Charpter III

Results

Standard Curve Determination

The data and typical standard curve for famotidine in 0.1 M phosphate buffer pH 4.5 were presented in Appendix 3 and 4 , respectively. The correlation coefficient of the standard curve was 0.9994.

The HPLC data and typical standard curve of lyophilized famotidine were presented in Appendix 5 and 6 , respectively. Chromatograms of famotidine and its degradation products were exibited in Figure 36. The correlation coefficient of the standard curve was 0.9999.

Selection of Appropriate Carrier System

1. Solubility Study of Physical Mixture.

Solubility studies were carried out on famotidine - carrier physical mixtures by adding a constant weight of famotidine powder to 10 ml water in 20 ml screw capped test tube. Before mounted on a top-to-bottom shaker, duplicate of test tubes were added with 40 mg of each type of carrier. Various carriers used were : PVP 12 PF, PVP 17 Pf, PEG 6000, xylitol, mannitol, glucose, sorbitol and mixed carrier systems. After 1 hour of equilibration at 37 $\pm$ 1 ° C, each test tube

was eye observed , any carrier system that could completely dissolved the drug was noted. The amount of carrier was gradually added to each test tube until clear solution was observed, and the drug carrier ratio was presented in Table 3 .

Table 3 Ratios of famotidine-carriers used when physically mixed in 10 ml water which completely dissolved the drug at 37 ± ° 0.5 C.

Drug carrier ratio *	
Famotidine-PVP 12 PF	1:30
Famotidine-PVP 17 PF	1:30
Famotidine-PEG 6000	1:22.5
Famotidine-Xylitol	>1:50
Famotidine-Mannitol	>1:50
Famotidine-Glucose	>1:50
Famotidine-Sorbitol	>1:50
Famotidine-PVP 12 PF-PEG 6000	1:12.5:12.5
Famotidine-PVP 17 PF-PEG 6000	1:12.5:12.5
Famotidine-PVP 17 PF-Mannitol	1:25:57.5
Famotidine-PVP 17PF-Glucose	1:25:30
Famotidine-PVP 17PF-Sorbitol	1:25:30
Famotidine-PVP 17PF-Xylitol	1:25:45
Famotidine-PVP 12PF-PVP 17 PF-PEG 6000	1:8.75:8.75:8.75

* average from two values (n=2)

The physical mixtures of famotidine with polymer carriers produced little effect on increasing the drug solubility. The ratiios of famotidine:PVP 12 PF , famotidine:PVP 17 PF and PEG 6000 were 1:30 , 1:30 and 1:22.5 , respectively . Both PVP 12 PF and PVP 17 PF demonstrated the same effect on the solubility of the drug.

The famotidine physically mixed with sugar carrier - xylitol , mannitol , glucose and sorbitol were fail to increase the solubility of the drug. All the drug-carrier ratios were used more than 1:50.

Combination of polymer and sugar carriers potentiated very slightly effect on increasing the solubility in the physical mixtures.

2. The Preparation of Solid Dispersions.

2.1 Solvent Method

The famotidine - solid dispersions prepared by solvent method were extremely difficult to find appropiate solvent system. As can be seen from Appendix 7 , famotidine can most highly soluble in dimethyl formamide (568 mg/ml). Unfortunately, there is no apparatus provided in the laboratory that can eliminate the solvent residual which is very hazardous to the body.

Glacial acetic acid is the second solvent that can most dissolved famotidine (498 mg / ml). However, almost all the preparations spent more than 7 days to be dried under the vacuum system. Besides, the famotidine-glucose solid dispersion was very moisten and sticky. (see Table 4)

Table 4 Duration used to obtain dry solid dispersions when prepared by solvent and fusion method.

Carrier	Method	
	Solvent *	Fusion
Mannitol	>1wk	immediately
Glucose	sticky	hygroscopic
Sorbitol	>1wk	1 day
Xylitol	>1wk	3 days
PEG 6000	>1wk	7 days
Citric acid anhydrous	viscous	viscous
PVP 12 PF	>1wk	wax-like
50 % Mannitol-50 % PEG 6000	>1wk	>1wk
50 % Mannitol-50 % PVP12 PF	>1wk	>1wk

* least amount of glacial acetic acid

2.2 Fusion Method

The discoloration of famotidine-PVP system was clearly observed before the melting occured. This can be attributed to the high melting point of PVP. At the temperature about 170 °C, the grey-brown mass was abtained.

After heating famotidine-PVP 12 PF physical mixture to about 168-169 ° C produced the pale-yellow melt with suspended particles. After cooling , the wax-like pale-yellow mass was abtained.

The famotidine-glucose physical mixture were melted at about 100 ° C and the yellow to brown masses were obtained. The dispersions absorpted moisture very quickly, and spent more than 7 days to be completely dried.

The mixture of famotidine-citric acid was obviously become viscous after a few minute heat. This made it more difficult for the obtained preparation to dry. Combinations of mannitol-PEG 6000 and mannitol-PVP 12 PF were also studied. Mannitol was molten out on the surface of solidified polymers. The preparations took more than a week to dry.

The physical mixture of famotidine and mannitol was molten at about 160 ° C and immediately dried after the preparation. Solid dispersions of famotidine-xylitol and famotidine-sorbitol were obtained after heating at the temperature close to 100 °C. The famotidine-sorbitol solid dispersions were white , while the famotidine-xylitol solid dispersions were glass-like transparent , and brittle. Famotidine-sorbitol molted dried within 1 day , while the xylitol system took a few more days to solidify.

3. Assay for Content of Famotidine in Dispersion Sytems

Typical calibration curves of famotidine in 0.1 M phosphate buffer pH 4.5 as determined using linear regression was presented in Appendix 4 .

The percentage content of famotidine in each dispersiion system obtained are shown in Table 5 . The famotidine content was between 93.58-104.12 % .

Table 5 The percentage content of famotidine in solid dispersion and physical mixture systems.

System	Percentage content *	
	Solid Dispersion	Physical mixture
1:1 Famotidine-Mannitol	93.58	101.34
1:2 Famotidine-Mannitol	94.67	99.31
1:5 Famotidine-Mannitol	96.58	99.87
1:10 Famotidine-Mannitol	95.43	103.31
1:20 Famotidine-Mannitol	94.15	102.11
1:30 Famotidine-Mannitol	95.53	98.81
1:40 Famotidine-Mannitol	93.97	100.02
1:1 Famotidine-Sorbitol	98.83	102.95
1:2 Famotidine-Sorbitol	97.05	97.99
1:5 Famotidine-Sorbitol	99.17	104.12
1:10 Famotidine-Sorbitol	96.43	101.29
1:20 Famotidine-Sorbitol	95.67	98.81
1:30 Famotidine-Sorbitol	98.21	100.14
1:40 Famotidine-Sorbitol	96.92	99.10
1:1 Famotidine-Xylitol	96.44	98.23
1:2 Famotidine-Xylitol	97.09	100.36
1:5 Famotidine-Xylitol	100.68	104.12
1:10 Famotidine-Xylitol	98.65	103.86
1:20 Famotidine-Xylitol	99.09	101.21
1:30 Famotidine-Xylitol	97.21	99.98
1:40 Famotidine-Xylitol	96.23	98.81

* average from three values (n=3)

From above data , the dispersions prepared in mannitol , sorbitol and xylitol by the fusion method were selected for the solubility studies. This was dued to their fine physical appearance , easily to prepare and still possessed high percentage content despite of high temperature when prepared by the fusion method.The results were also confirmed by the HPLC method and no peak of degradation product was observed.

4. Solubility Determination

Solubility data of famotidine in mannitol , sorbitol and xylitol was shown in Appendix 8-10. The solubility curves was presented in Figure 9-12. The solubility of famotidine in water was 1.1 mg/ml. Famotidine-mannitol dispersion system had the highest solubility followed by sorbitol and, finally xylitol. The solid dispersion of 1:10 famotidine with mannitol , sorbitol and xylitol could markedly increase the solubility about 260 %, 54 % and 25 % of the pure drug , respectively.

5. Physicochemical Properties Studies

5.1 Morphology of Powder

Photomicrographs of pure drug , carriers and all types of 1:10 dispersion systems (physical mixtures and solid dispersions), were presented in Figure 13 -15 with magnifications X 350. The general shape and surface topography could be observed.

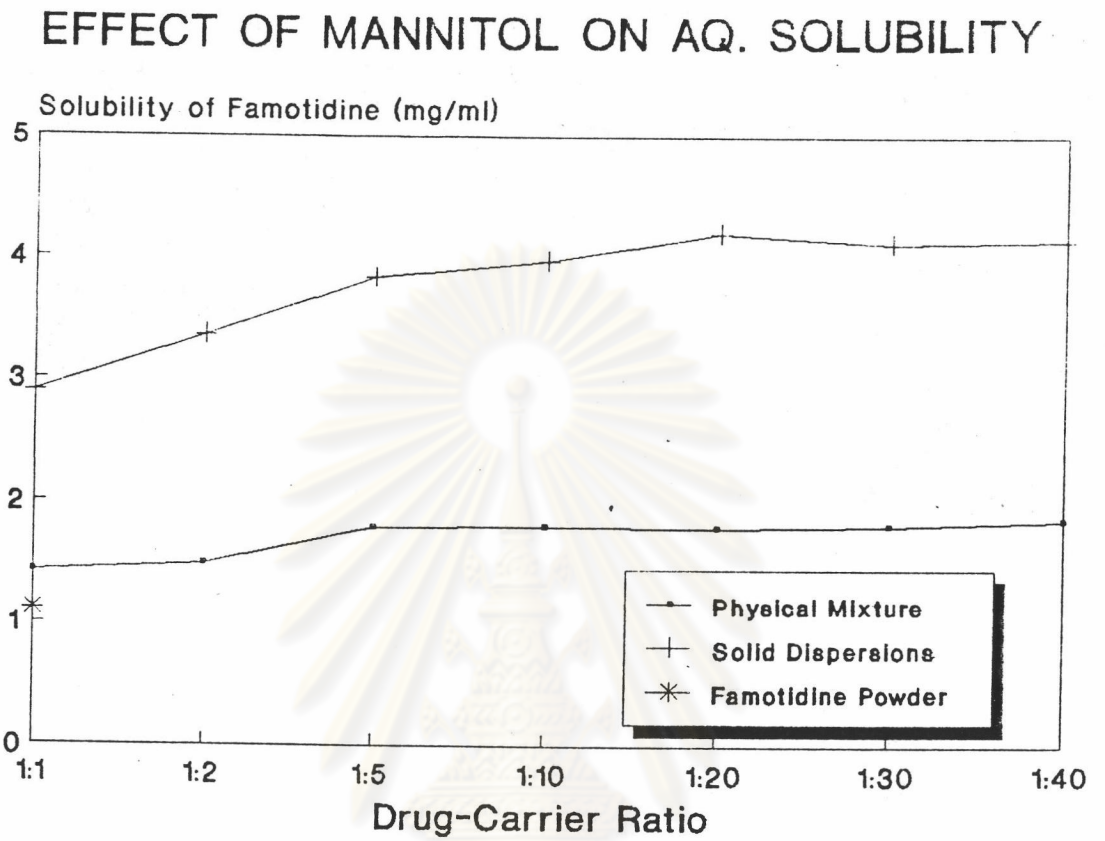


Figure 9 The aqueous equilibrium solubilities of famotidine from powder of pure famotidine, famotidine-mannitol physical mixture and solid dispersions at 37 ± 0.5° C.

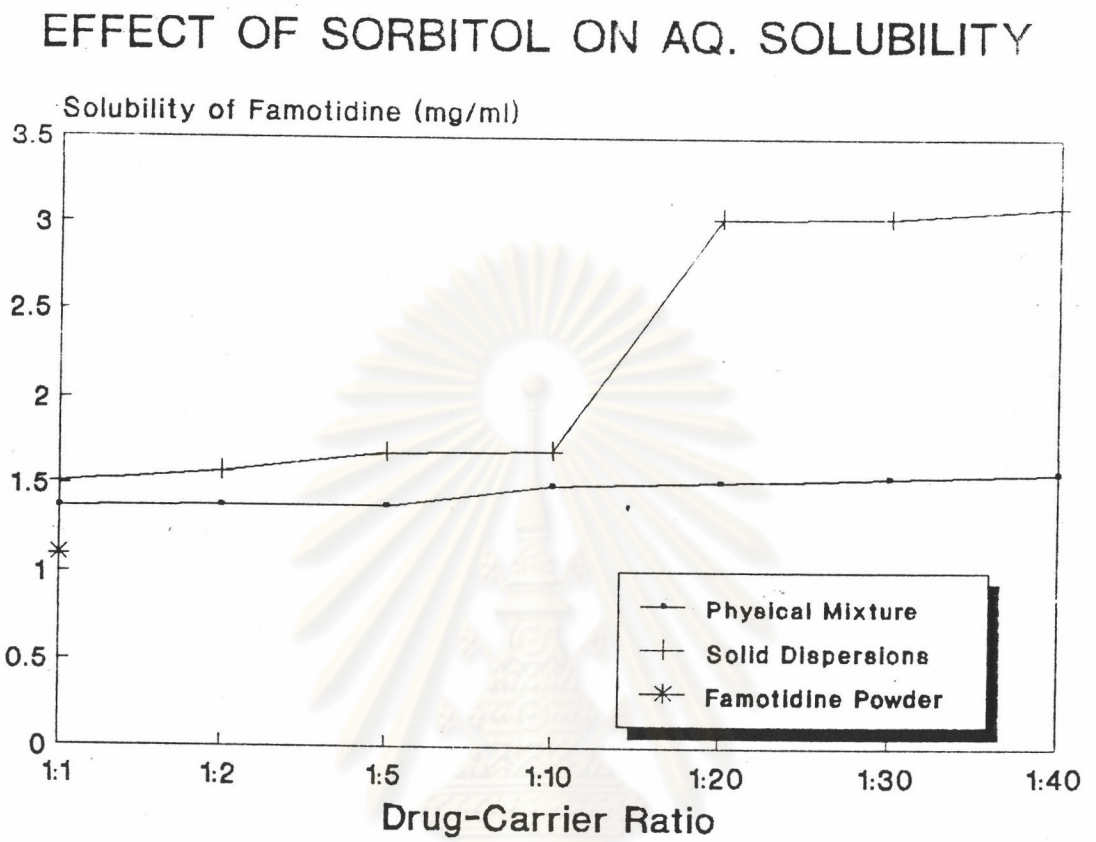


Figure 10 The aqu eous equilibrium solubilities of famotidine from powder of pure famotidine, famotidine-sorbitol physical mixture and solid dispersions at 37 ± 0.5° C.

