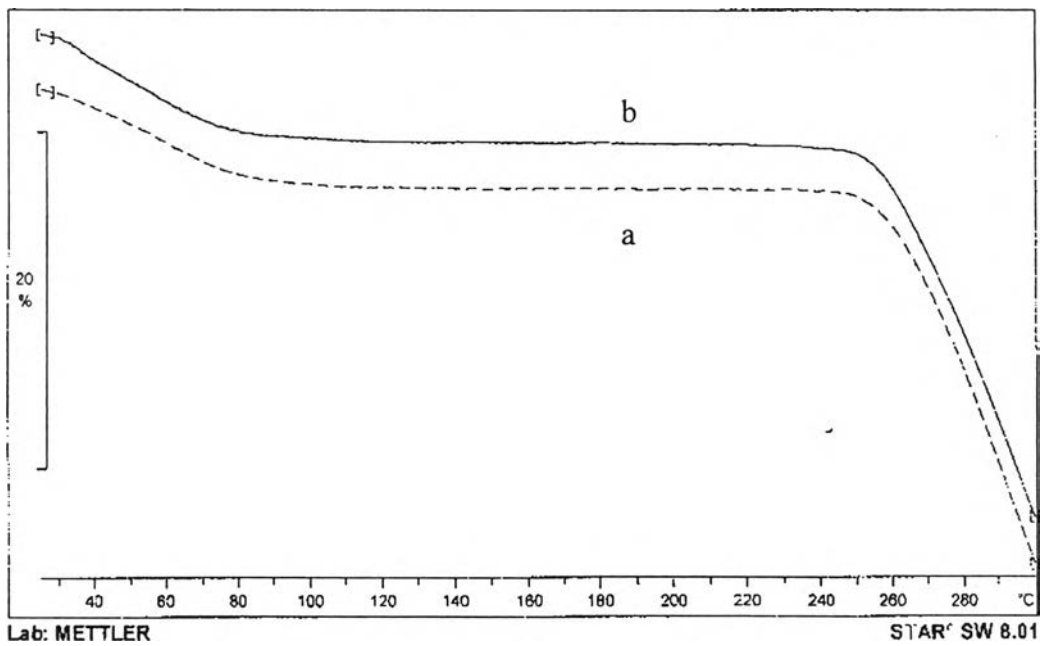

Figure 27 TGA thermogram of asiaticoside at the scanning rate of 5°C/min, from 25-300°C (a = asiaticoside I , b = asiaticoside II)

4. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a method which measures the difference in energy between reference material and the sample. DSC is useful for the study of polymorphs, solvates, hydrates for their thermal interconversions.

DSC thermograms of asiaticoside I and asiaticoside II had onset melting points of 229.85 and 230.79 °C, respectively (Figure 28). This data conformed to the melting points which was observed under the microscope with equipped hot stage. The thermograms of both samples had broad endothermic peaks at the immediate starting temperatures similar to that obtained from TGA thermograms and did not find any sharp endothermic peaks indicating solvates or hydrates.

XRPD patterns of 2 polymorphs are different and DSC and TGA thermograms of asiaticoside I and asiaticoside II are also different at temperature range of 40 - 70°C. This information may be due to a slight differences in molecular arrangements within the solid structure (shown by XRPD) which had minor impact on thermal analysis by DSC show at 40 - 70°C (Figure 28 b). The minor difference in endothermic peaks of DSC at 40 - 70°C implied that asiaticoside II may rearranged their internal structure and recrystallized to asiaticoside I. However, the large and broad desolvation peak interfered with recrystallization peak of asiaticoside II and obscured the event which could be seen otherwise. This result agreed well with XRPD pattern which found that the transformation of asiaticoside II to asiaticoside I occurred after isothermal heating heat at 105°C for 3 hours (shown in Figure 26).

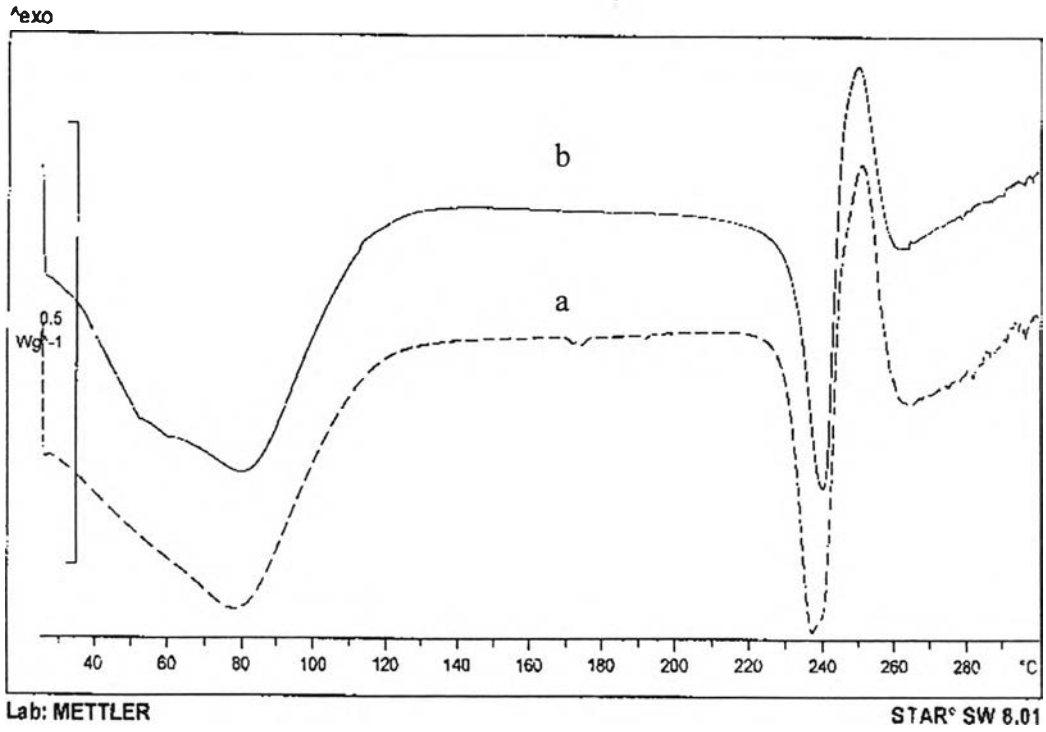

Figure 28 DSC thermograms of asiaticoside at the scanning rate of 5°C/min, from 25-300°C (a = asiaticoside I , b = asiaticoside II)

5. Karl Fischer titration

The water content of asiaticoside I and asiaticoside II by Karl Fisher titration was shown in Table 4

Table 4 The water content in asiaticoside I and asiaticoside II

Substance	Weight of Sample (g)	Used Karl Fisher reagent (ml)	Water content (%)	average	SD
Asiaticoside I	0.0325	0.36200	5.7174	5.9122	0.17
	0.0393	0.45800	5.9820		
	0.0352	0.41400	6.0371		
Asiaticoside II	0.0262	0.35600	6.9746	6.8357	0.18
	0.0280	0.36200	6.6362		
	0.0335	0.45000	6.8951		

The water content in asiaticoside I and asiaticoside II were 5.9122 and 6.8357 %, respectively. This result agreed with wieght loss(%) in thermogravimetric analysis (see Appendix) which implied that the lossing water was only water surface.

5. Gas chromatography

Gas chromatography is a useful method to identify and determine amount of volatile substances. Because of minor difference in DSC thermogram at temperature approximately 40 – 70 °C, this result imply that it has other solvent which boiling point about 40 – 70°C volatile from the sample.

From GC chromatograms as shown in Figure 29, the retention time of the major peak of standard methanol, volatile substance from asiaticoside I and volatile substance from asiaticoside II are 2.253, 2.210 and 2.283, respectively. So the residual volatile substance on the surface of both forms was methanol. However, the broad endothermic DSC peaks at ~ 40 - 120°C was due to the loss of water and possibly the loss of minute amount of methanol. This result was confirmed by Karl Fascher experiment which shown to exhibit approximately 6-7 %w/w of water in both asiaticoside I and asiaticoside II. Althought GC chromatogram of volatile substance from asiaticoside I had smaller peak at the retention time about 2.692 minutes, it may

be due to other solvents which was used by the manufacture to increase recrystallization yeild.

Thus, GC analysis confirmed that the only volatile substance liberted at approximately 60°C in DSC analysis was only methyl alcohol. The result proved that temperature fluctuations between 40– 70°C was highly possibly due to phase transformation from asiaticoside I and asiaticoside II as was found by XRPD.

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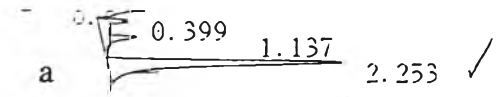
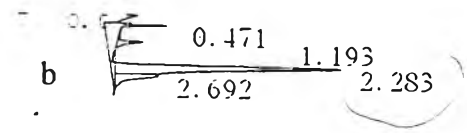


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.399	1807	53	S		17.187	
	3	1.137	480	48	T		4.561	
	4	2.253	8229	440	T		78.253	
		TOTAL	10516	541			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=3 DATA=1:@CHRM1.C00 05 12/08 13:10:34



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.471	2645	113	S		26.295	
	2	1.193	440	47	T		4.376	
	3	2.283	5837	421	T		58.028	
	4	2.692	1137	77	TV		11.302	
		TOTAL	10060	658			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=2 DATA=1:@CHRM1.C00 05 12 08 13:04:12

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.493	670	64	S		0.403	
	2	1.179	312	36	T		0.187	
	3	2.21	165216	11603	T		99.41	
		TOTAL	166197	11704			100	

Figure 29 GC chromatogram of standard methanol and volatile substance from asiaticoside (a = standard methanol, b = asiaticoside I, c = asiaticoside II)

IV Physicochemical studies

1. Solubility studies

The solubility of a new drug substance in water is most important in the preformulation study because many pharmaceutical solids exhibits more than one polymorph which is different in solubility such as acetazolamide, paclitaxel, picotamide, piretanide etc. (Chikaraishi,Otsuka and Matsuda, 1996; Griesser,Burger and Mereiter, 1997; Liggins,Hunter and Burt,1997; Bettinetti, 1999).The rate of solution and the solubility of a solid in water or other solvents are important aspects in studying the solid-state chemistry of drugs. The dissolution rate and solubility of different polymorphs, solvates and amorphous forms of the same drug was known to be different (Byrn,Pfeiffer and Stowell, 1999).

The solubility profiles of asiaticoside I and asiaiticoside II are shown in Figure 30. The solubility profile of asiaticoside I present an increasing asiaticoside concentration and became stable at 60 minutes. For asiaticoside II , the profile showed highest solubility at 20 mins and decresed until stable at about 240 mins with the same plateau concentration as asiaticoside I. This data implied that asiaticoside II in water was transformed to asiaticoside I which is more stable than asiaticoside II. In addition, the result of solubility testing correlates well with XRPD result that asiaticoside II can change to asiaticoside I which was more thermodynamically stable.

2. Stability studies of asiaticoside

Solid state reaction can be categorized either as a physical transformation or as chemical reaction. The physicochemical transformation affects the pharmaceutically important physical properties such as solubility, flowability, density. Different polymorphs lead to very different physicochemical properties. The chemical stability is also important to shelf life of pharmaceutical products. Pharmaceutical preformulation should study both of these topic.

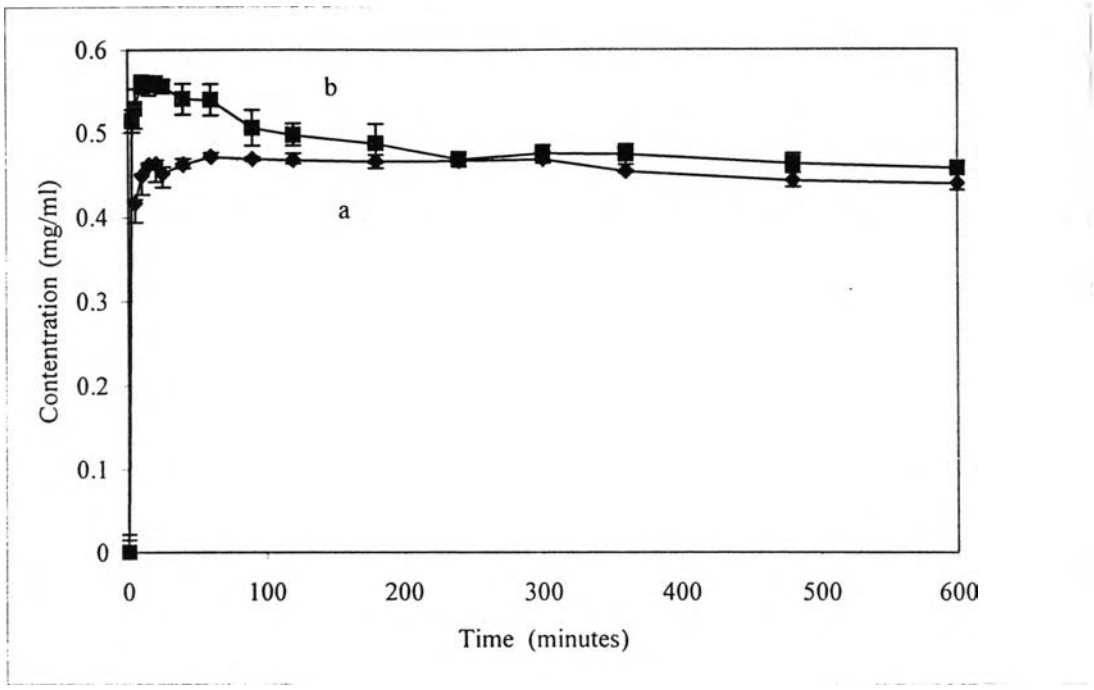


Figure 30 Solubility Profile of asiaticoside in 37 ± 2 °C (a = asiaticoside I , b = asiaticoside II)

2.1 Solid State Stability of Asiaticoside

2.1.1 Effect of temperature

Asiaticoside I and II were stored under 40 °C, 50 °C and 60 °C at 55 – 65 %RH and observed for any polymorphic transformation using XRPD. The initial intention of this experiment was to store the samples for 16 weeks but X-ray powder diffractometer was out of order in the last phase of experiment. The XRPD patterns obtained of the final points must use different X-ray powder diffractometer (See Appendix). Thus,the discussion will not include these final data points.

The XRPD patterns of asiaticoside I after stored at 40 °C, 50 °C, 60 °C and asiaticoside II under 40 °C in period 2, 4, 6 and 8 weeks did not change from the original sturcture (Figures 31-35). However, the pattern of asiaticoside form II at 50 °C found peak at 2θ about 28.54, 29.12 and 29.60 since the second week of storage.

For the pattern at 60 °C, It had 2 changing points similar to 50 °C and found peak shift from tripplet peak to 3 separate peaks at 2θ about 8.78, 9.66 and 10.34 since the second week also (Figures 35 – 36).

The overview of asiaticoside XRPD pattern may imply that transformation process of asiaticoside form II to asiaticoside form I can be accellerated by temperature. At higher temperature asiaticoside form II will change to form I more rapidly than at low temperature.

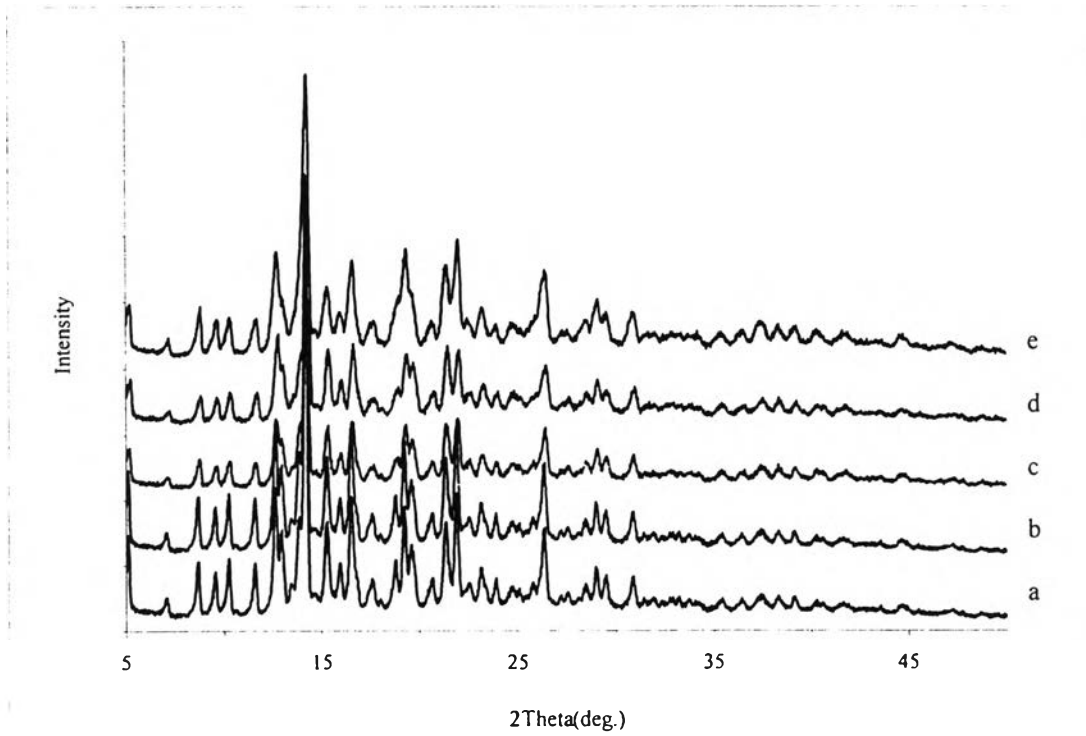


Figure 31 : XRPD pattern of asiaticoside I after stored in 40 °C and 62 %RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

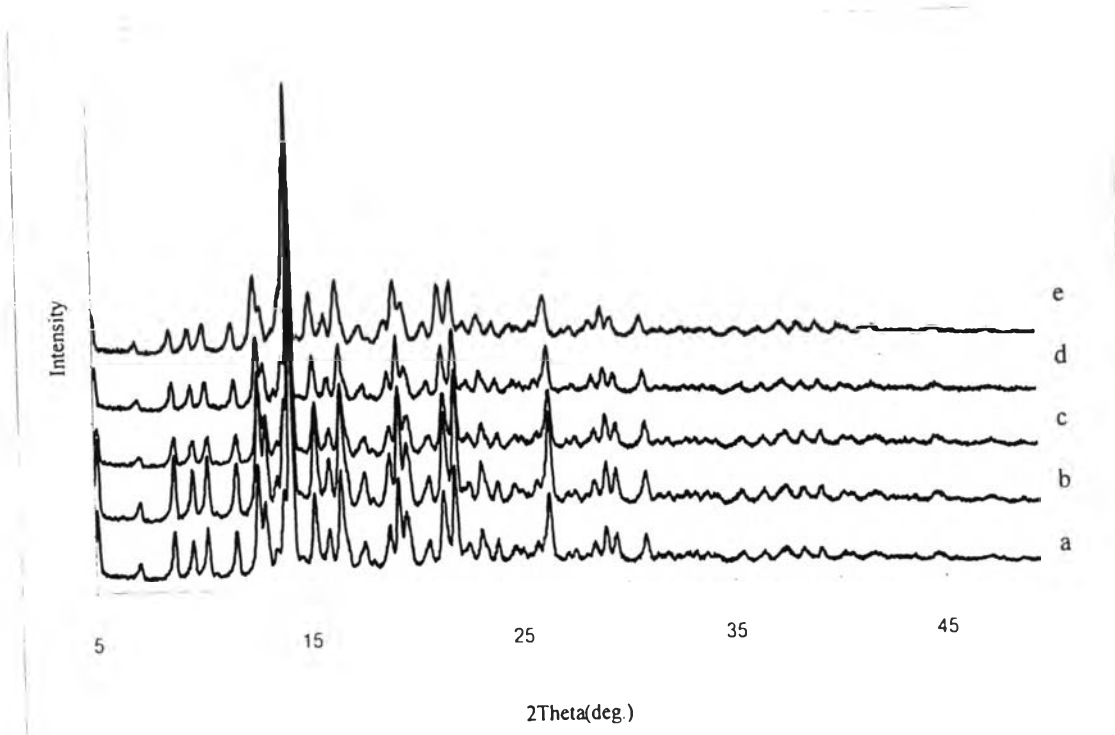


Figure 32 : XRPD pattern of asiaticoside I after stored in 50 °C and 59 %RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

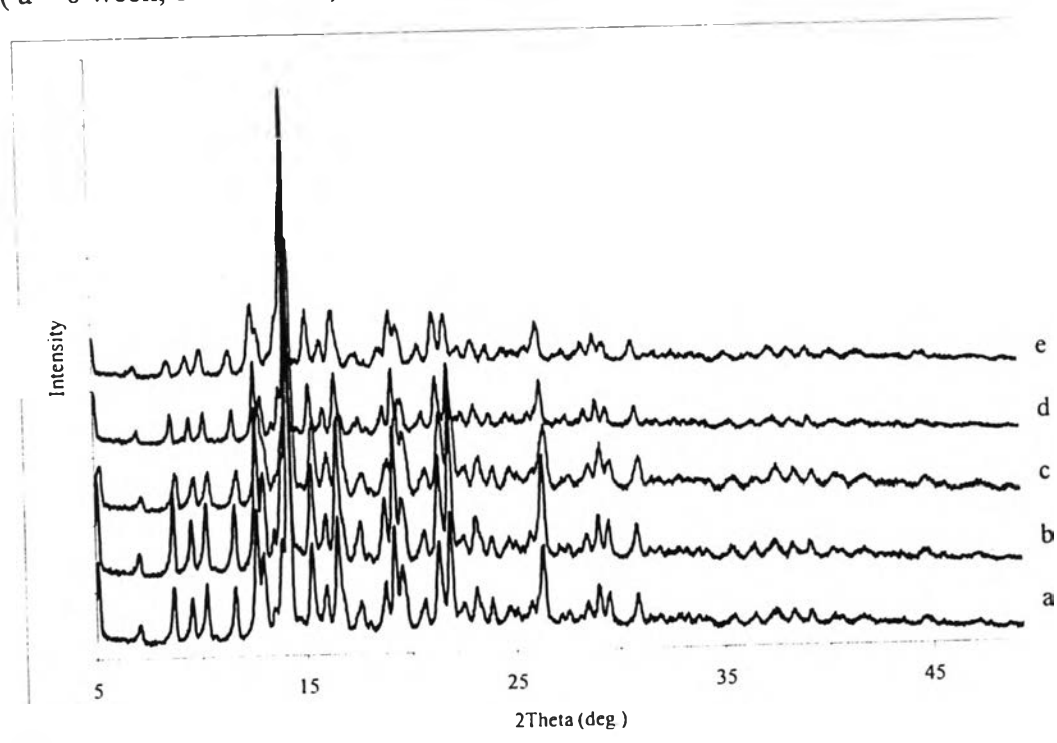


Figure 33 : XRPD pattern of asiaticoside I after stored in 60 °C and 59 %RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

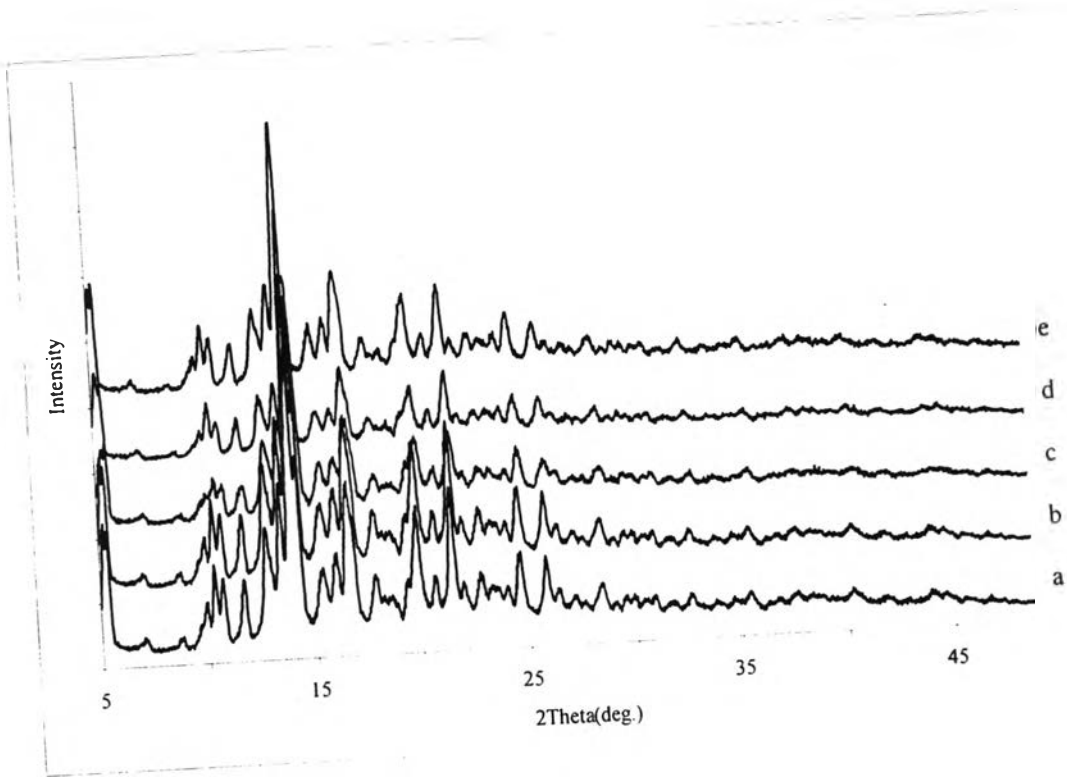


Figure 34 : XRPD pattern of asiaticoside II after stored in 40 °C and 62 % RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