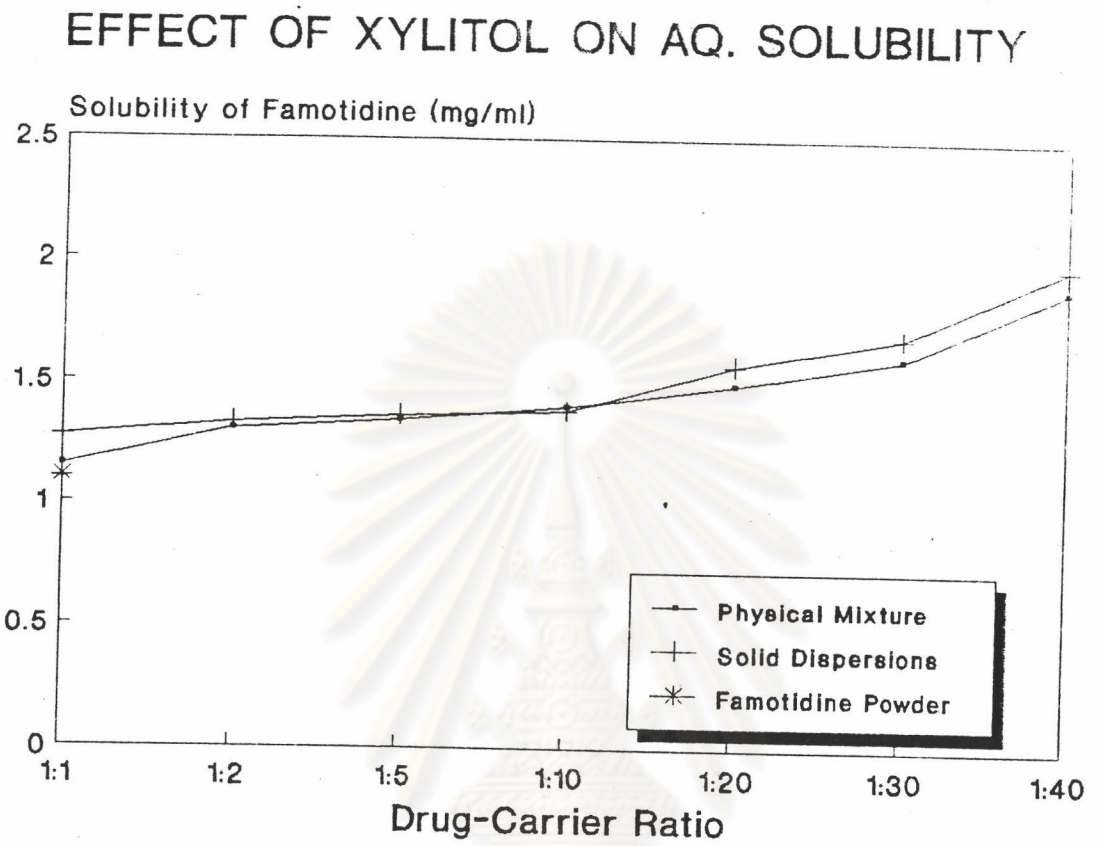


Figure 11 The aqueous equilibrium solubilities of famotidine from powder of pure famotidine, famotidine-xylitol physical mixture and solid dispersions at 37 ± 0.5° C.

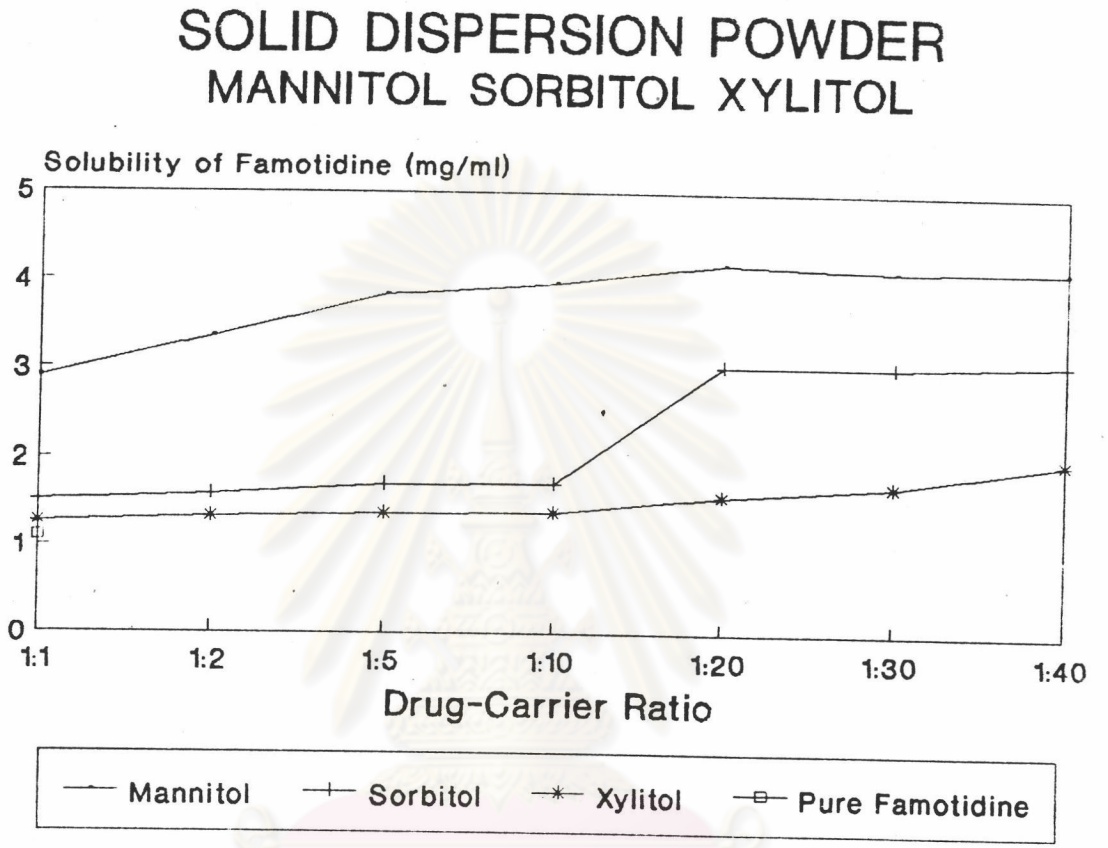
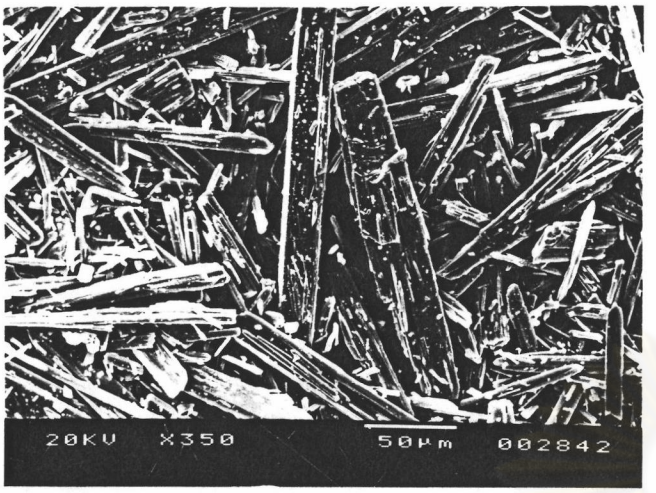
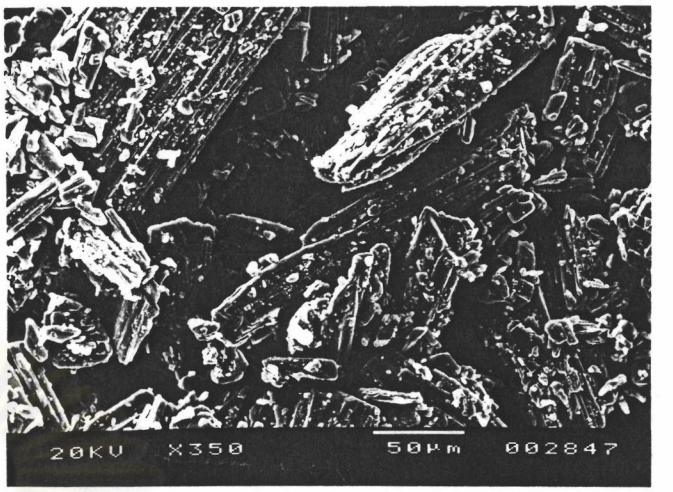


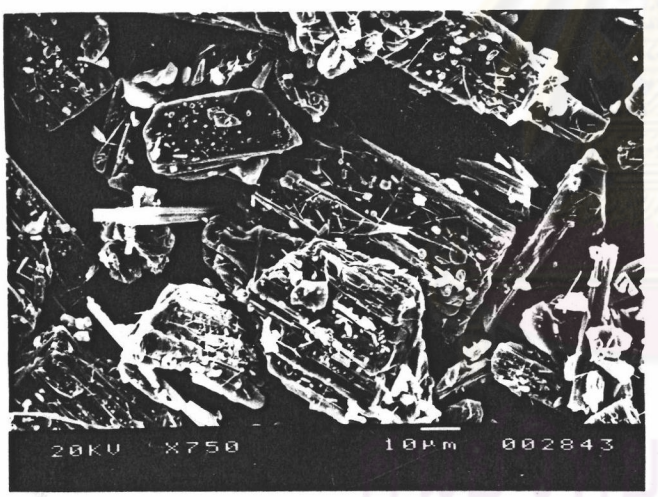
Figure 12 Comparative aqueous equlibrium solubilities of famotidine from different solid dispersion ratios using various types of sugar carriers.



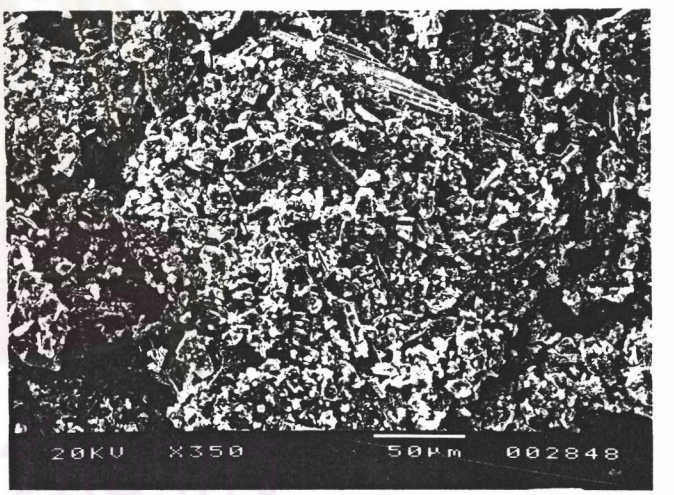
A



B



C



D

Figure13 SEM photomicrographs of pure famotidine (A);mannitol(B); 1:10 famotidine-mannitol physical mixture (C); and 1:10 famotidine-mannitol solid dispersions (D) in magnifications x350.

5.1.1 Pure Famotidine Powder

The microscopic appearance of pure famotidine powder were shown in Figure 13 - 13 . Pure famotidine powder composed of needle shape in various width, and the surface of powder was smooth.

5.1.2 Pure Mannitol Powder

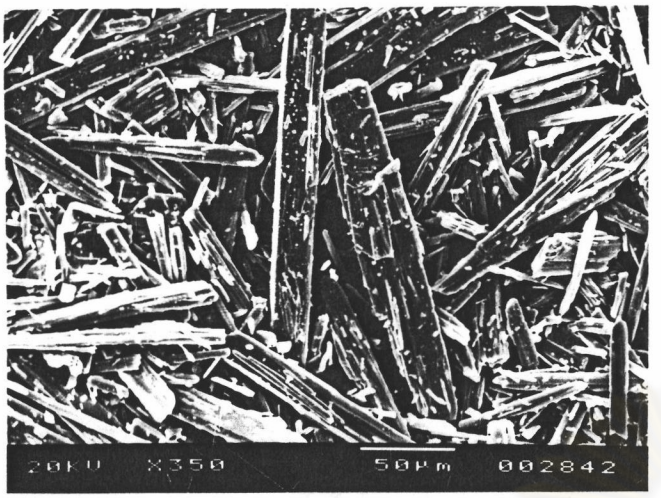
The microscopic image of mannitol powder were depicted in Figure 13 . Mannitol powder composed of irrigular shape particles in various size.

5.1.3 Physical Mixture of 1:10 Famotidine-Mannitol

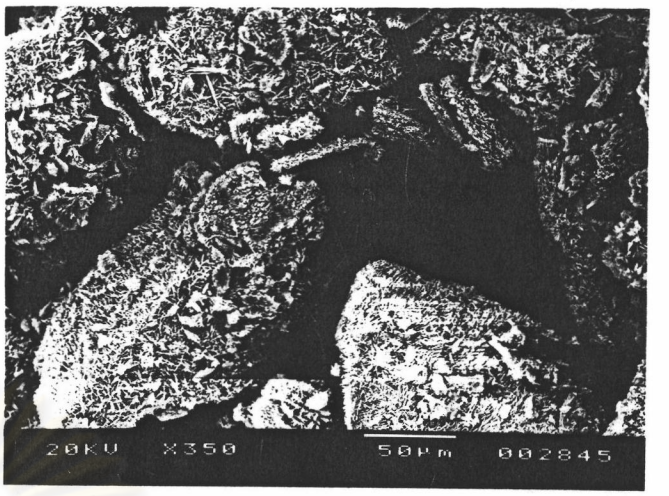
The SEM photomicrography of 1:10 famotidine-mannitol physical mixture was shown in Figure 13. Most needle shape particle of famotidine mostly adhered on the surface of mannitol particles.

5.1.4 Solid Dispersion of 1:10 Famotidine-Mannitol

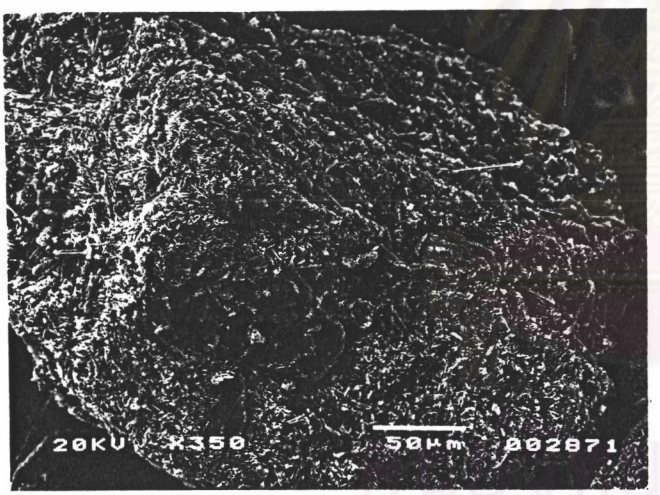
Photomicrograph of 1:10 famotidine - mannitol solid dispersion was exhibited in Figure 13 . The shape of particles seen were almost totally different from pure drug and carrier. It showed fine and irregular shape particles.



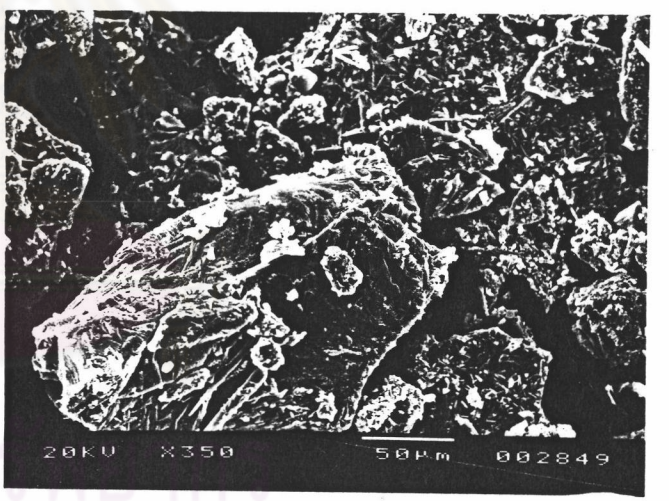
A



B



C



D

Figure 14 SEM photomicrographs of pure famotidine (A);sorbitol(B); 1:10 famotidine-sorbitol physical mixture (C); and 1:10 famotidine-sorbitol solid dispersions (D) in magnifications x350.

5.1.5 Pure Sorbitol Powder

The microscopic appearances of pure sorbitol powder was introduced in Figure 14. Pure sorbitol powder composed of agglomerative fine needle-liked particles.

5.1.6 Physical Mixture of 1:10 Famotidine Sorbitol

The microscopic image of 1:10 famotidine-sorbitol physical mixture was presented in Figure 14 . It presented very fine paticle of sorbitol, included with some needle shaped particles of famotidine sticked on the surface .