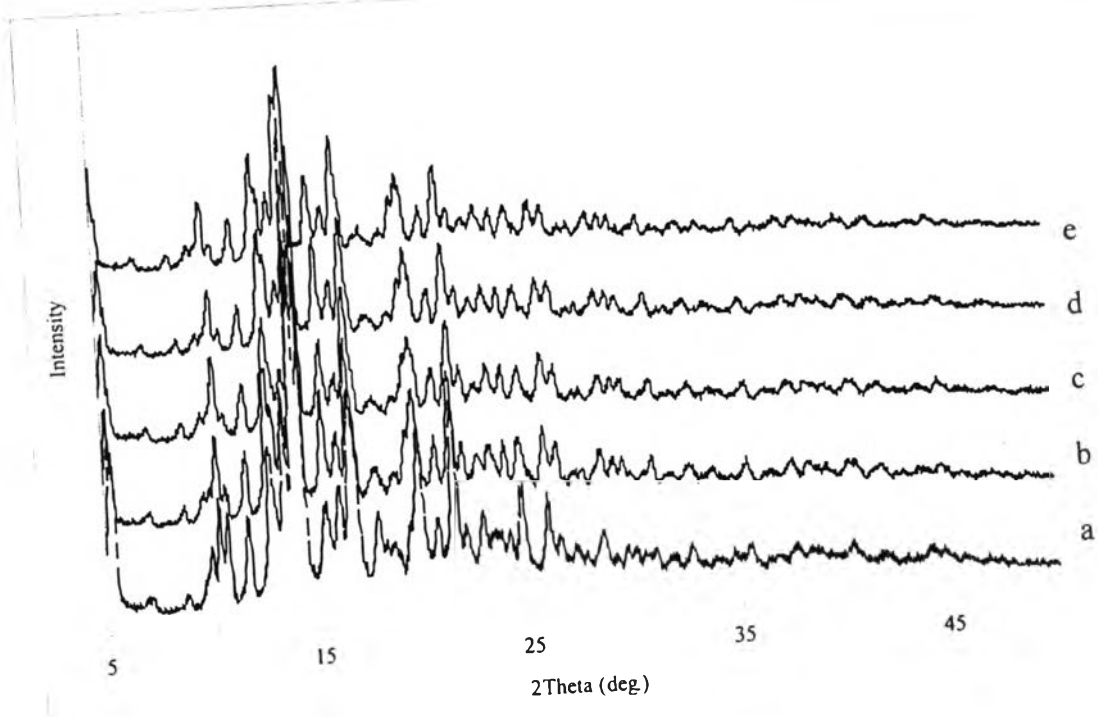


Figure 35 : XRPD pattern of asiaticoside II after stored in 50 °C and 59 %RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

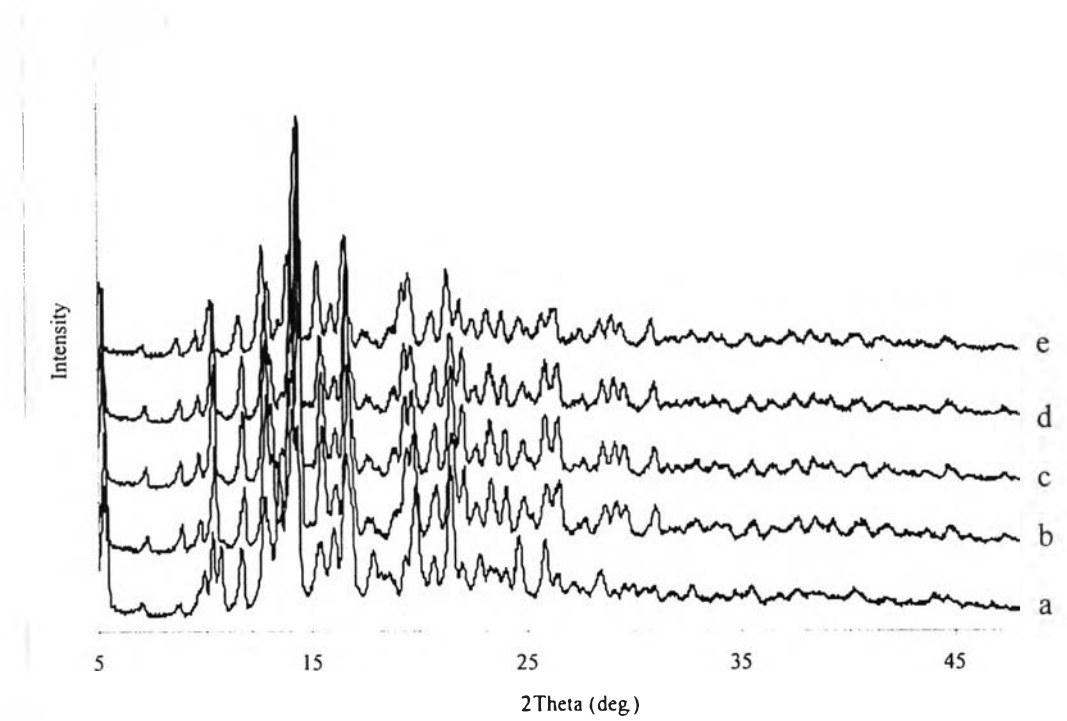


Figure 36 : XRPD pattern of asiaticoside II after stored in 60 °C and 59 %RH (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

2.1.2 Effect of Relative Humidity

The XRPD patterns of asiaticoside I which stored at under 42%RH, 62%RH ,75%RH and asiaticoside II under 62 %RH and 75%RH at 40°C in the period 2, 4, 6 and 8 weeks did not change from the original structure (Figures 31,37-38,40). However,the pattern of asiaticoside II at 42%RH had a changed to the pattern which found the peaks at 2θ about 28.54, 29.12 and 29.60 since the second 2 week (Figure 39) .

The data of XRPD patterns of asiaticoside I and II in various relative humidity which asiaticoside II can convert to form I and the low relative humidity is more effective on this transformation than at higher relative humidity .

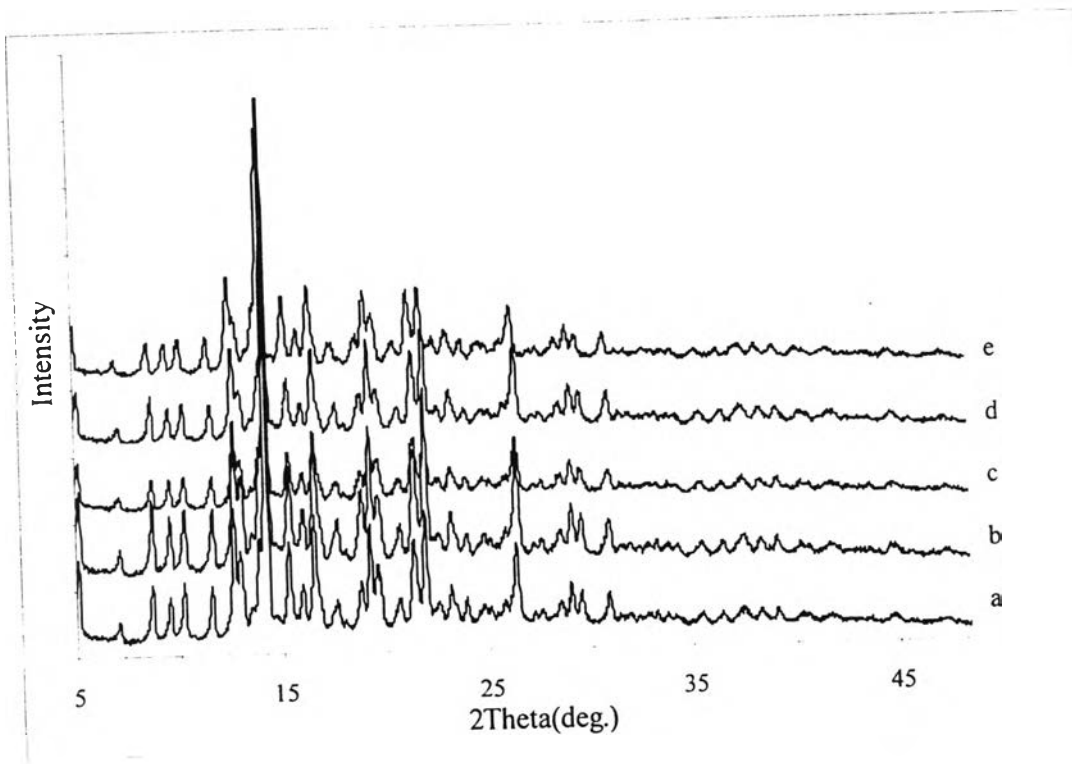


Figure 37 : XRPD pattern of asiaticoside I after stored in 42%RH and 40°C (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

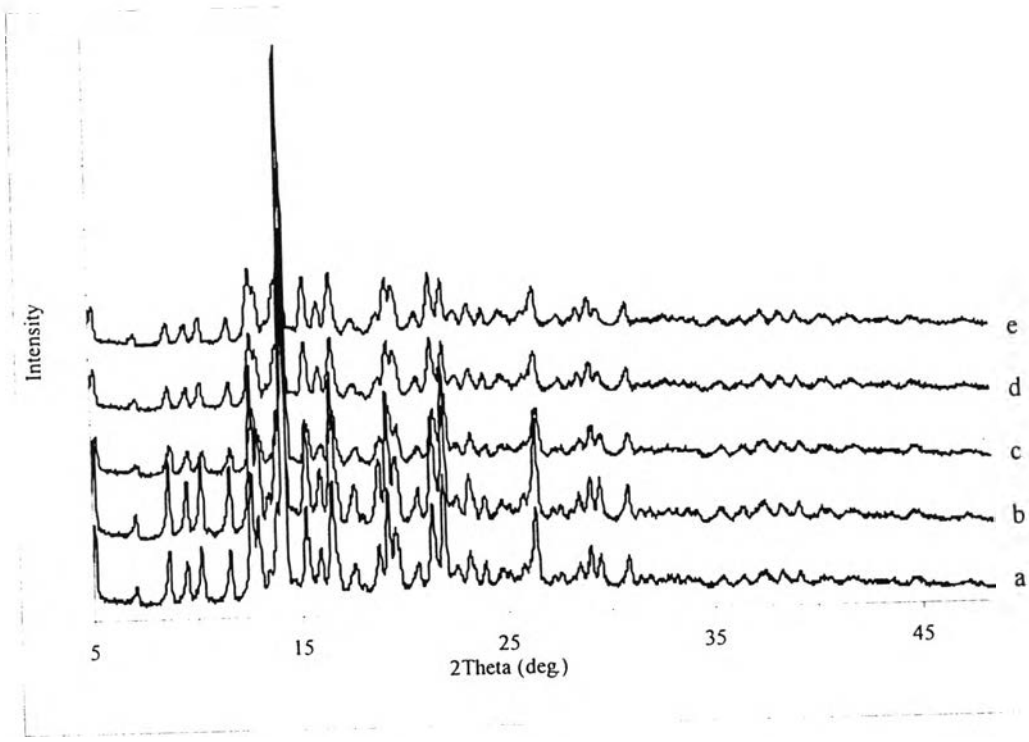


Figure 38 : XRPD pattern of asiaticoside I after stored in 75%RH and 40°C (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

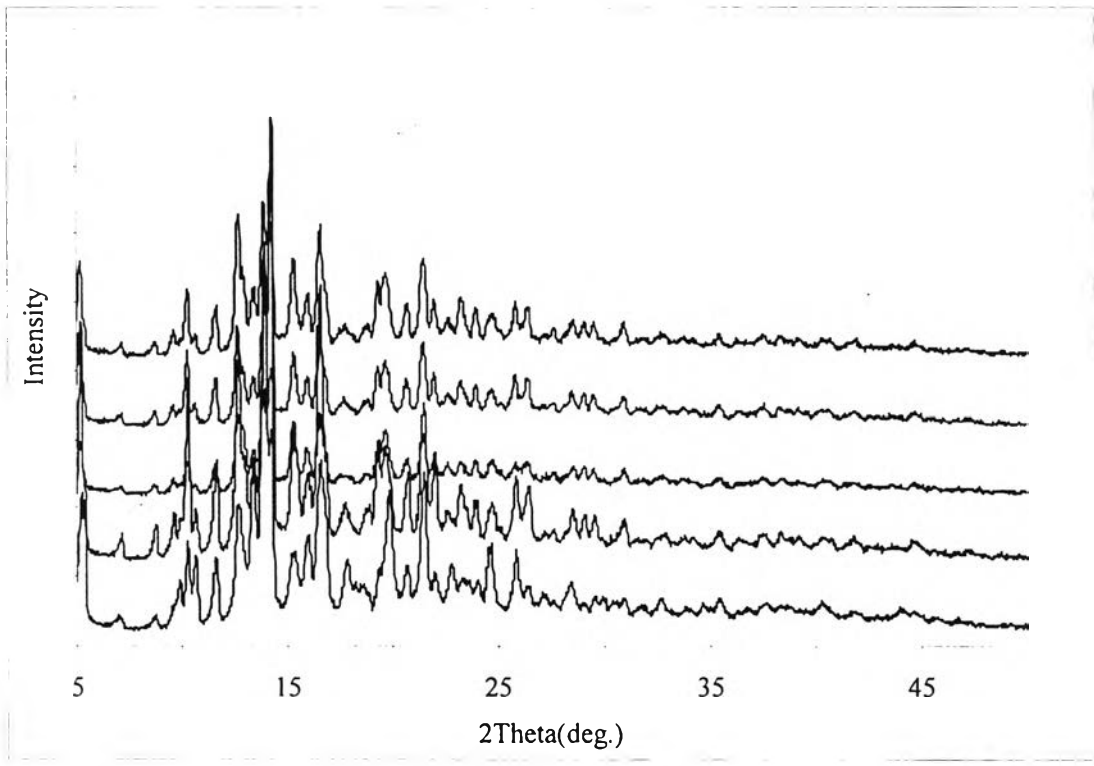


Figure 37 XRPD pattern of asiaticoside II after stored in 42%RH and 40°C (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

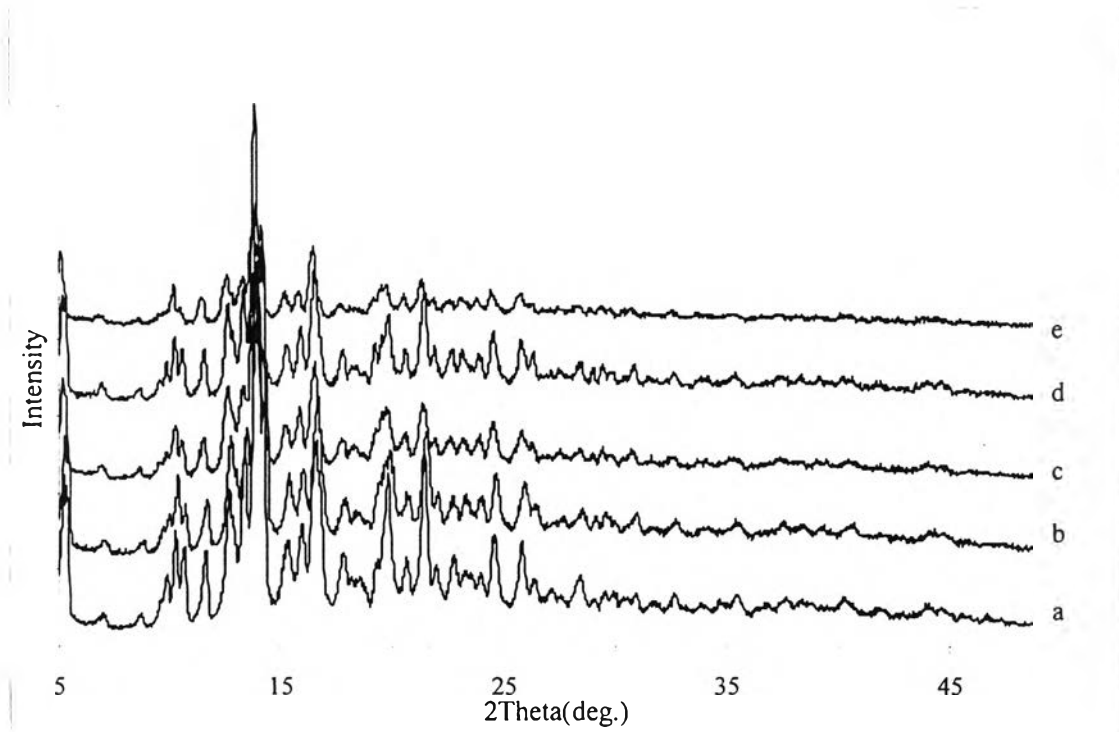


Figure 38 XRPD pattern of asiaticoside II after stored in 75%RH and 40°C (a = 0 week, b = 2 weeks, c = 4 weeks, d = 6 weeks , e = 8 weeks)

Chemical stability

Chemical stability study is one of the most important part in pharmaceutical preformulation. The study should be designed to represent various stress conditions for handling, formulation, production and storage. Because of the limited time, this investigation was aimed to examine decomposition kinetics of asiaticoside I and II under 2 stress factors, temperature and relative humidity.

The common reaction kinetics used to predict the decomposition rate of the substances as below:

Zero order reaction :

$$C_0 - C_t = - k_0 t$$

First order reaction :

$$\ln(C_0/C_t) = - k_1 t$$

Second order reaction :

Case 1 : $a = b = C_0$

$$x/a(a-x) = k_2 t$$

Case 2 : $a \neq b$

$$1/(a-b) \ln b(a-x)/a(b-x) = k_2 t$$

Where Co = concentration at t = 0

Ct = concentration at t = t

x = concentration of product

k = rate constant

t = time

a = initial concentration of substance A

b = initial concentration of substance B

Asiaticoside I and asiaticoside II were treated under various condition and determine amount of asiaticoside . After that plot amount of asiaticoside versus time according to each kinetic reaction equation. To determine which kinetic equations can be fitted, the correlation of determination (r^2) was used. The rate constants and the correlation of determination calculated form each equation show in table 6

Effect of temperature, The results from Table 6 found that when the equation was fitted with decomposition amounts of asiaticoside I and II, it correlated with many kinetic equations which found the correlation of determination (r^2) of nearly 1. Therefore, the data can assume that the decomposition of asiaticoside may occur by complex mechanism.

Effect of relative humidity, when the equation was fitted according to the decomposed amounts of asiaticoside I and II, the correlation was not found. This results may be assumed that the decomposition of asiaticoside by various of relative humidities was complex and could not be explained by individual simple kinetic equations.

The reaction rate constants of asiaticoside I and II tend to increased when stored at high temperature (Figures 41,42) and high r^2 values. However, the affect of relative humidity on of the reaction rate constants of asiaticoside I was not conclusive due to very low correlation coefficient (r^2)(Figure 43) while humidity only slightly affect the stability of asiaticoside II (Figure 44).

Table 6 The rate constants and the correlation of determination of asiaticoside I and asiaticoside II from stability studies

Substance	Condition	Zero order kinetic		First order kinetic		Second order kinetic	
		k_0	r^2	k_1	r^2	k_2	r^2
Asiaticoside I	40°C,55-65%RH	0.1632	0.9721	0.0018	0.9675	2×10^{-5}	0.9626
	50°C,55-65%RH	0.2357	0.9658	0.0027	0.9595	3×10^{-5}	0.9526
	60°C,55-65%RH	0.3278	0.9897	0.0041	0.9873	5×10^{-5}	0.9799
	40°C,42%RH	0.0115	0.170	0.0002	0.1779	1×10^{-6}	0.1840
	40°C,62%RH	0.1632	0.9721	0.0018	0.9675	2×10^{-5}	0.9626
	40°C,75%RH	0.0770	0.6182	0.0016	0.6214	8×10^{-6}	0.6308
Asiaticoside II	40°C,55-65%RH	0.0443	0.8801	0.0009	0.8817	5×10^{-6}	0.8865
	50°C,55-65%RH	0.1583	0.9031	0.0033	0.9018	2×10^{-5}	0.8983
	60°C,55-65%RH	0.2946	0.9136	0.0065	0.9110	4×10^{-5}	0.9037
	40°C,42%RH	0.0493	0.7388	0.0005	0.7440	5×10^{-6}	0.7492
	40°C,62%RH	0.0443	0.8801	0.0009	0.8817	5×10^{-6}	0.8865
	40°C,75%RH	0.0384	0.8435	0.0004	0.8460	4×10^{-6}	0.8484

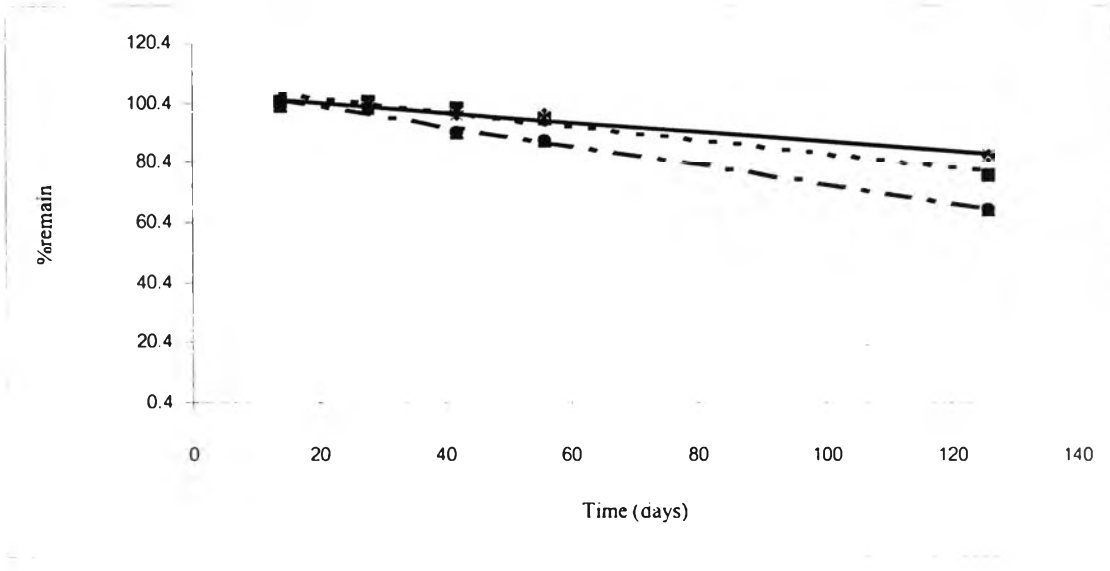


Figure 41 Zero order kinetic of asiaticoside I at various temperatures at 55 – 65 %RH(— = 40°C , - - - = 50°C , — - — = 60°C)