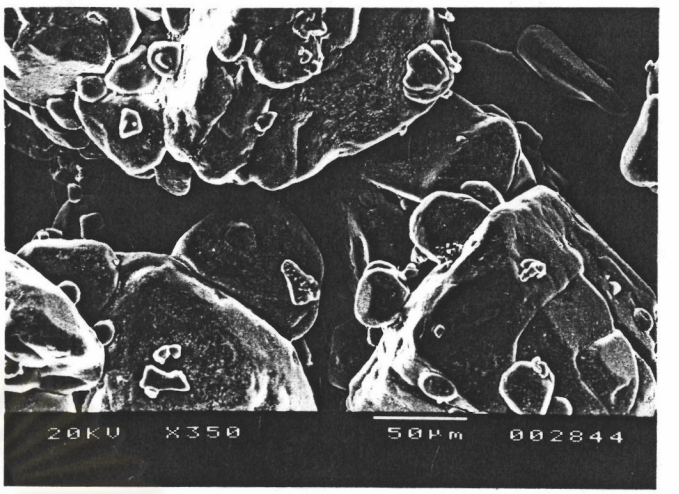
5.1.7 Solid Dispersions of 1:10 Famotidine-Sorbitol

The SEM photomicrograh of 1:10 famotidine-sorbitol solid dispersions was displayed in Figure 14 .Irregular big particles, with rough suface were obtained.

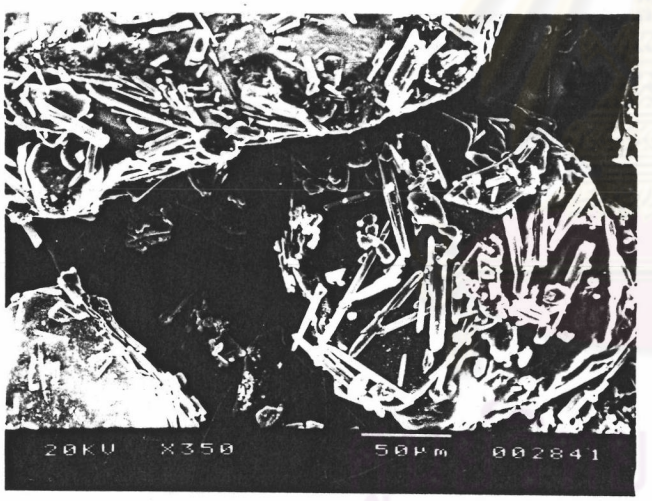
5.1.8 Pure Xylitol Powder

Xylitol powder was shown in Figure 15. The particles constitued of irregular shaped and size with smooth surface.

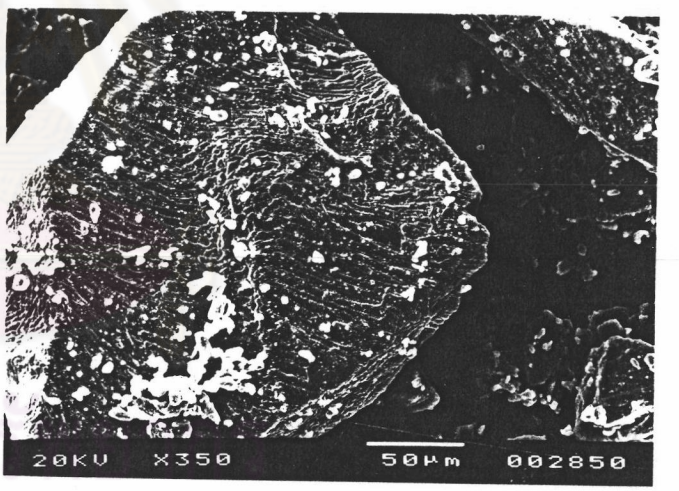
A



B



C



D

Figure 15 SEM photomicrographs of pure famotidine (A);xylitol(B); 1:10 famotidine-xylitol physical mixture (C);and 1:10 famotidine-xylitol solid dispersions (D) in magnifications x350.

5.1.9 Physical Mixture of 1:10 Fmotidine-Xylitol

Famotidine - xylitol systems (Figure 15), consisted of particles in two types of shapes ; spherical and needle-like . The needle-like particles of famotidine were bound to the spherical of xylitol.

5.1.10 Solid Dispersion of 1:10 Famotidine-Xylitol

Photomicrograph of 1:10 famotidine- xylitol solid dispersion was exhibited in Figure 15 . The two components were fused together to the big irregular particles with rough surface.

5.2 Infrared Spectra

The IR spectra of pure famotidine , carriers , physical mixture and solid dispersions , using three types of sugar carriers- mannitol, sorbitol and xylitol were presented in Figure 16 - 18. At the bottom of every IR spectra system , it showed the peak of famotidine. The peaks of sulfonamides appeared at 1325 and 1140 cm^{-1} , resulted from S=O stretching , and the other two peaks at 3500 and 3300 cm^{-1} resulted from N-H stretching. Peaks between 1690-1580 cm^{-1} resulted from C=N stretching. At 3200 , 2900 and 1640 cm^{-1} resulted from =CH stretching, C-H stretching and C=C stretching of thiazole ring, respectively. The second upper from the bottom, were the spectra of sugar carrier. Peak at 3320 cm^{-1} resulted from C-O stretching of the primary alcohol.

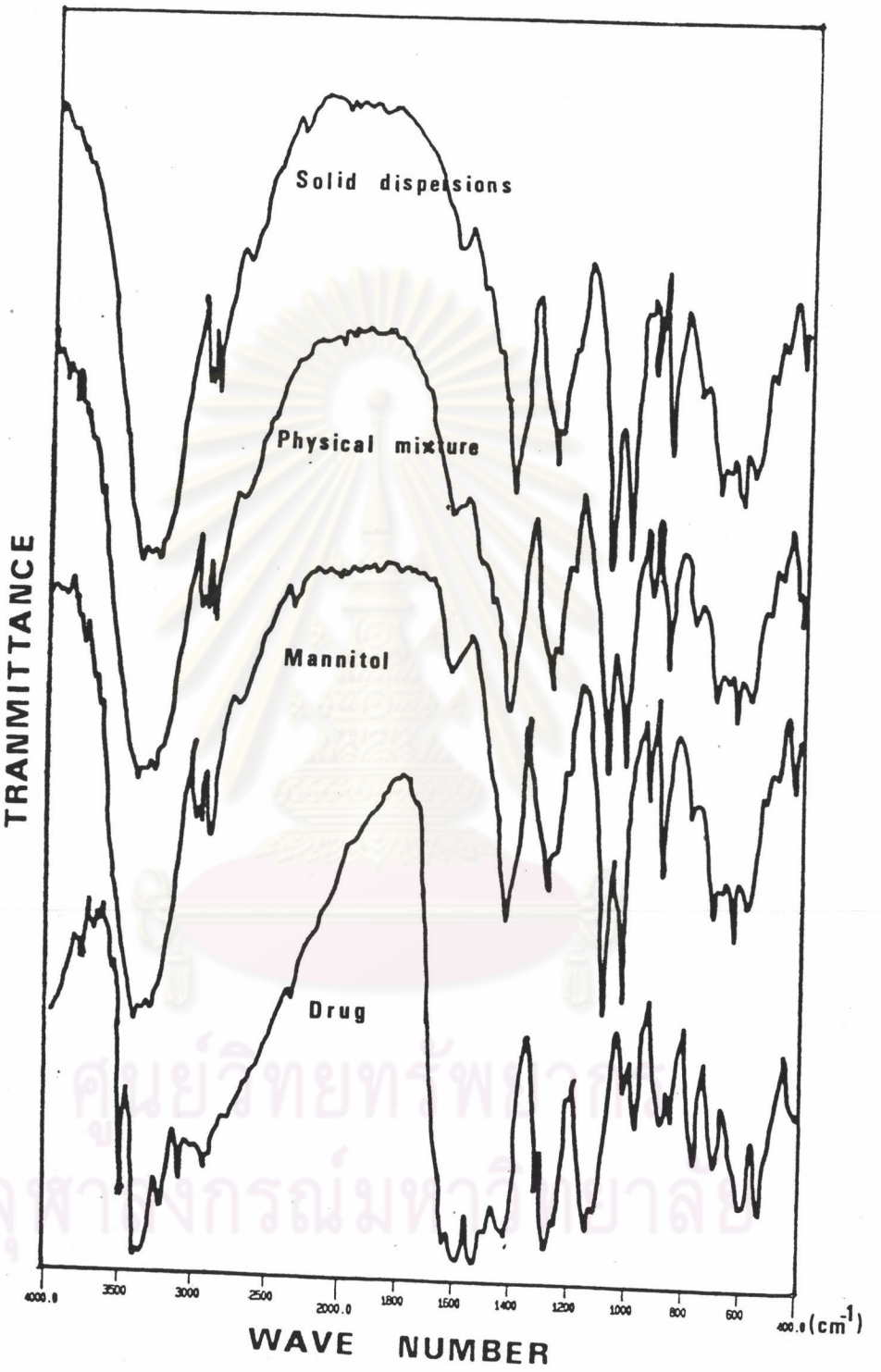


Figure16 IR spectra of pure famotidine; mannitol; 1:10 famotidine-mannitol physical mixture and 1:10 famotidine-mannitol solid dispersions.

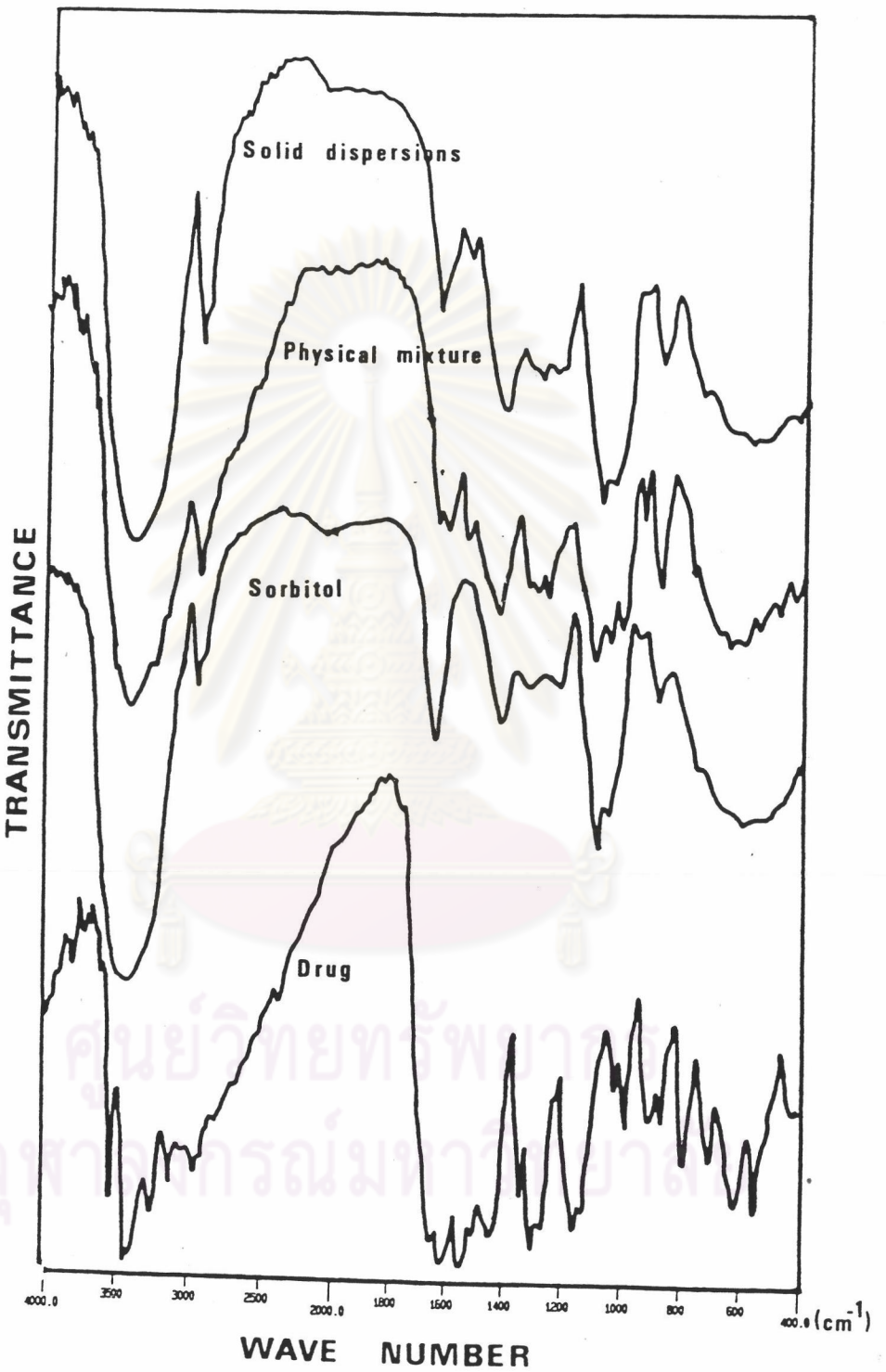


Figure 17 IR spectra of pure famotidine; sorbitol; 1:10 famotidine-sorbitol physical mixture and 1:10 famotidine-sorbitol solid dispersions.

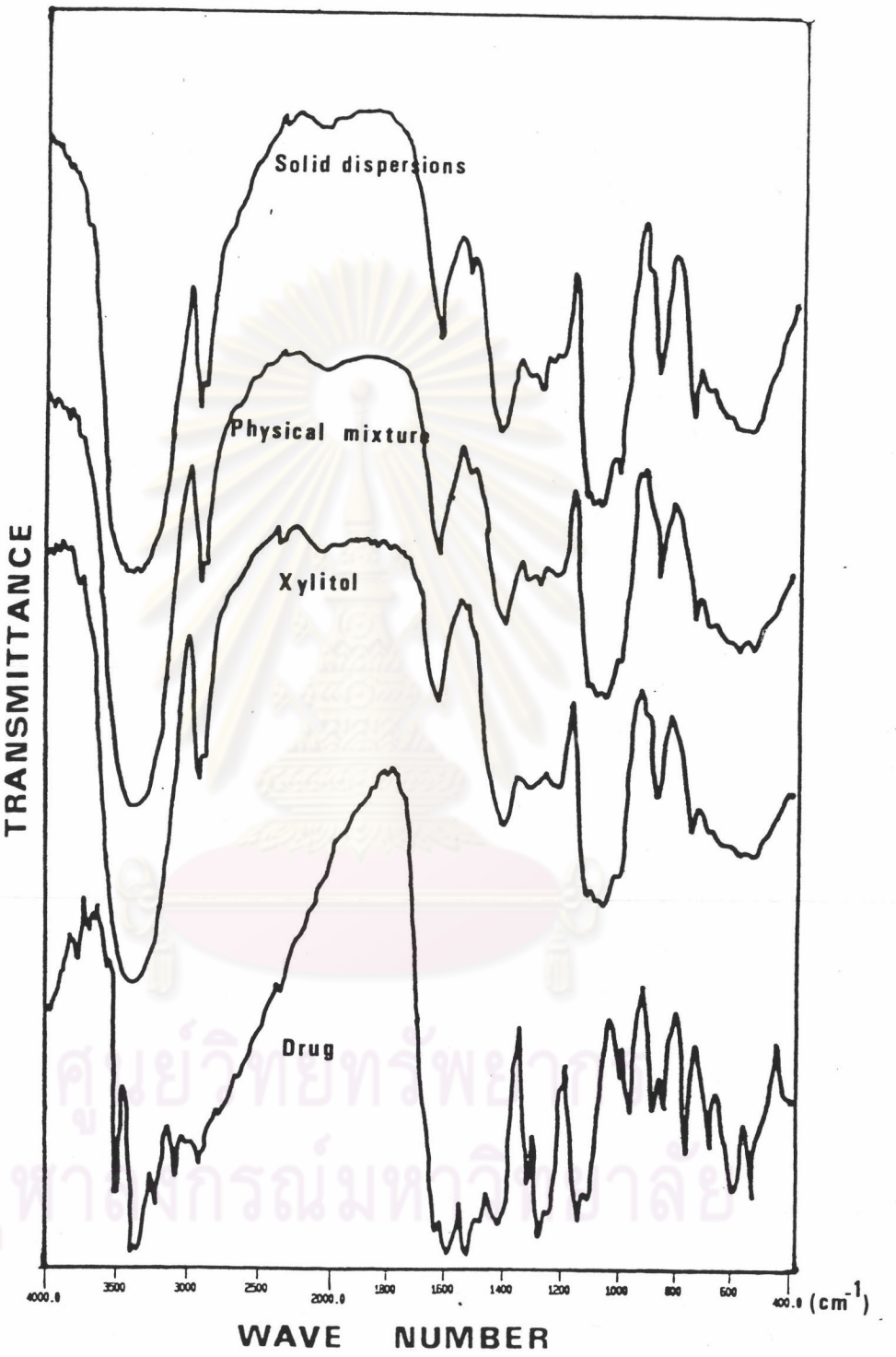


Figure 18 IR spectra of pure famotidine; xylitol; 1:10 famotidine-xylitol physical mixture and 1:10 famotidine-xylitol solid dispersions.

The absorption band characteristics of the physical mixture systems exhibited very similar band to those of the sugar carriers, and some peaks of the drug could also be seen in the system contained famotidine-sorbitol physical mixture. The IR spectra of the solid dispersion still not much presented the main absorption band of the sugar carrier, no peak of the drug could be seen. In the famotidine-sorbitol solid dispersion system , some peaks were weaker or disappear .

5.3 DTA Thermograms

Thermograms of pure famotidine , carrier , physical mixtures and solid dispersions were shown in Figure 19 - 24 . The thermogram of pure drug and mannitol characteristic melting endotherm at 161 °C, while sorbitol and xylitol exhibited the melting endotherm at 100 °C and 98 °C , respectively. Both 1:5 and 1:20 drug-carrier dispersion systems presented very similar endothermic thermogram pattern.

5.3.1 Famotidine-Mannitol

Thermograms of famitidine - mannitol dispersion appeared in Figure 19-20. Both 1:5 and 1:20 famotidine-mannitol physical mixtures combined the features of the thermograms of each component. The solid dispersion system showed only the features of mannitol thermograms.

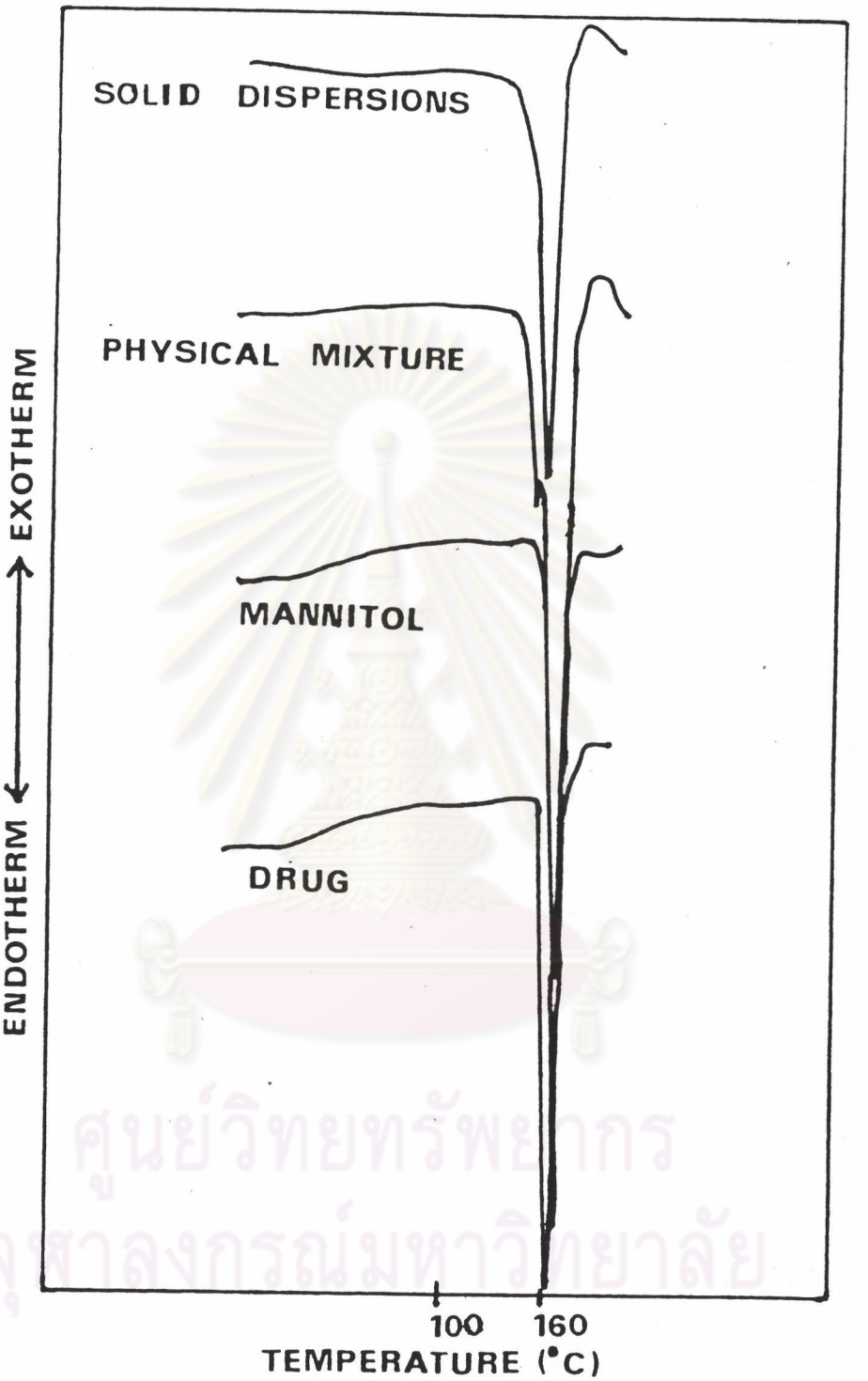


Figure 19 DTA thermograms of pure famotidine ; mannitol ; 1:5 famotidine-mannitol physical mixture and 1:5 famotidine-mannitol solid dispersions.

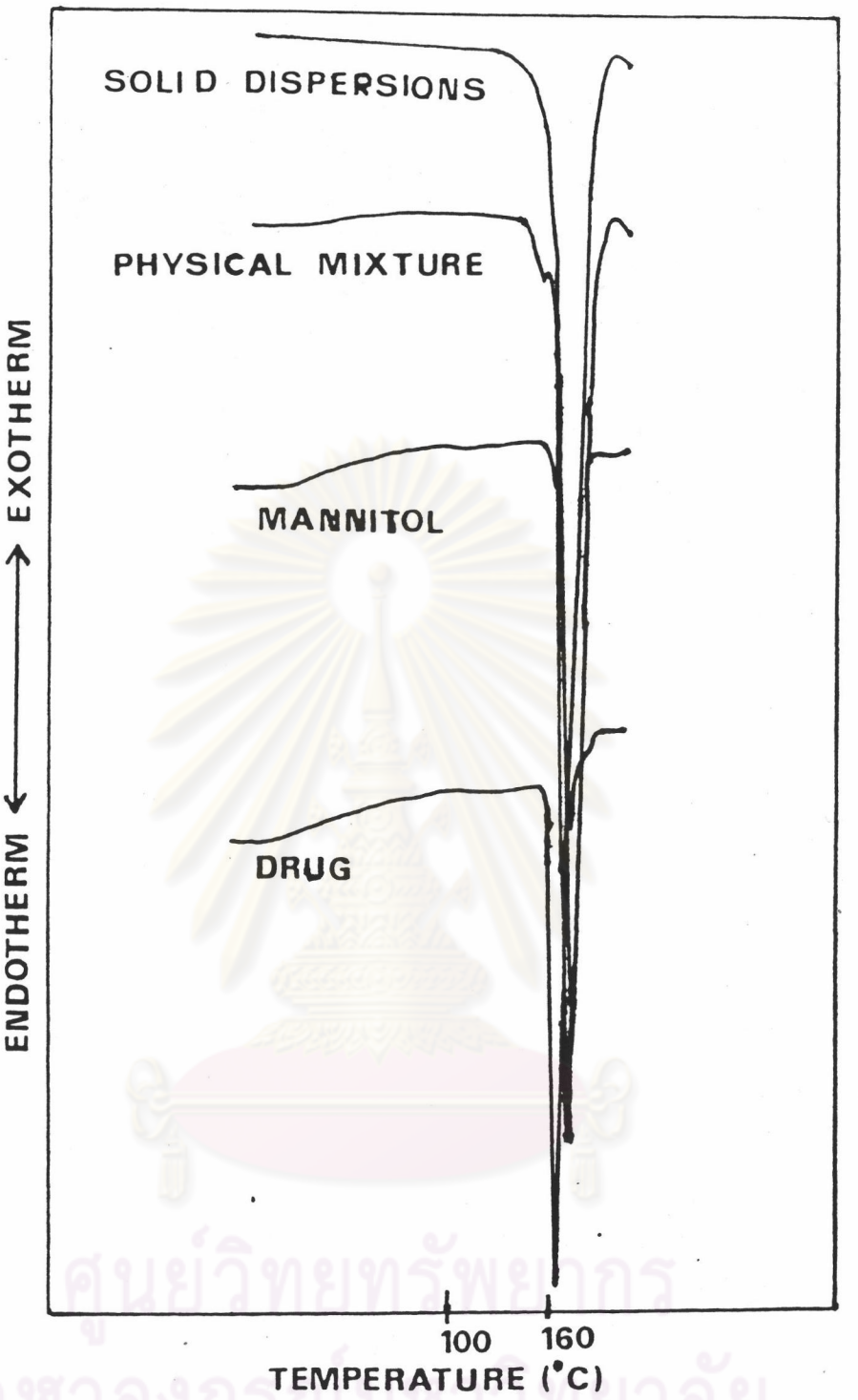


Figure 20 DTA thermograms of pure famotidine ; mannitol ; 1:20 famotidine-mannitol physical mixture and 1:20 famotidine-mannitol solid dispersions.

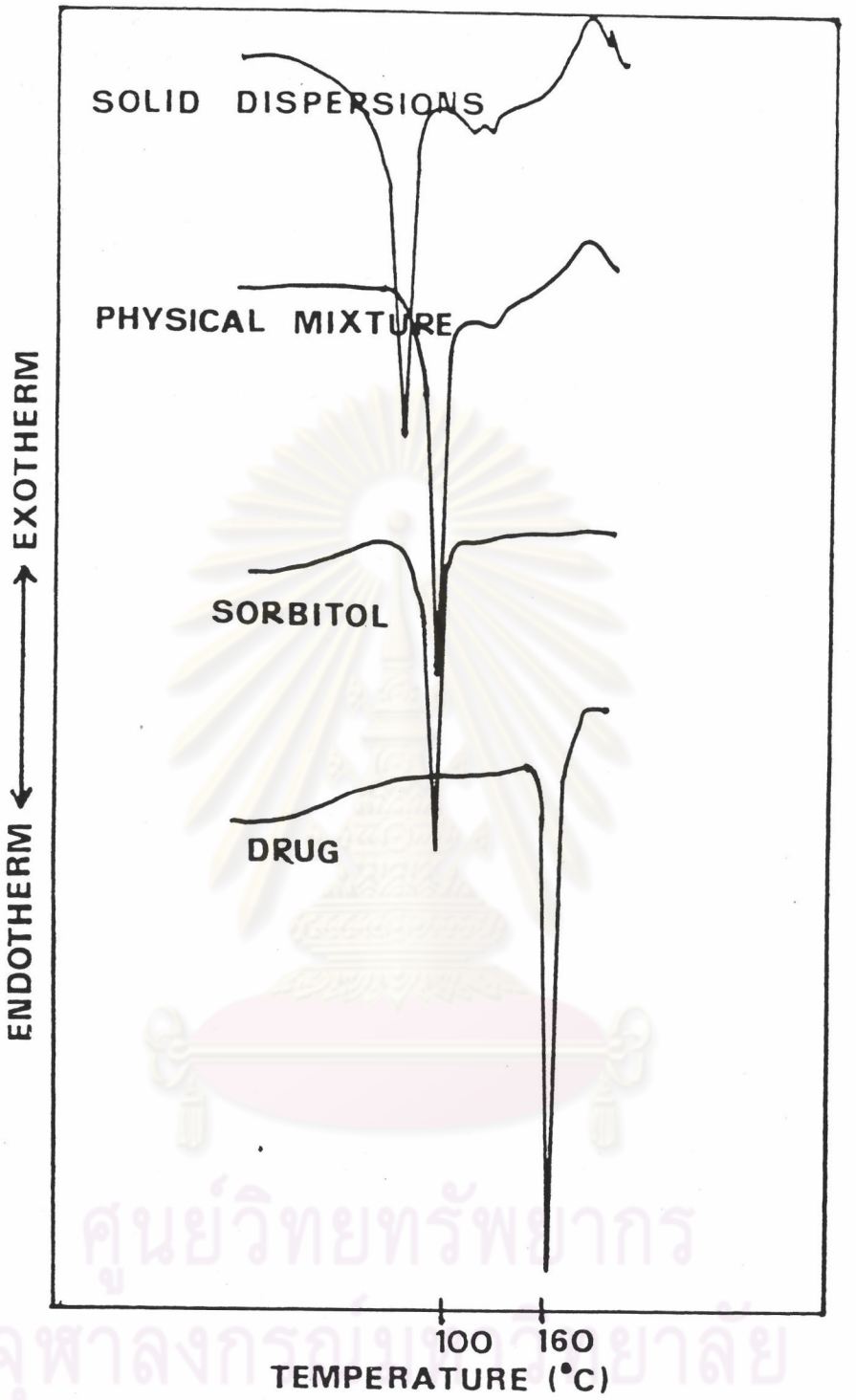


Figure 21 DTA thermograms of pure famotidine ; sorbitol; 1:5 famotidine-sorbitol physical mixture and 1:5 famotidine-sorbitol solid dispersions.