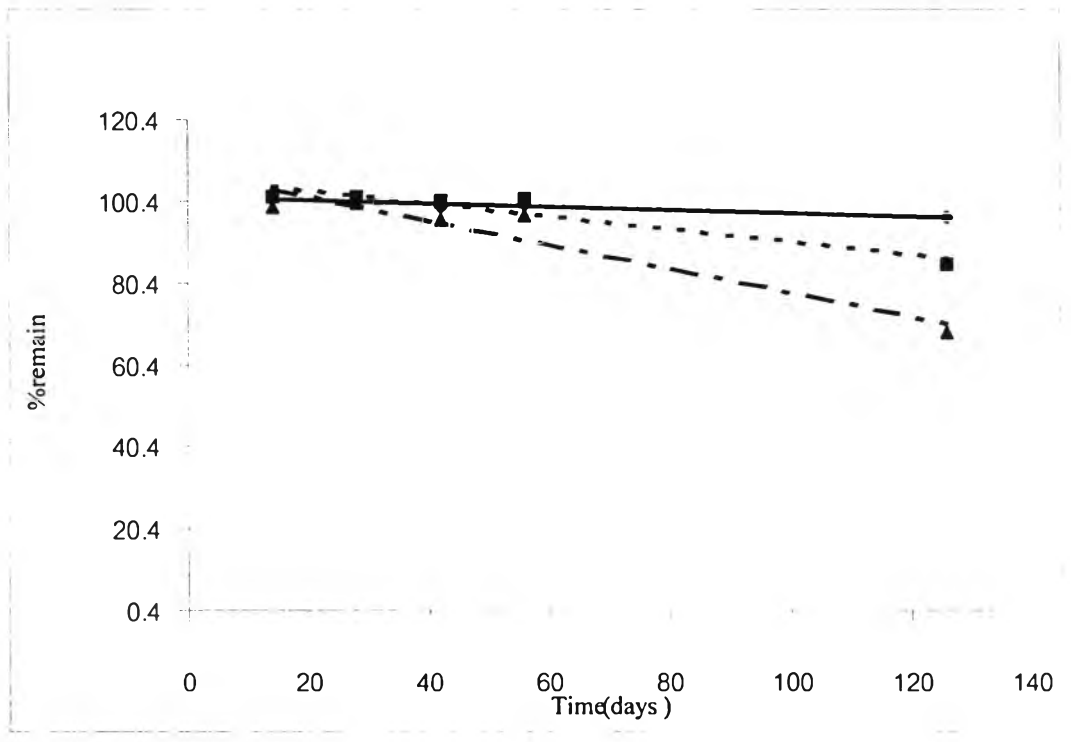


Figure 42 Zero order kinetic of asiaticoside II at various temperatures at 55 – 65 %RH(— = 40°C , - - - = 50°C , — - — = 60°C)

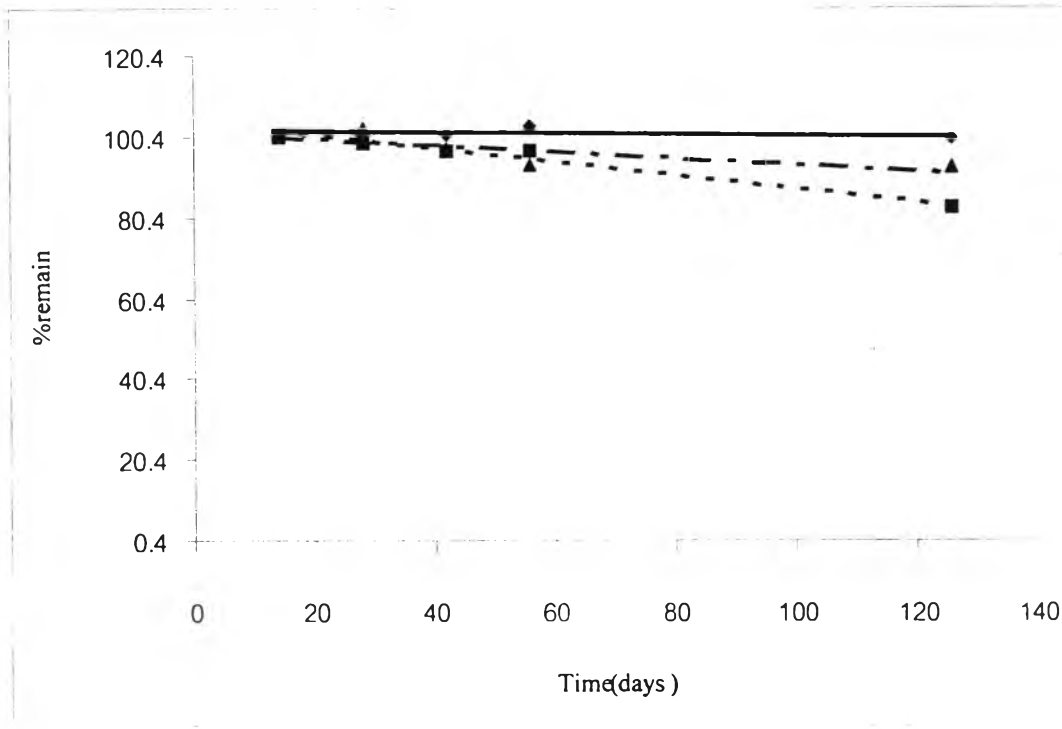


Figure 43 Zero order kinetic of asiaticoside I at various relative humidities at 40°C (— = 42%RH , - - - = 62 %RH and — - — = 75%RH)

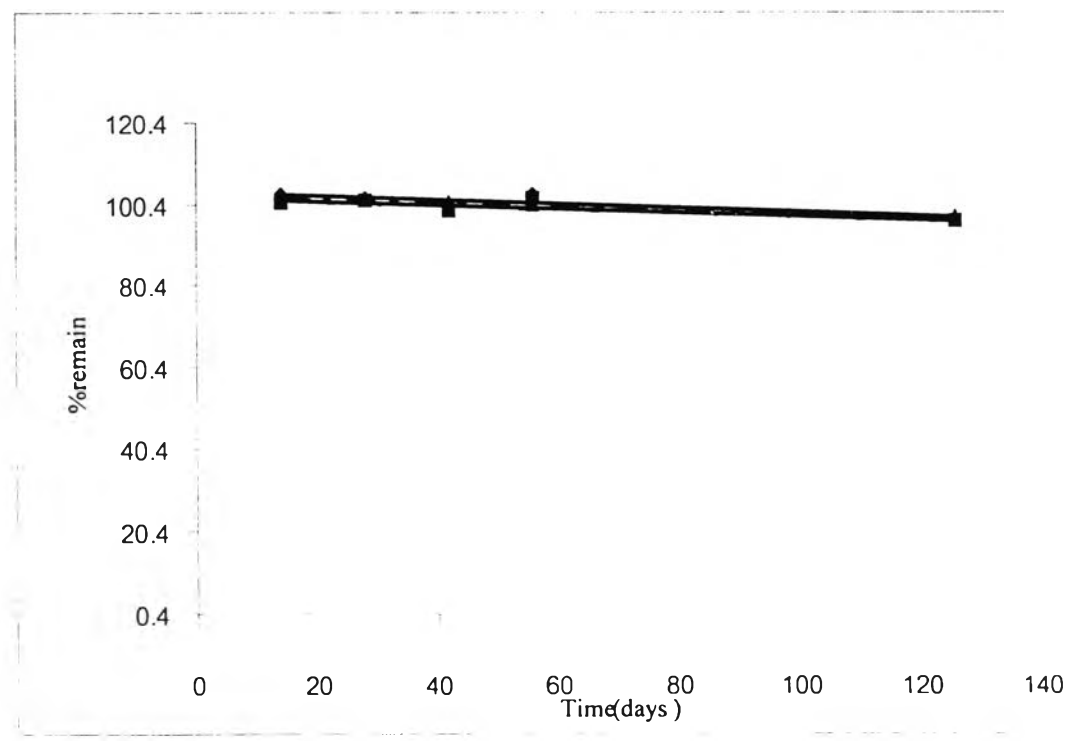


Figure 44 Zero order kinetic of asiaticoside II at various relative humidities at 40°C (— = 42%RH , - - - = 62 %RH and — - — = 75%RH)

3. Incompatibility study

The solid-solid compatibility tests play very important role in the preformulation studies of solid oral dosage form. Problems arise because of the interaction with other drug substances and with preservatives, stabilizers, dyes and flavors (Goto,Kim and Hirakawa, 1995).

Mixtures of asiaticoside I and II with spray-dried lactose, pregelatinized strach , dibasic calcium phosphate, talcum, silicon dioxide and magnesium stearate were stored under 40 °C at 75 %RH and observed for polymorphic transformation by XRPD. The studies designed for this experiment was to store the sample 16 weeks but X-ray power diffractometer was out of survice in the last phase of experiment. Thus, XRPD patterns of the final point (16 weeks) were detected using other X-ray powder diffractometer which in this discussion will not include this last data point at 16 weeks.

Solid State Stability of Asiaticoside Mixture

The XRPD patterns of asiaticoside mixed with diluents, ie., spray-dried lactose, pregellatinized strach , dibasic calcium phosphate in the ratio of 1 : 20 (Figure 45-47,51-53) found a low intensity of active ingredient, so the discussion of this section will point to the effect of asiaticoside on the transformation of diluents.The XRPD patterns of mixtures of asiaticoside with diluents after stored under stressed conditions for 8 weeks were found to remain the same.

The XRPD patterns of mixture asiaticoside with talcum, silicon dioxide and magnesium stearate in the ratio of 1:1(Figure 48-50,54-56) were found to remain unchanged.

The DSC thermograms of all mixtures (Figure 109-120) show the endothermic peaks and exothermic peaks of active ingredients and excipients which were separated and could not found signs of interference due to incompatability.

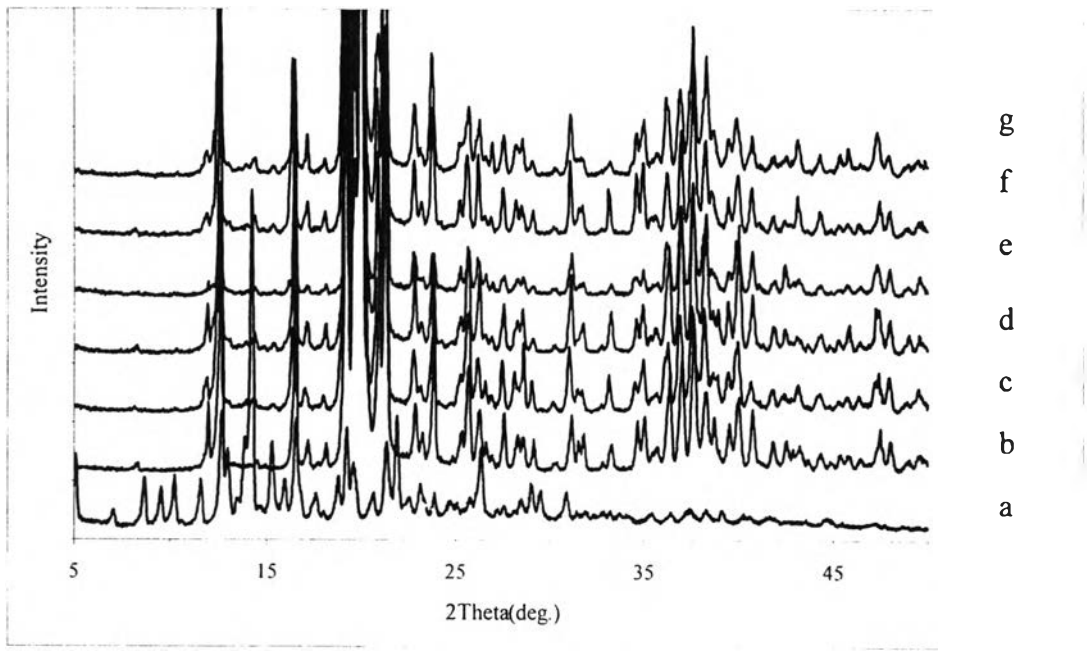


Figure 45 The XRPD patterns of mixture of asiaticoside I with spray-dried lactose (a = asiaticoside I , b = spray-dried lactose , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

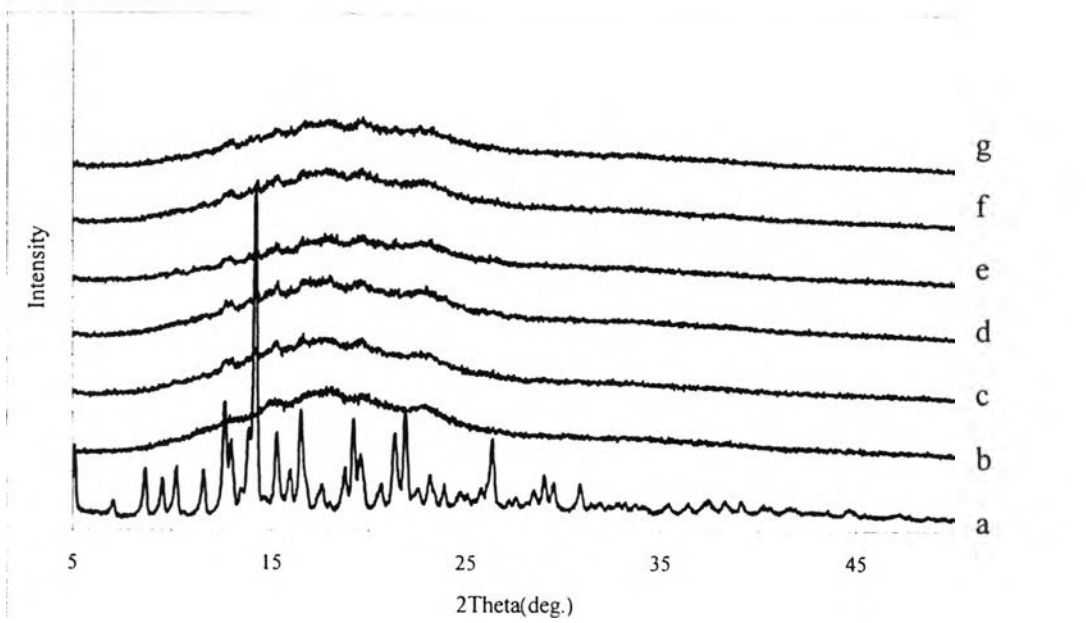


Figure 46 The XRPD patterns of mixture of asiaticoside I with pregelatinized starch (a = asiaticoside I , b = pregelatinized starch , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