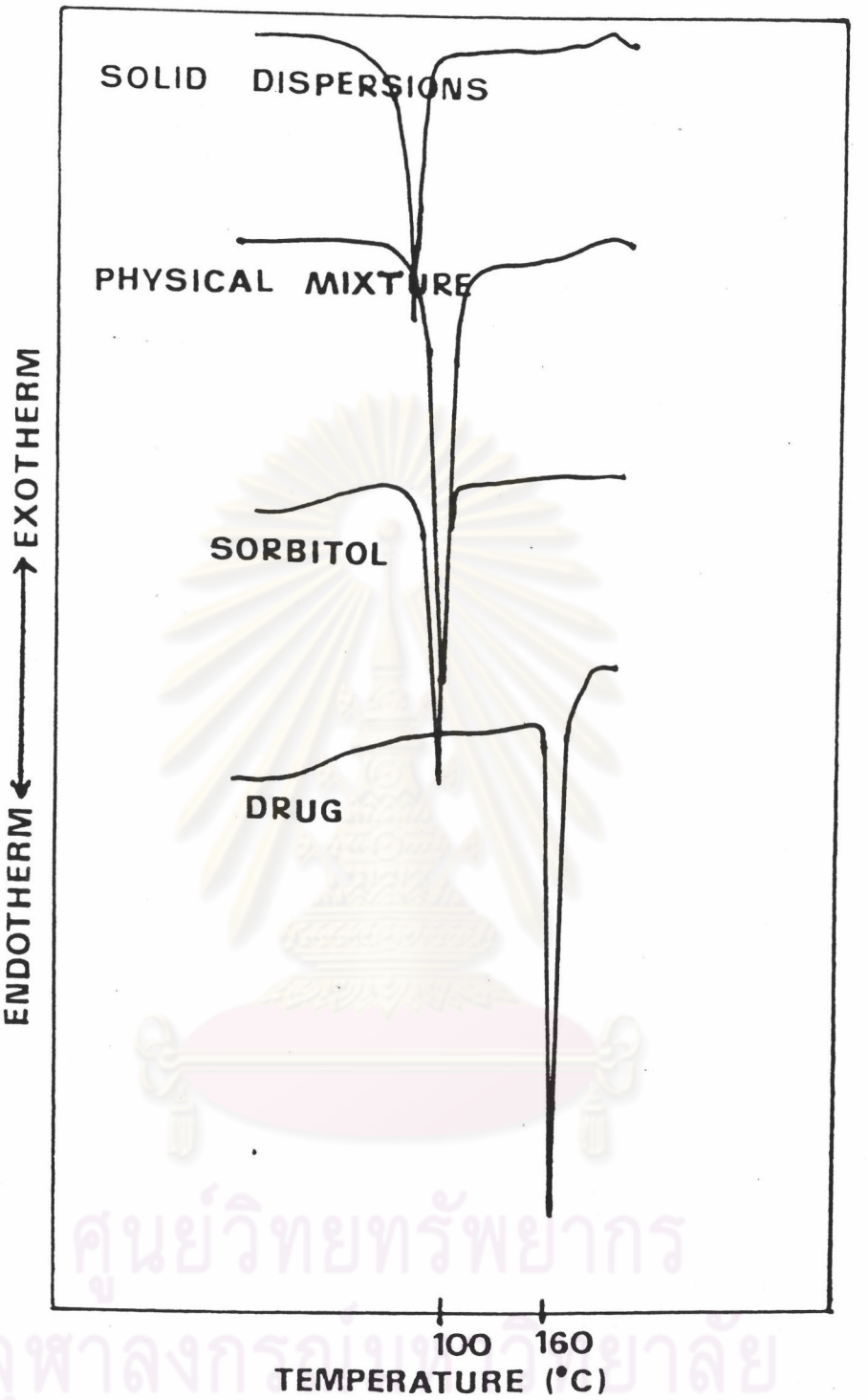


Figure 22 DTA thermograms of pure famotidine ; sorbitol ; 1:20 famotidine-sorbitol physical mixture and 1:20 famotidine-sorbitol solid dispersions.

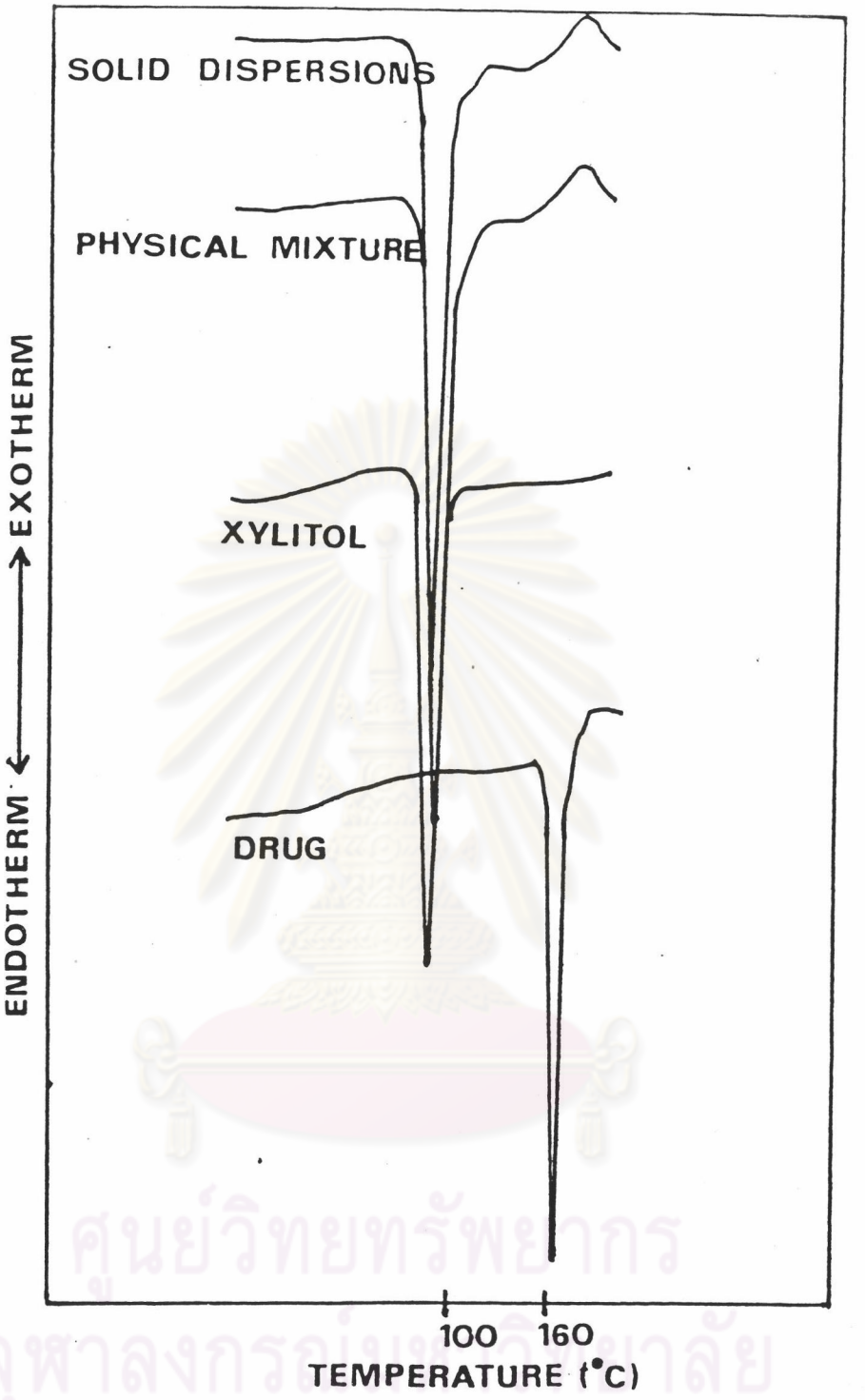


Figure 23 DTA thermograms of pure famotidine ; xylitol ; 1:5 famotidine-xylitol physical mixture and 1:5 famotidine-xylitol solid dispersions.

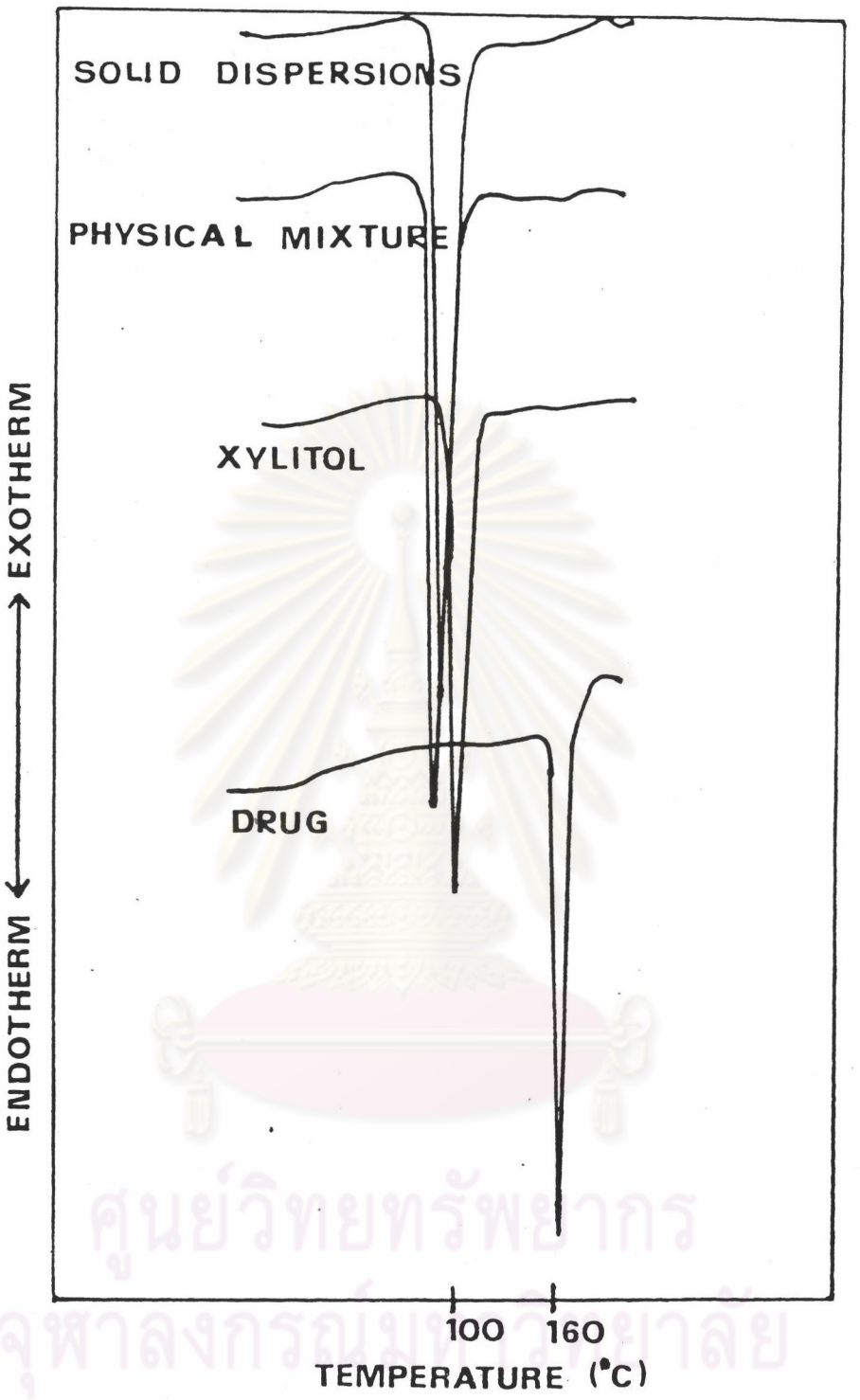


Figure 24 DTA thermograms of pure famotidine ; xylitol ; 1:20 famotidine-xylitol physical mixture and 1:20 famotidine-xylitol solid dispersions.

5.3.2 Famotidine-Sorbitol

Solid dispersion thermograms of 1:5 and 1:20 famotidine-sorbitol (Figure 21-22) , shift from 100 °C to about 90 °C,eventhough the baseline of famotidine endothermic peak was still occurred in 1:5 solid dispersion. The famotidine thermogram was not showed in 1:20 solid dispersion. Thermogram of the physical mixture from 1:5 and 1:20 drug-carrier ratio appeared similar to that of the carrier.

5.3.3 Famotidine-Xylitol

Thermograms of 1:5 famotidine-xylitol physical mixtures and solid dispersion (Figure 23-24) revealed the characteristic melting point of xylitol. Similar thermogram pattern also appeared in 1:20 solid dispersion system.

5.4 X-Ray Diffraction Spectra

X - ray diffraction patterns for pure famotidine ,carriers , physical mixtures and solid dispersions were shown in Figure 25 - 27. Many investigators , such as Hajratwala and Ho (1981) and Simonelli et al. (1969) , have stated that weak diffraction or scattering spectrum indicates the presence of substance in amorphous or extremely fine dispersed form while the diffraction peaks reveal the existence of crystallinity.

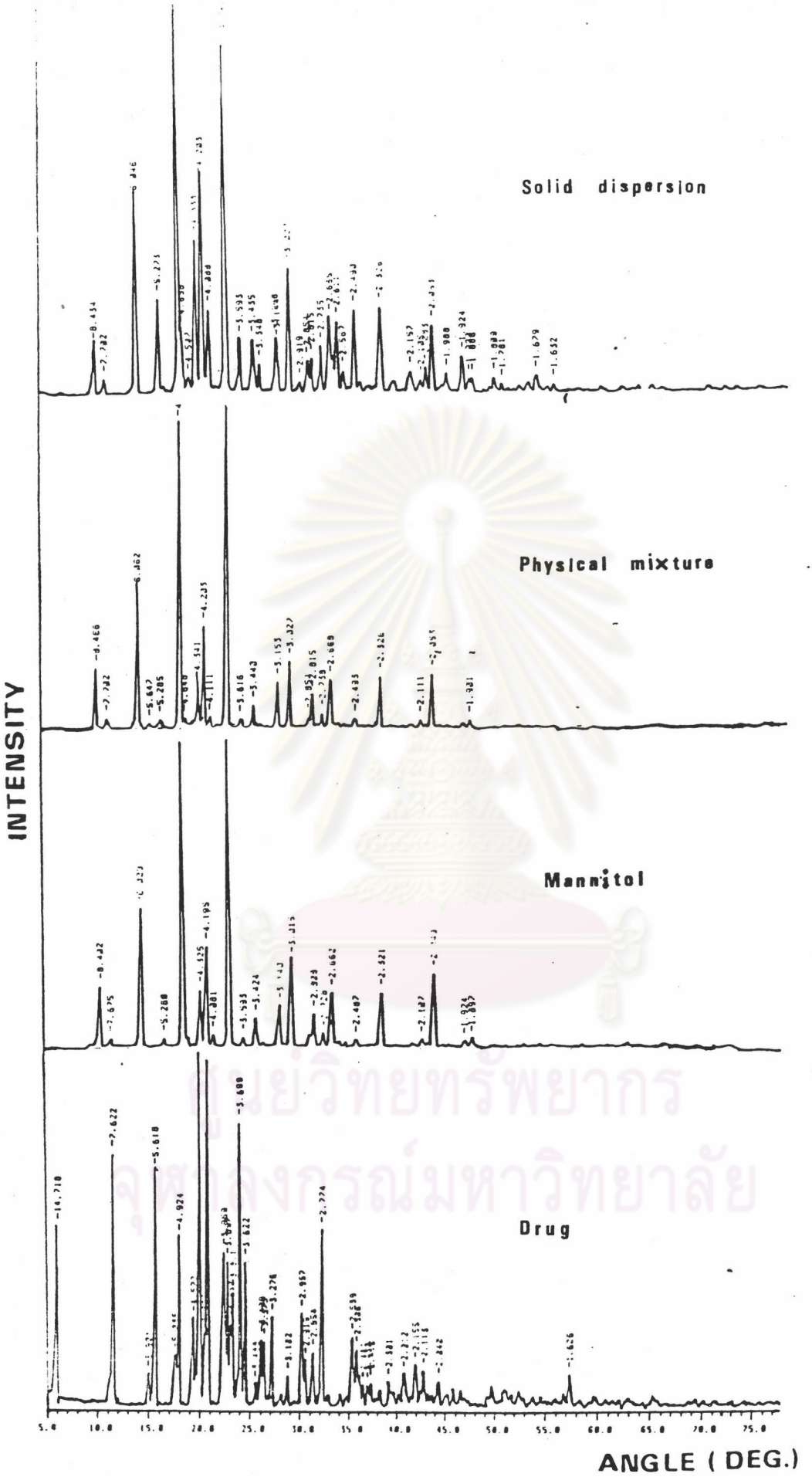


Figure 25 X-ray diffractograms of pure famotidine ; mannitol ; 1:10 famotidine-mannitol physical mixture and 1:10 famotidine-mannitol solid dispersion .

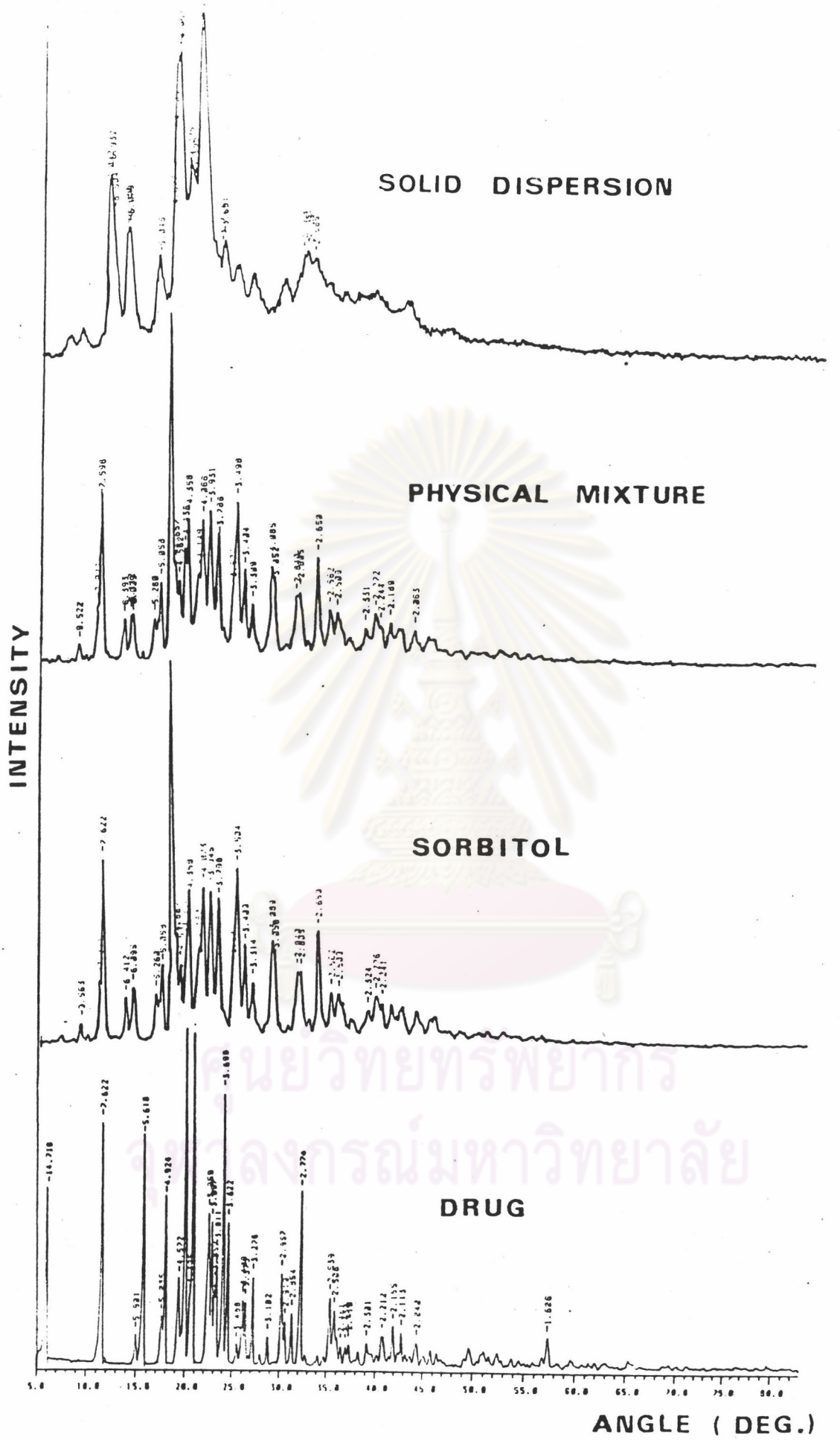


Figure 26 X-ray diffractograms of pure famotidine ; sorbitol ; 1:10 famotidine-sorbitol physical mixture and 1:10 famotidine-sorbitol solid dispersion.

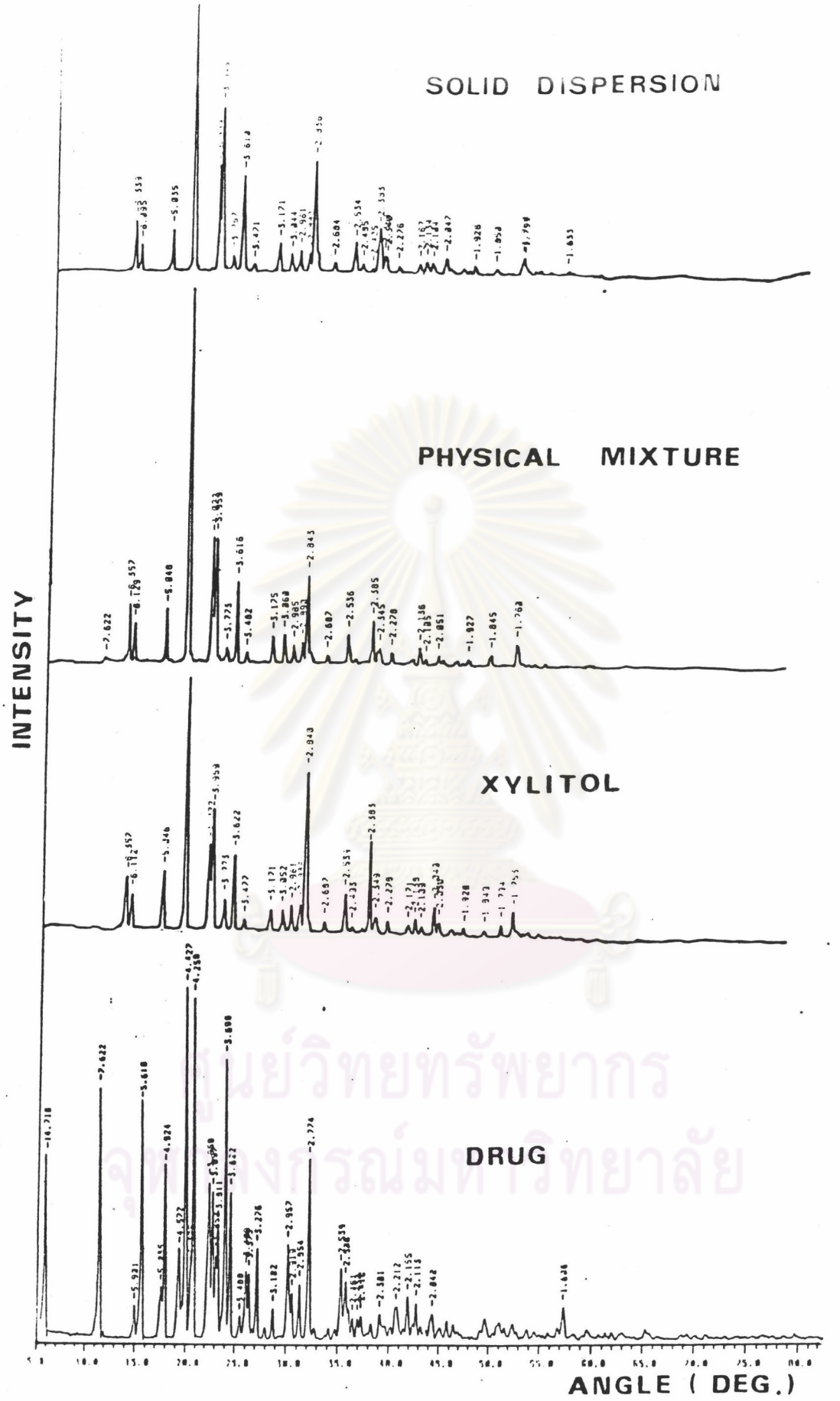


Figure 27 X-ray diffractograms of pure famotidine ; xylitol ; 1:10 famotidine-xylitol physical mixture and 1:10 famotidine-xylitol solid dispersion.

X-ray diffraction patterns of pure famotidine and pure mannitol showed characteristic diffraction peaks. The spectrum of famotidine:mannitol (1:10) solid dispersion still exhibited some characteristic mannitol peaks , while general absence of crystalline famotidine peaks was observed. This was also occured in the diffraction pattern of famotidine:xylitol (1:10) solid dispersion .The x-ray diffraction pattern of famotidine:sorbitol exibited absence of some crystalline famotidine peaks.

From data mentioned earlier, mannitol was proved to be a fine carrier in the preparation of famotidine solid dispersions that could most dramatically increase the solubility of the drug. Consequently, it was selected as the most suitable carrier to be fomulated in the production of famotidine tablets for further studies.

5.5 Tablet Evaluations

Famotidine-mannitol physical mixture and solid dispersions were produced as tablets by direct compression method in the formula mentioned earlier (page 48). Together with three other commercial famotidine tablet products, the prepared tablets were observed for their physical properties as follows :

5.5.1 Weight Variation

The average weight and standard deviations of the prepared tablets and commercial products were presented in Table 6. In all cases, the data were complied with USP. requirement.

5.5.2 Hardness

The results of average hardness and standard deviations of famotidine tablets were shown in Table 6 .The average hardness of physical mixture and solid dispersion tablets were 3.86 and 7.35 Kp, respectively. While the data of average hardness from all tested commercial tablets were more than 10 Kp. Moreover, the average hardness of brand A tablets was exceeded 20 Kp.

5.5.3 Thickness

The data of average thickness and standard deviations were displayed in Table 6 . The thickness of commercial products was in the range of 4.40-4.43 mm, except for that of

brand C which was 3.51 mm. Where as the average values of solid dispersion tablets appeared very close to those of commercial products.

5.5.4 Disintegration Time

The average d isintegration time of famotidine tablets was presented in Table 6 . In all cases, the disintegration times were about 60 seconds.Tablets from physical mixture exhibited the least disintegration time due to their lower hardness .

5.5.5 Content Uniformity of Tablet

The data of content uniformity of the prepared and commercial famotidine tablets were given in Table 7. It was discovered that the data of all cases met the USP. requirement . The standard curve of famotidine in 0.1 M phosphate buffer pH 4.5 was shown in Appendix 4 .

5.5.6 Dissolution of Tablet

The dissolution profiles and data of pure famotidine powder and prepared tablets were displayed in Figure 28 . The solid dispersion tablets were initially dissolved slowly but gave the highest amount of famotidine dissolved after a few minutes passed. Tablets produced from famotidine-mannitol physical mixture exhibited the second fastest dissolution rate where as the pure drug appeared the slowest. However, all of them possessed

the dissolution studies in the limit of the USP. standard of which the time requires for 75 % of famotidine to dissolved was 30 minutes.

The dissolution profiles of three commercial tablet products from various sources of manufacture were illustrated in Figure 29 . Tablets that performed the fastest dissolution rate were brand A, which displayed about the same rate as tablets prepared by 1:10 famotidine-dispersion, followed by dissolution of tablets brand C and B. Tablets prepared by 1:10 famotidine-mannitol physical mixture had faster dissolution rate than famotidine powder alone which presented the slowest dissolution rate. All the experimental samples exibited the dissolution rate within the USP. requirement.

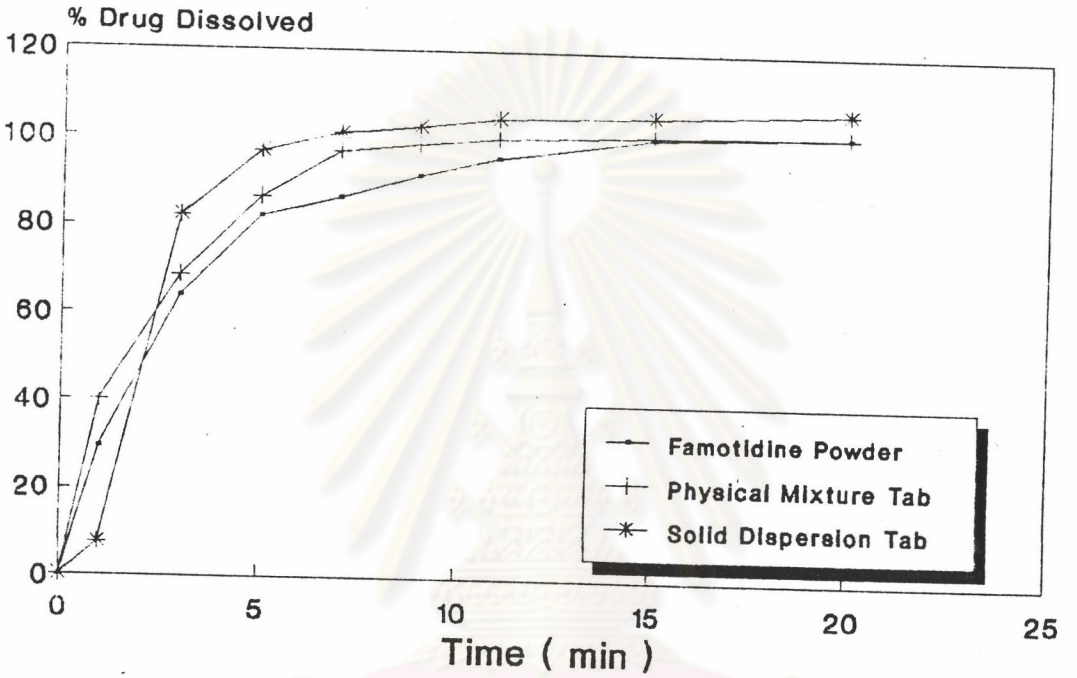


Figure 28 Dissolution profiles of famotidine from famotidine powder and tablets of 1:10 famotidne-mannitol physical mixture and solid dispersions in 0.1 M phosphate buffer pH 4.5 at 37 ± 0.5° C.

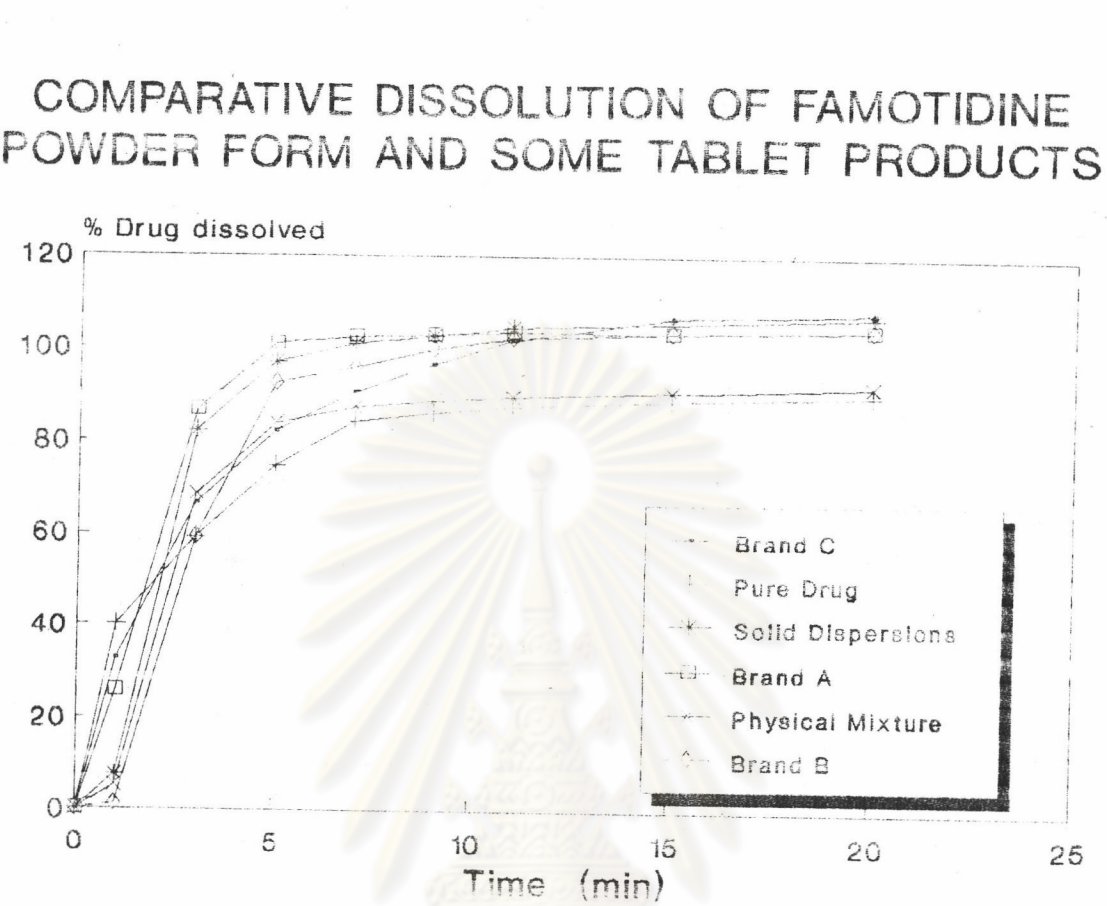


Figure 29 Dissolution profiles of famotidine from famotidine powder and tablets of 1:10 famotidne-mannitol physical mixture and solid dispersions, and some commercial tablet products in 0.1 M phosphate buffer pH 4.5 at 37 ± 0.5° C.