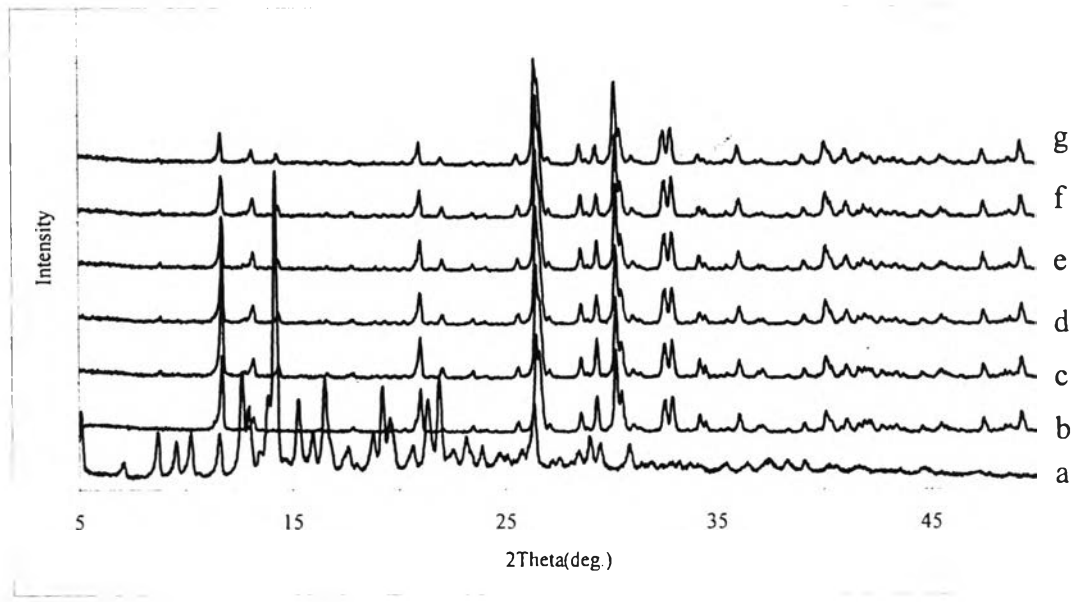


Figure 47 The XRPD patterns of mixture of asiaticoside I with dibasic calcium phosphate (a = asiaticoside I , b = dibasic calcium phosphate , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

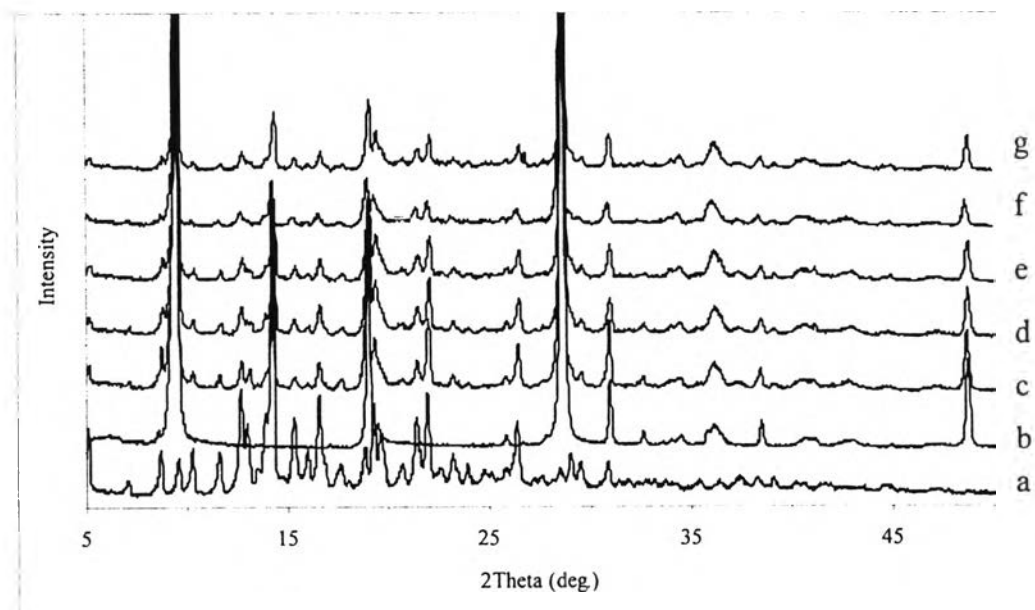


Figure 48 The XRPD patterns of mixture of asiaticoside I with talcum (a = asiaticoside I , b = talcum , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

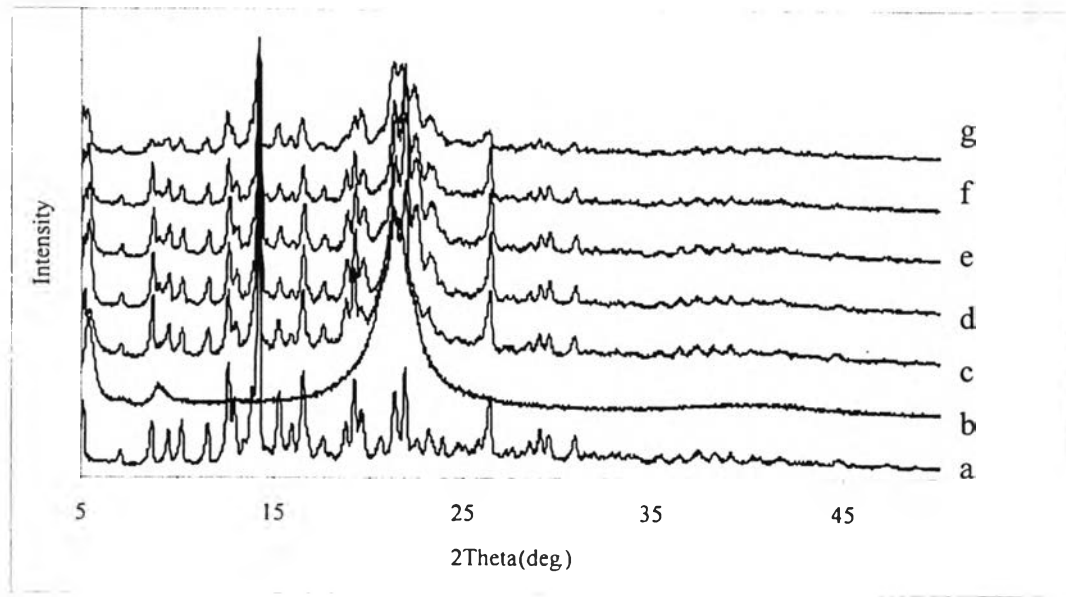


Figure 49 The XRPD patterns of mixture of asiaticoside I with magnesium stearate (a = asiaticoside I , b = magnesium stearate , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

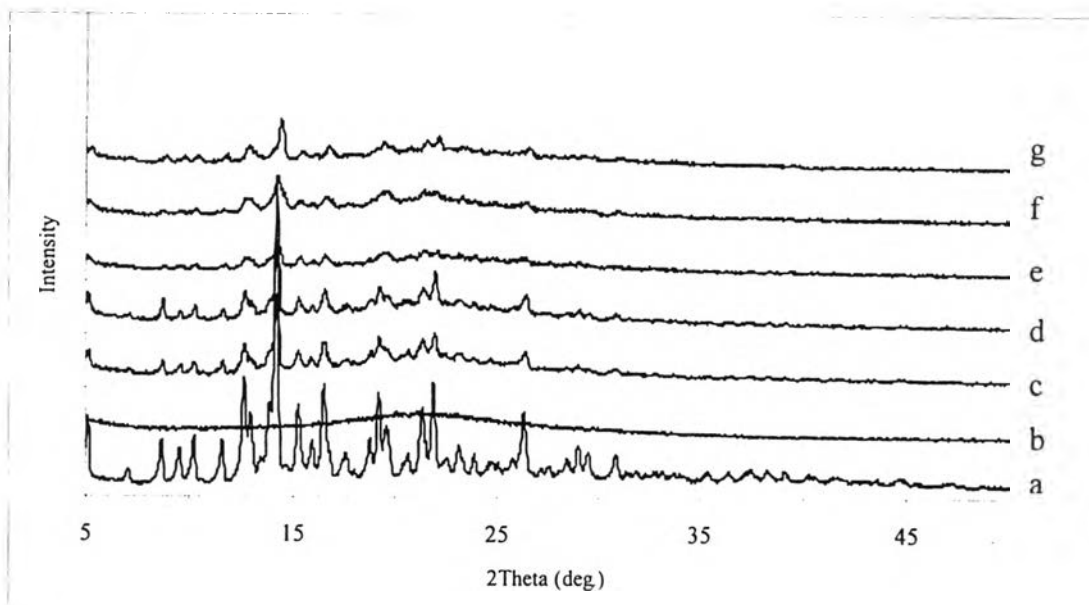


Figure 50 The XRPD patterns of mixture of asiaticoside I with silicon dioxide (a = asiaticoside I , b = silicon dioxide , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

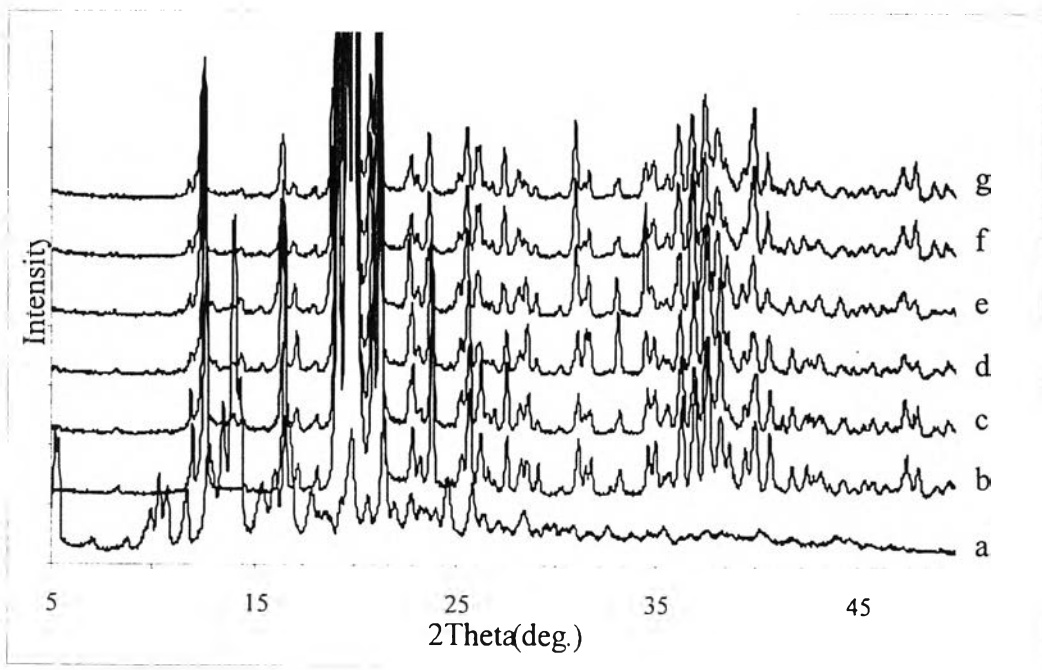


Figure 51 The XRPD patterns of mixture of asiaticoside II with spray-dried lactose (a = asiaticoside II , b = spray-dried lactose , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

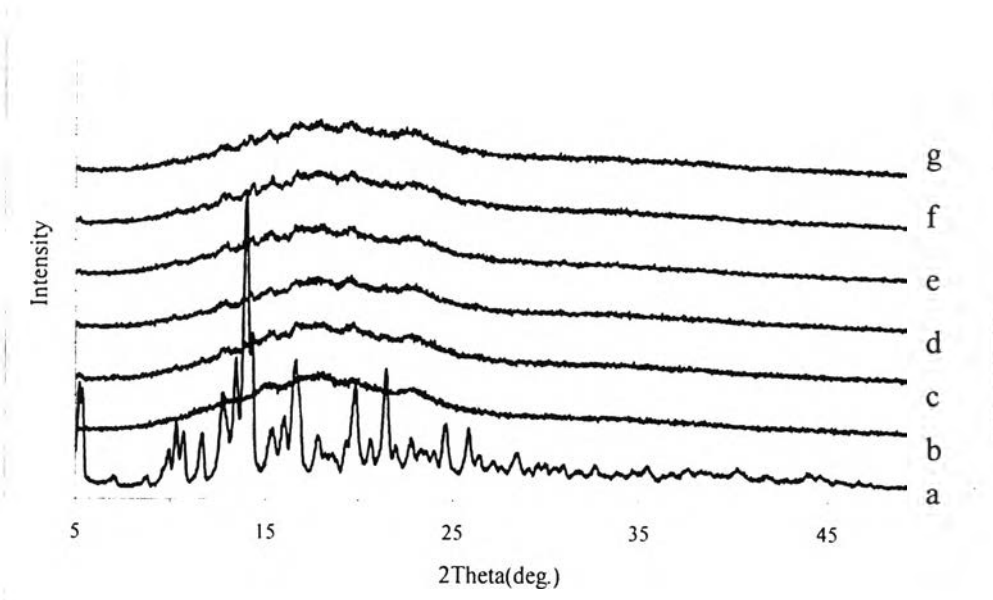


Figure 52 The XRPD patterns of mixture of asiaticoside II with pregellatinized starch (a = asiaticoside II , b = pregelatinized starch , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

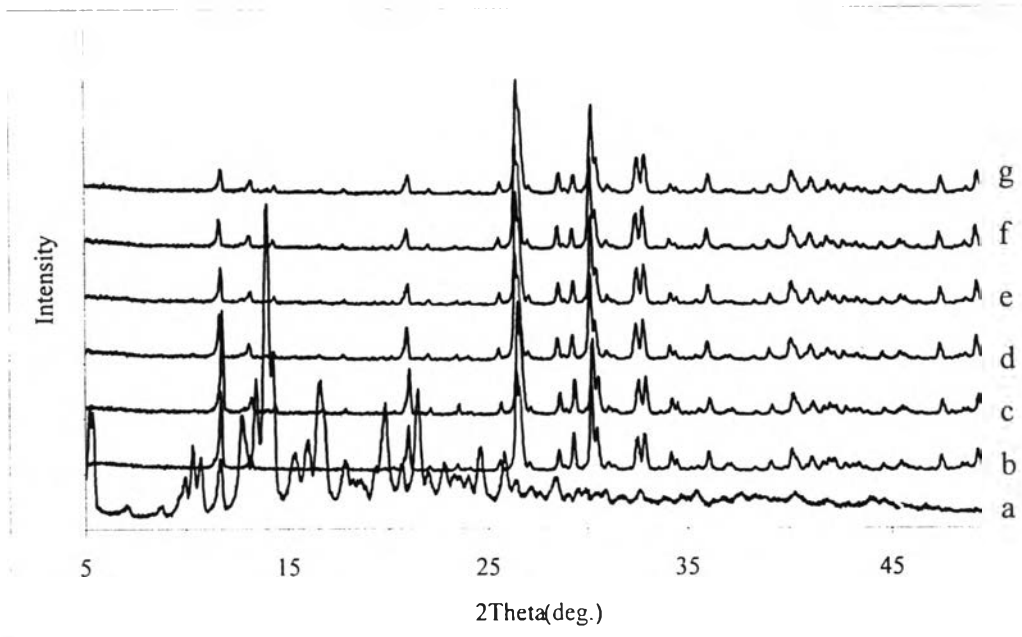


Figure 53 The XRPD patterns of mixture of asiaticoside II with dibasic calcium phosphate (a = asiaticoside II , b = dibasic calcium phosphate , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

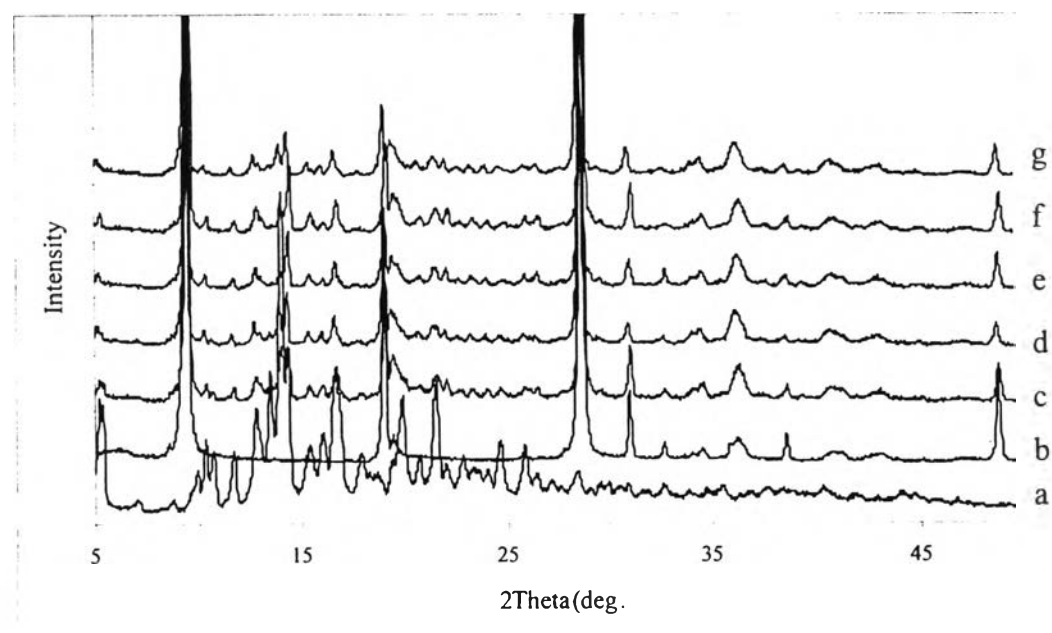


Figure 54 The XRPD patterns of mixture of asiaticoside II with talcum (a = asiaticoside II , b = talcum , c = mixture 0 week , d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

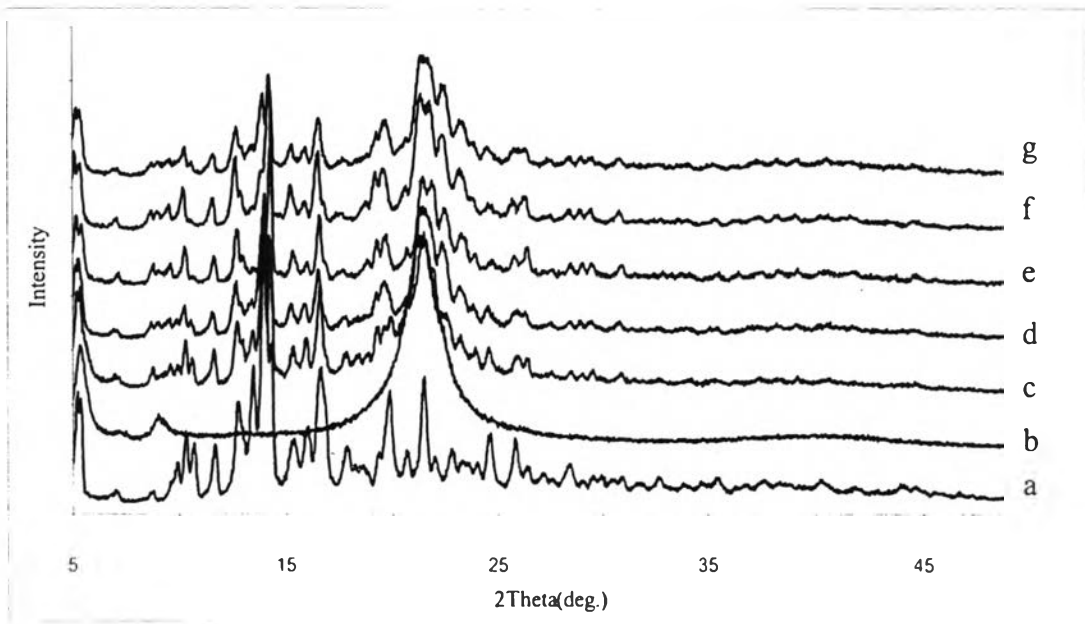


Figure 55 The XRPD patterns of mixture of asiaticoside II with magnesium stearate (a = asiaticoside II, b = magnesium stearate, c = mixture 0 week, d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

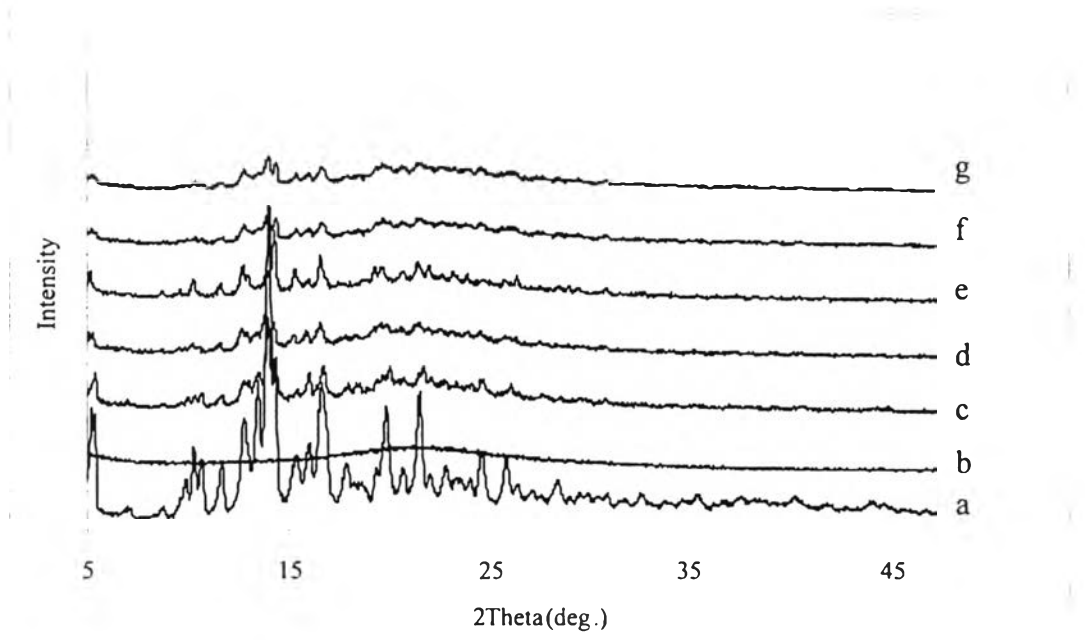


Figure 56 The XRPD patterns of mixture of asiaticoside II with silicon dioxide (a = asiaticoside II , b = silicon dioxide, c = mixture 0 week, d = mixture 2 weeks, e = mixture 4 weeks, f = mixture 6 weeks, g = mixture 8 weeks)

Chemical Stability of Asiaticoside Mixture

The collected asiaticoside mixtures from the compatibility study at different time intervals were assayed by High Pressure Liquid Chromatography. The chromatograms found peak of Asiaticoside at retention time of about 6.0 mins with no interfering peaks.

The percent remaining amount of asiaticoside in each mixtures are shown in Figure 57-62. From the data, the amount of asiaticoside in the mixtures were shown to fluctuate especially in mixtures of asiaticoside with spray dried lactose, pregelatinized starch and dibasic calcium phosphate in the ration of 1:20. This ratio ingredients in the mixture was very difficult to homogeneously mixed together. The percent remaining amount of asiaticoside II in mixture of talcum, magnesium stearate and silicon dioxide seemed to decreased when stored at accelerated condition, 40°C and 75% RH, for 18 weeks. On the other hand, The mixture of asiaticoside I was not found to decreased. This result implied that asiaticoside II was incompatible with excipients chosen and are stable a less extent than asiaticoside I.