Table 6 Physical properties of prepared and some famotidine commercial tablet products.

Physical properties of tablets	Type of famotidine tablet products				
	Sol.Disp	Phy.Mix	BrandA	BrandB	Brand C
Weight (mg.)	249.01	249.89	212.73	215.68	150.33
Standard deviation	0.88	0.32	1.46	0.84	4.18
Relatived Standard Deviation	0.35	0.13	0.69	0.39	2.78
Maximum Deviation (%)	0.64	0.24	0.79	0.47	2.58
Minimum Deviation (%)	0.81	0.23	0.48	0.45	2.88
Hardness (kp.)	7.35	3.86	>20	13.75	11.25
Standard Deviation	0.50	0.21	-	1.20	1.06
Thickness (mm.)	4.03	3.95	4.43	4.40	3.51
Standard Deviation	0.016	0.019	0.025	0.021	0.065
Disintegration Time (sec.)	53.16	18.33	56.00	63.50	60.00
Standard Deviation	2.64	0.82	3.60	11.10	10.03

Table 7 Content uniformity of prepared and some commercial famotidine tablet products.

sample number	% Drug in tablet				
	Sol.Disp	Phy.Mix	Brand A	Brand B	Brand C
1	102.38	97.43	102.06	99.67	98.47
2	104.65	101.67	101.34	101.04	102.34
3	99.42	102.59	103.58	98.84	101.02
4	105.26	98.34	99.46	102.36	99.36
5	101.73	99.86	102.54	101.42	102.23
6	98.87	100.63	103.72	100.73	100.04
7	104.62	97.85	101.53	99.62	101.37
8	101.91	102.54	103.62	98.86	99.84
9	100.23	101.03	100.37	101.92	101.94
10	99.65	100.58	102.42	102.68	102.76
Mean	101.87	100.25	102.06	100.71	100.94
S.D	2.35	1.86	1.42	1.41	1.44
% C.V	2.30	1.85	1.39	1.40	1.43

Selection for Appropriate Lyophilized Famotidine Product.

Famotidine lyophilized products of 1:1 and 1:2 drug-carrier ratios were prepared, using various carrier---PVP 12 PF, PEG 6000, glycine, mannitol, sorbitol, xylitol, glucose and citric acid. Various types of vehicles that could be applied in parenteral formulation had been tested to use the higher pH solution (famotidine is a basic drug and decomposes quickly in acidic environment), and the least volume as possible that could completely dissolve the drug in order to stabilize the drug and shorten the duration applied in the dried stage of the lyophilization process.

1. Vehicle System

Some physicochemical properties of famotidine lyophilized product made in acetate, phosphate buffer and L-aspartic acid, varing acidity were presented in Table 8 to 12 , respectively.

Most of the products produced by using acetate buffer pH 5.5 and phosphate buffer pH 4.5, spent more than 5 minutes to reconstituted. Therefore, they were not suitable to be applied in the pharmaceutical manufacture.

Lyophilized products made in 5 ml of L-aspartic acid pH 3.7, utilized more than 48 hours to dry, while other systems applied less time. As a result, the two vehicle systems: 2 ml of acetate buffer pH 3.7 and 2 ml of L- aspartic acid pH 3.3 were selected to produce the famotidine lyophilized product for further study.

Table 8 Some physicochemical properties of famotidine lyophilized product using various carriers prepared in 6 ml acetate buffer pH 5.5.

Carrier	acetate buffer pH 5.5, 6 ml		
	Appearance	Reconstitution Time (min)	pH
PVP 12 PF	not dry	2	6.6
PEG 6000	not dry	>5	6.9
Glycine	very bulky	>5	6.7
Mannitol	very bulky	>5	6.6
Sorbitol	not dry	>5	6.6
Xylitol	slightly bulky	>5	6.5
Glucose	not dry	1	6.5
Citric acid	not dry	>5	4.9

Table 9 Some physicochemical properties of famotidine lyophilized product using various carriers prepared in 2 ml acetate buffer pH 3.7.

Carrier	acetate buffer pH 3.7, 2 ml		
	Appearance	Reconstitution Time (min)	pH
PVP 12 PF	not dry	1	5.8
PEG 6000	not dry	40 sec.	6.4
Glycine	bulky	1	6.5
Mannitol	very bulky	15 sec	5.7
Sorbitol	slightly bulky	15 sec	6.0
Xylitol	slightly bulky	20 sec	6.2
Glucose	not dry	1	6.0
Citric acid	not dry	1	3.7

Table 10 Some physicochemical properties of famotidine lyophilized product using various carriers prepared in 5 ml phosphate buffer pH 4.5.

Carrier	phosphate buffer pH 4.5, 5 ml		
	Appearance	Reconstitution Time (min)	pH
PVP 12 PF	slightly bulky	4	6.2
PEG 6000	slightly bulky	>5	6.1
Glycine	bulky	4	6.2
Mannitol	bulky	3	6.2
Sorbitol	slightly bulky	>5	6.4
Xylitol	slightly bulky	>5	6.2
Glucose	slighty bulky	>5	6.1
Citric acid	slightly bulky	>5	4.0

Table 11 Some physicochemical properties of famotidine lyophilized product using various carriers prepared in 5 ml L-aspartic acid pH 3.7.

Carrier	L-aspartic acid pH 3.7, 5 ml		
	Appearance	Reconstitution Time (min)	pH
PVP 12 PF	not dry	2	4.8
PEG 6000	slightly bulky	2	4.8
Glycine	very bulky	30 sec	4.9
Mannitol	very bulky	30 sec	4.7
Sorbitol	bulky	2	4.8
Xylitol	slightly bulky	2	4.8
Glucose	not dry	2	4.8
Citric acid	not dry	1	3.7

Table 12 Some physicochemical properties of famotidine lyophilized product using various carriers prepared in 2 ml L-aspartic acid pH 3.3.

Carrier	L-aspartic acid pH 3.3, 2 ml		
	Appearance	Reconstitution Time (min)	pH
PVP 12 PF	slightly bulky	2	4.5
PEG 6000	bulky	2	4.6
Glycine	very bulky	20 sec	4.5
Mannitol	very bulky	20 sec	4.4
Sorbitol	bulky	2	4.5
Xylitol	bulky	2	4.4
Glucose	slightly bulky	2	4.3
Citric acid	slightly bulky	1	3.9

2. Carrier

2.1 Carrier Ratio

From the experimental results , it was discovered that products prepared by 1:2 drug carrier ratio were more bulky and spent less time to reconstitute than those of 1:1. Consequently, the 1:2 drug carrier ratio was selected for the following studies.

2.2 Carrier Type

Concerning the two vehicles : 2 ml of acetate buffer pH 3.7 and L-aspartic acid pH 3.3, the physical appearance and reconstitution time of the lyophilized product prepared from each carrier type were compared. The products prepared in PVP 12 PF, PEG 6000, glycine, mannitol, sorbitol, xylitol glucose and citric acid were not dried when produced in 2 ml of acetate buffer pH 3.3. Thus, the undried products leaded to a longer time for reconstitution comparing to products from other carriers.

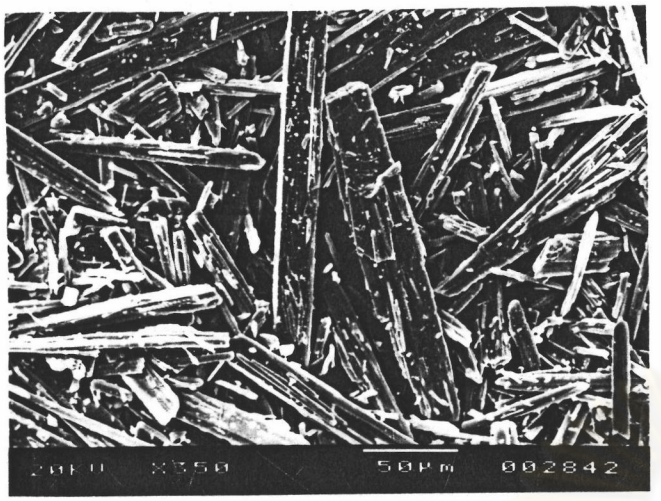
3. pH of Reconstituted Solution

The pH value of prepared lyophilized powder after diluted with 5 ml of water was approximately in the range of 4-6, about one pH unit was shift up from that of the initial pH value of vehicle. Freezed dried products prepared in citric acid played an important role in acidifying the solution, its pH value was in the range of about 3-4 which considered too acidic conditioning for the parenteral preparation and the stability of famotidine.

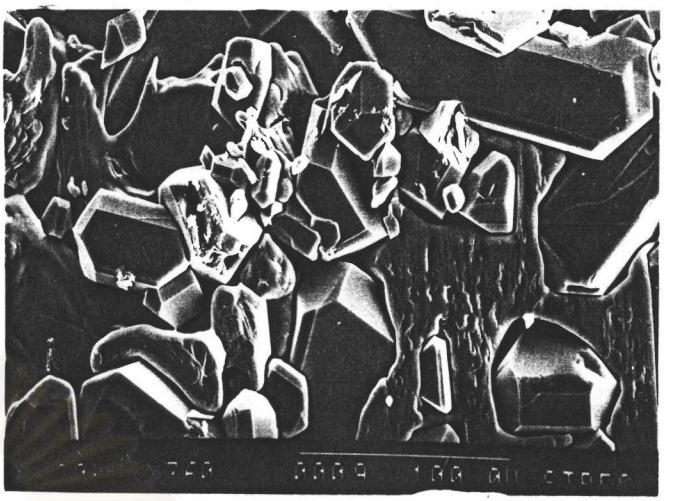
4. Morphology of Lyophilized Product

SEM photomicragraphs of lyophilized powder of famotidine -glycine produced in L-aspartic acid, famotidine-mannitol made in acetate buffer and famotidine-mannitol prepared in L-aspartic acid were shown in Figure 32 - 35 , respectively. The microscopic images of all systems exhibited very fine particles , and products from 1:2 drug-carrier ratio presented even finer particles.

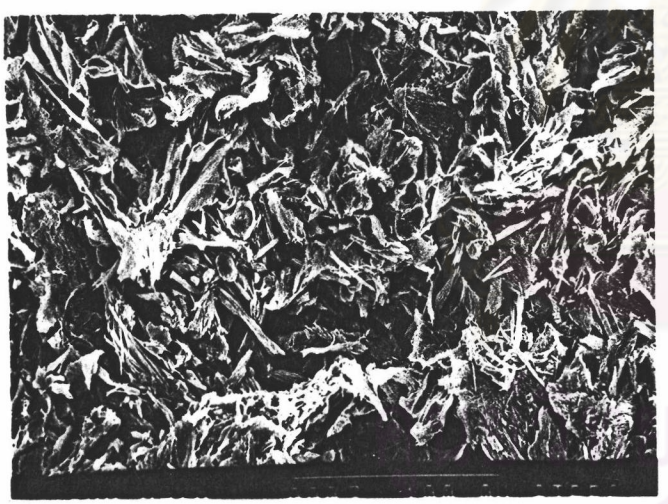
Concerning with the less reconstitution time and the fine microscopic appearance , 1:2 famotidine-mannitol was then selected as the most suitable system to produce the lyophilized powder as final product for the stability testing.



A



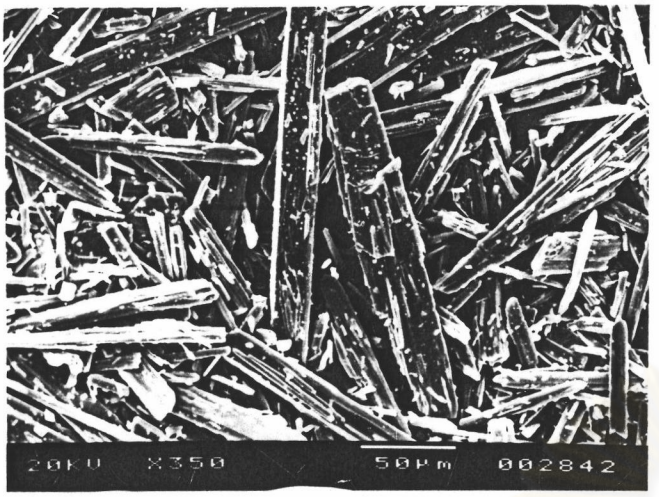
B



C

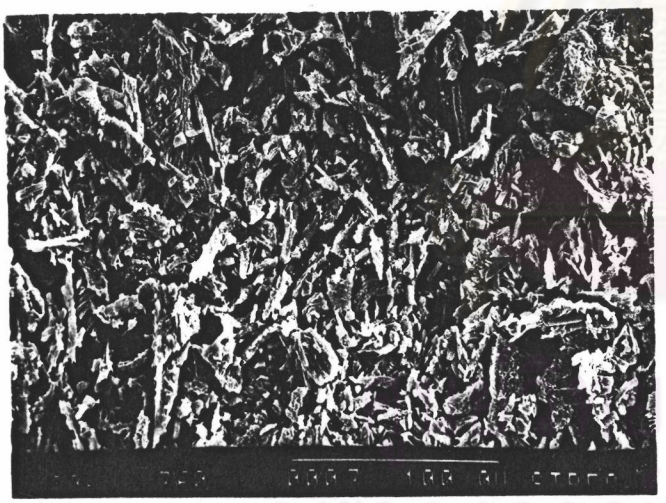
D

Figure30 SEM photomicrographs of pure famotidine(A);glycine(B); 1:1 famotidine-glycine lyophilized powder produced in acetate buffer (C) and 1:2 famotidine-glycine lyophilized powder made in acetate buffer (D) in magnifications x350.