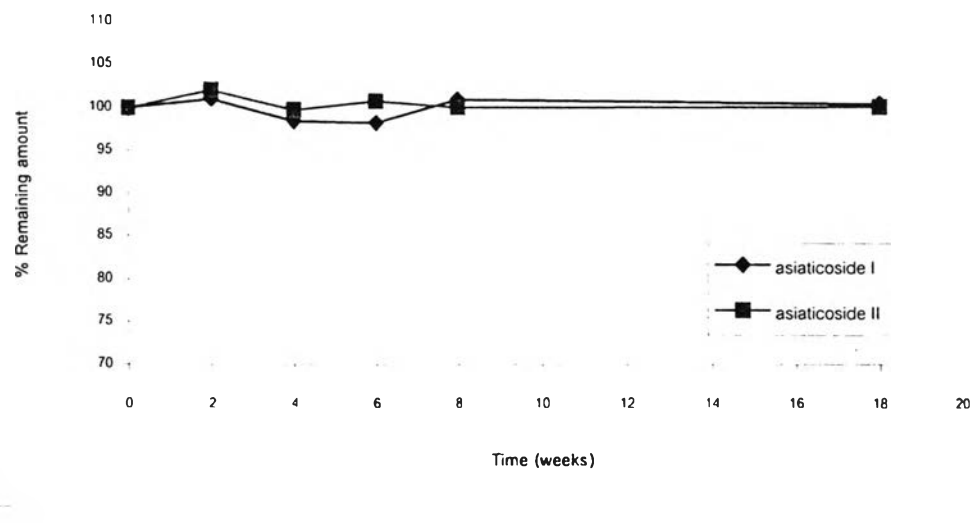


Figure 57 Incompatibility studies showing the percent remaining of mixture asiaticoside and spray dried lactose (1:20)

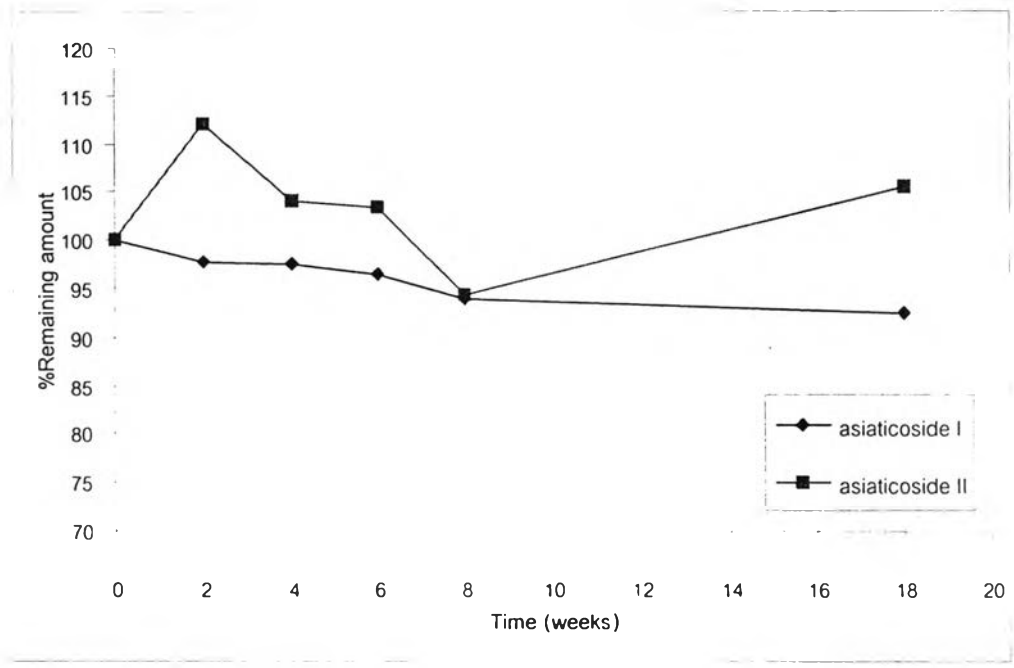


Figure 58 Incompatibility studies showing the percent remaining of mixture asiaticoside and pregelatinized starch (1:20)

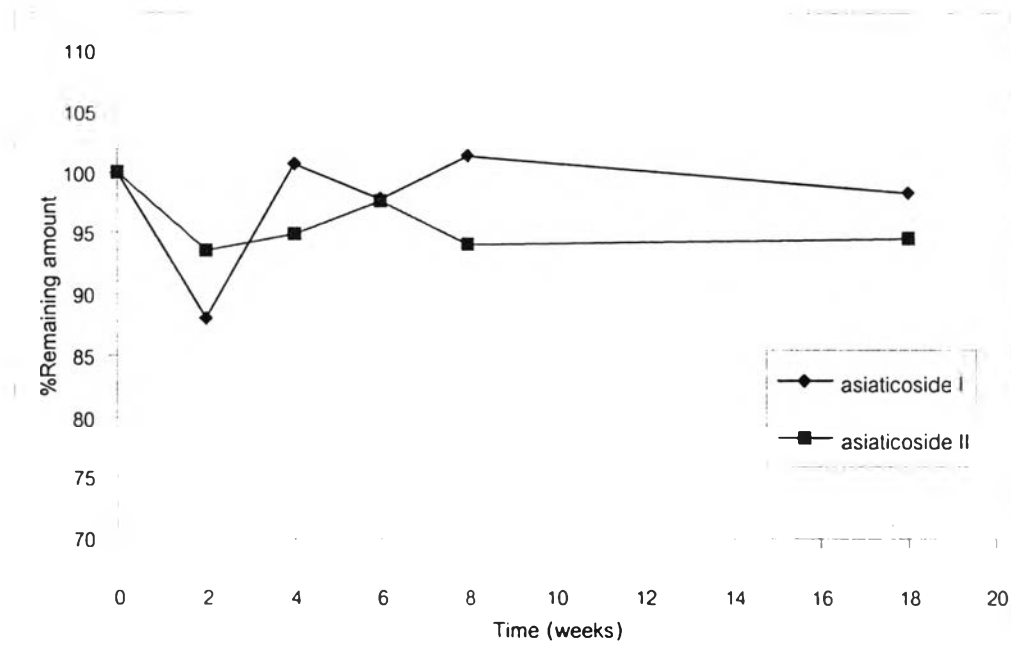


Figure 59 Incompatibility studies showing the percent remaining of mixture asiaticoside and dibasic calcium phosphate (1:20)

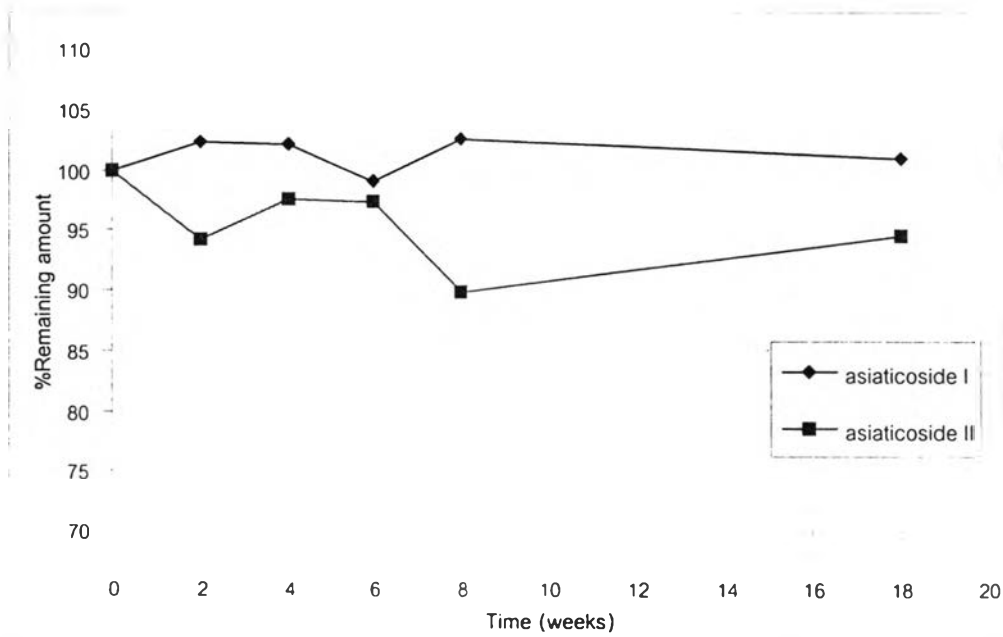


Figure 60 Incompatibility studies showing the percent remaining of mixture asiaticoside and talcum (1:1)

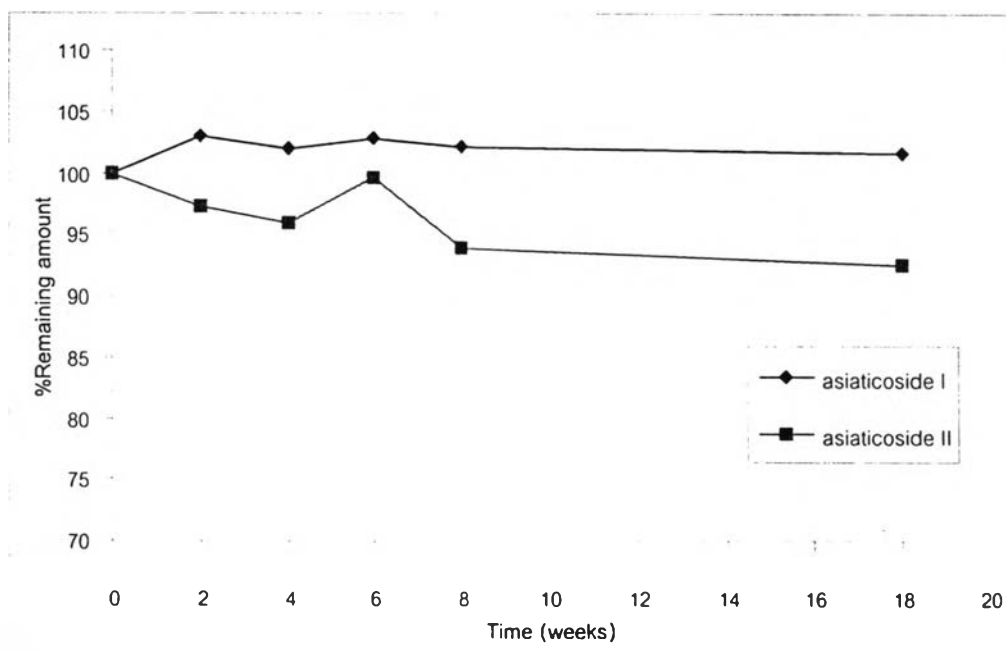


Figure 61 Incompatibility studies showing the percent remaining of mixture asiaticoside and magnesium stearate (1:1)

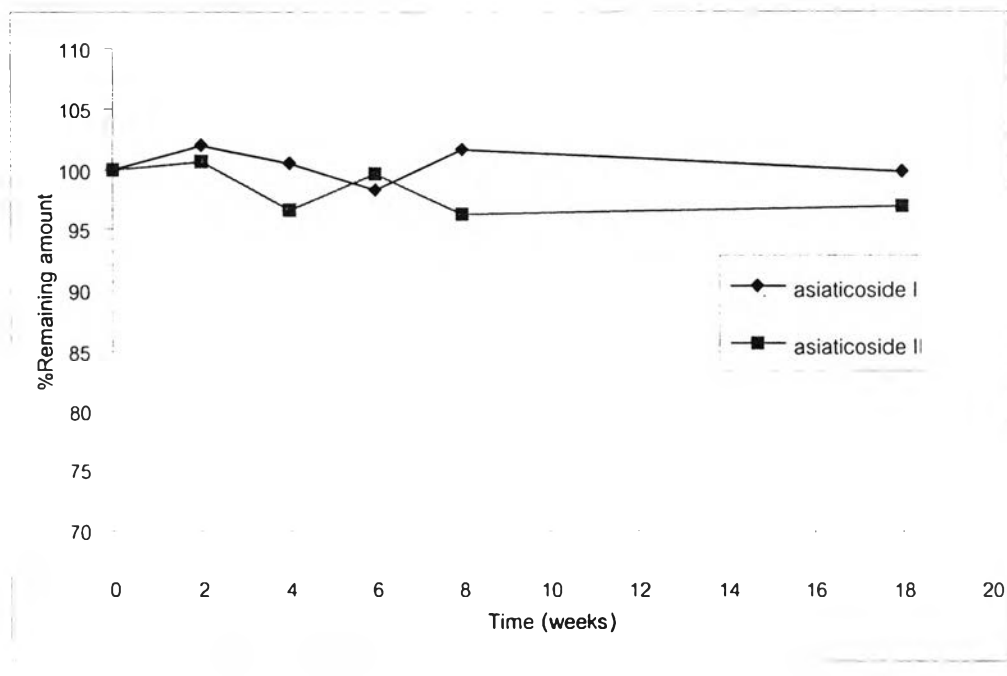


Figure 62 Incompatibility studies showing the percent remaining of mixture asiaticoside and silicon dioxide (1:1)