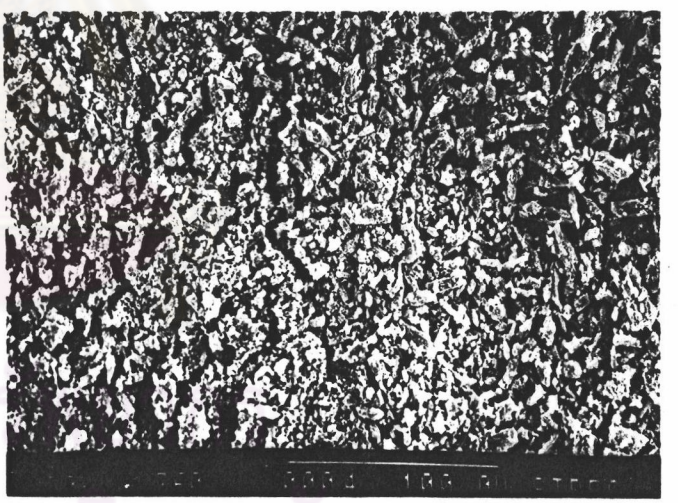


A

B



C

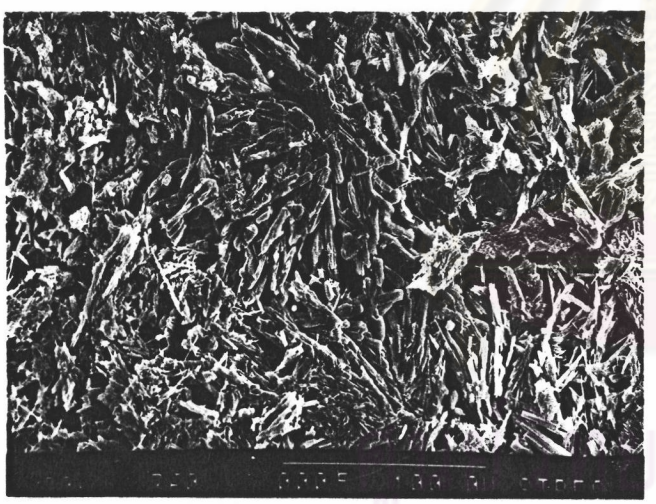


D

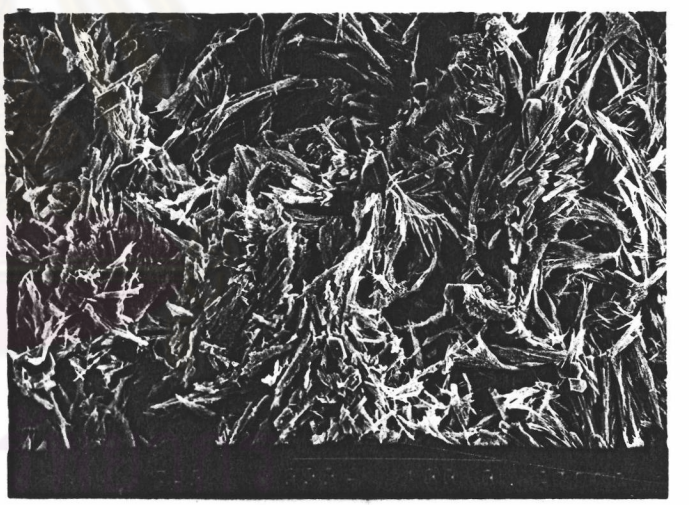
Figure 31 SEM photomicrographs of pure famotidine (A); glycine (B); 1:1 famotidine-glycine lyophilized powder produced in L - aspartic acid (C); and 1:2 famotidine - glycine lyophilized powder produced in L - aspartic acid (D) in magnifications x 350.

A

B

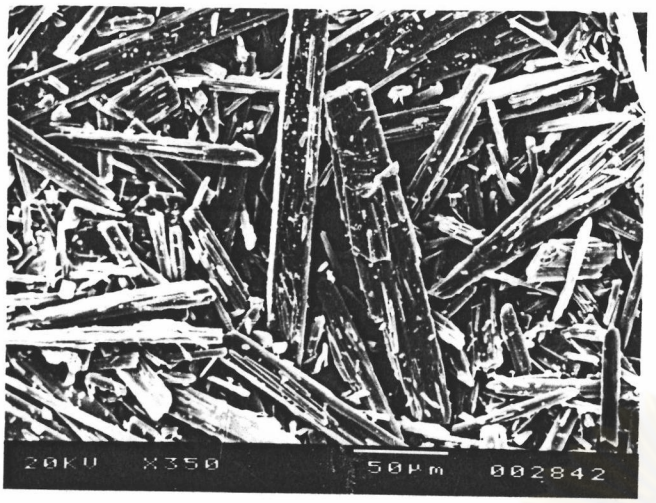


C



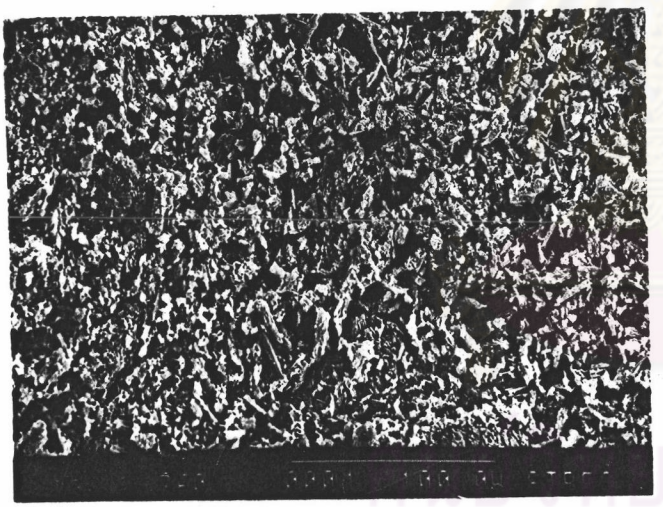
D

Figure 32 SEM photomicrographs of pure famotidine(A);mannitol(B); 1:1 famotidine-mannitol lyophilized powder produced in acetate buffer (C); and 1:2 famotidine-mannitol lyophilized powder made in acetate buffer (D) in magnifications x350.

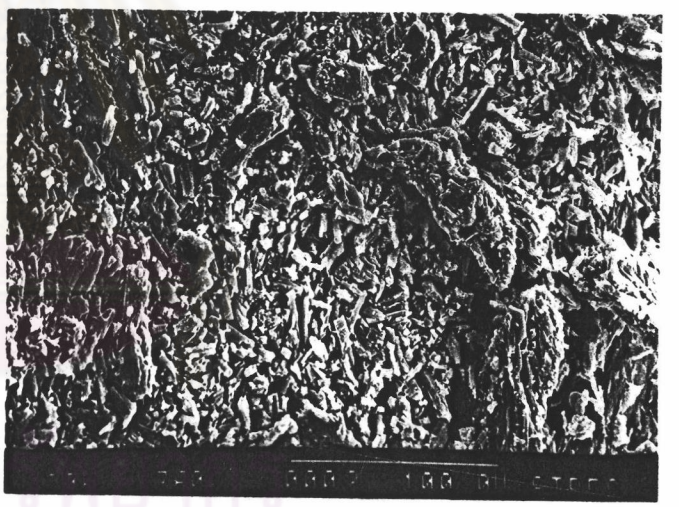


A

B



C



D

Figure 33 SEM photomicrographs of pure famotidine (A); mannitol (B); 1:1 famotidine-mannitol lyophilized powder produced in L - aspartic acid (C); and 1:2 famotidine - mannitol lyophilized powder produced in L - aspartic acid (D) in magnifications x 350.

Evaluation of Lyophilized Product

1. Reconstitution Time , pH Solution and Osmolarity Determination

The reconstitution time , pH value and osmolarity of solution at different temperature condition were exibited in Figure 34 to 36, respectively. The three parameters were all slightly increased.

Code of famotidine lyophilized products was composed of 3 parts :

- DP
- letter A or L or Br
- number 45 or RT

DP was abbreviated from dried powder form, letter A and L represented the vehicle in which the lyophilized powder produced in : acetate buffer pH 3.7 and L-aspartic acid pH 3.3 , respectively. Besides, Br A was stood for commercial lyophilized product brand A. Lastly, number 45 or RT resembled the temperature which the freezed dried powder were kept during the stability study : 45 °C , 75 % RH and room temperature , respectively.

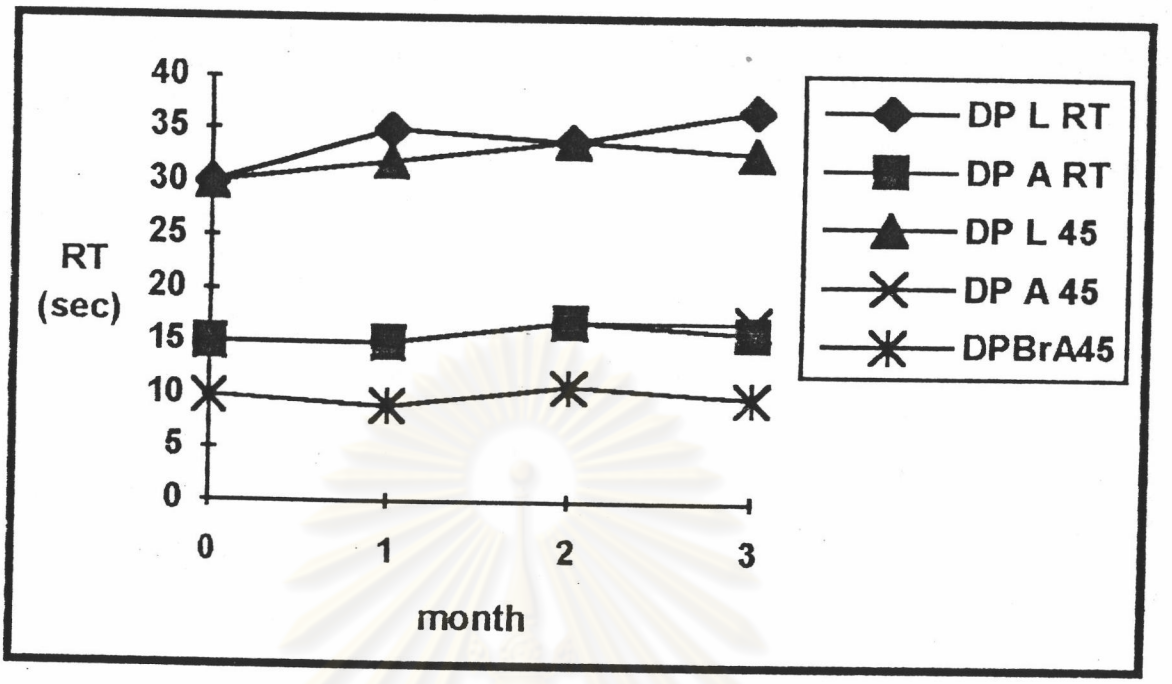


Figure 35 Reconstitution time changes of lyophilized famotidine powder kept at various conditions for three months .

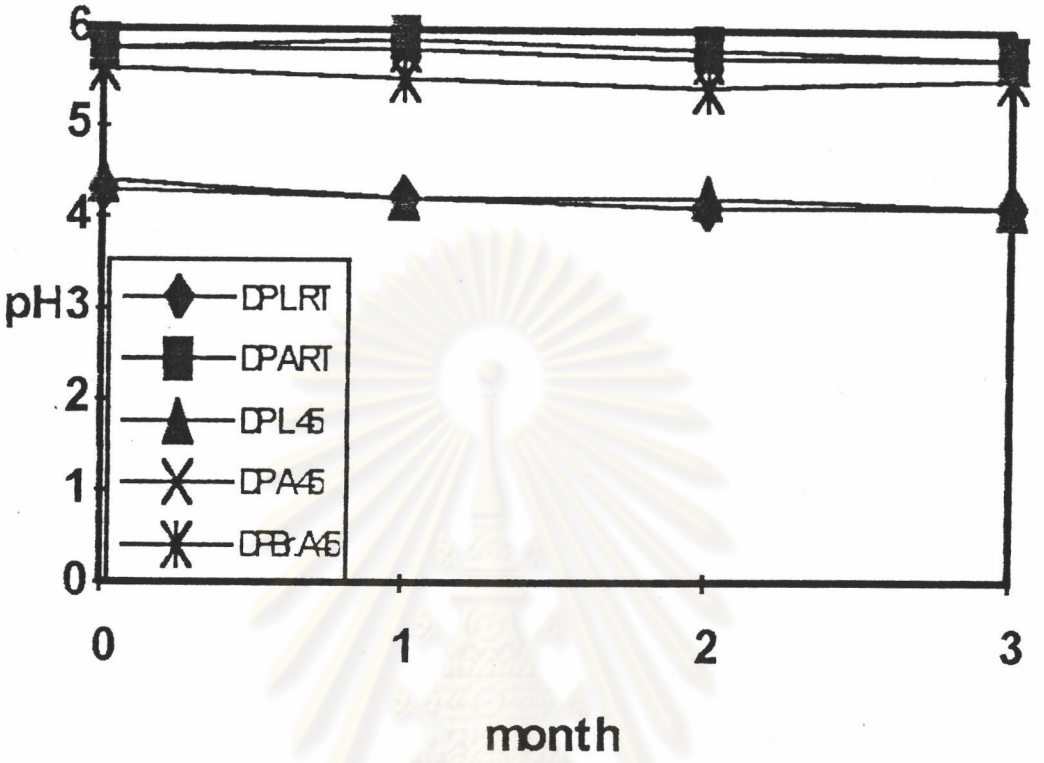


Figure 35 pH of famotidine lyophilized powder kept at various conditions for three months.

Table 13 pH change of lyophilized famotidine powder produced in L-aspartic acid after reconstituted with water and kept as solution at various conditions.

Day	pH of reconstituted solution at *			
	Room Temp	45 ° C	55 ° C	65 ° C
Initial	4.3	4.3	4.3	4.3
1	4.3	4.3	4.4	4.3
2	4.4	4.4	4.4	4.2
3	4.3	4.3	4.3	4.2
4	4.2	4.3	4.3	4.1
6	4.2	4.2	4.3	4.2
8	4.3	4.3	4.2	4.1
10	4.3	4.3	4.1	4.1
15	4.3	4.2	4.2	4.0
25	4.3	4.2	4.2	4.0
45	4.2	4.0	4.1	4.1
60	4.2	4.0	4.0	4.0
90	4.2	3.9	4.1	3.9

* average of two values (n=2)

Table 14 pH change of lyophilized famotidine produced in acetate buffer pH 3.7 after reconstituted with water and kept as solution at various conditions.

Day	pH of reconstituted solution at *			
	Room Temp	45 ° C	55 ° C	65 ° C
Initial	5.9	5.8	5.9	5.8
1	5.9	5.8	5.8	5.8
2	5.8	5.9	5.8	5.7
3	5.7	5.8	5.7	5.7
4	5.7	5.7	5.7	5.6
6	5.8	5.7	5.8	5.7
8	5.9	5.7	5.8	5.8
10	5.8	5.8	5.8	5.8
15	5.8	5.7	5.8	5.8
25	5.8	5.7	5.8	5.8
45	5.8	5.6	5.7	5.7
60	5.7	5.5	5.6	5.6
90	5.7	5.5	5.6	5.6

* average of two values (n=2)

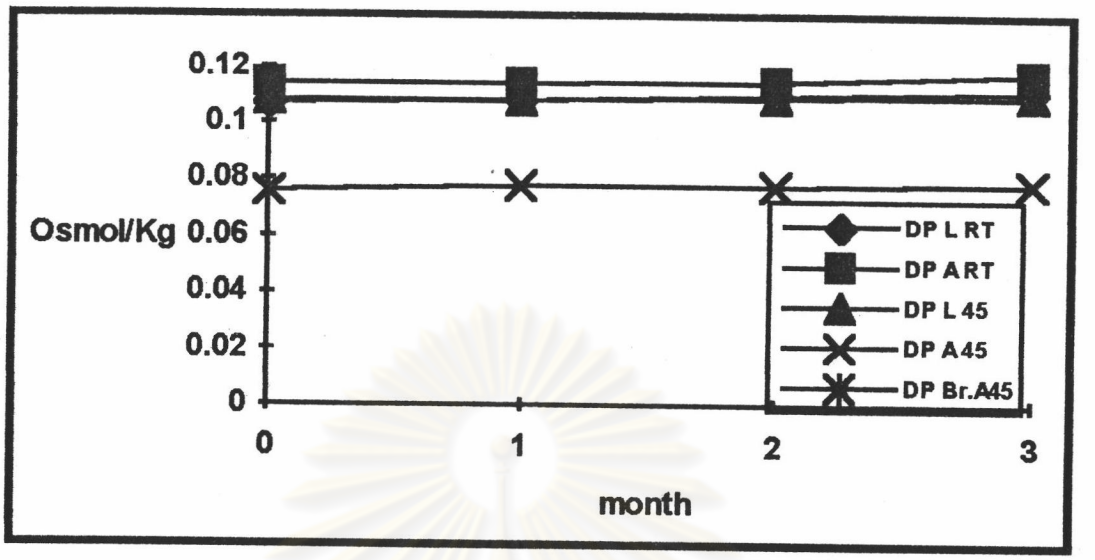


Figure 36 Osmolarity of famotidine lyophilized powder after kept at RT or 45 ° C , 75 % RH, and reconstituted with water.

2. Stability Study

Chromatograms of famotidine and its degradation product were presented in Figure 37. The retention time of famotidine and sulfamerazine (the internal standard) was about 9 and 13 minutes, respectively.

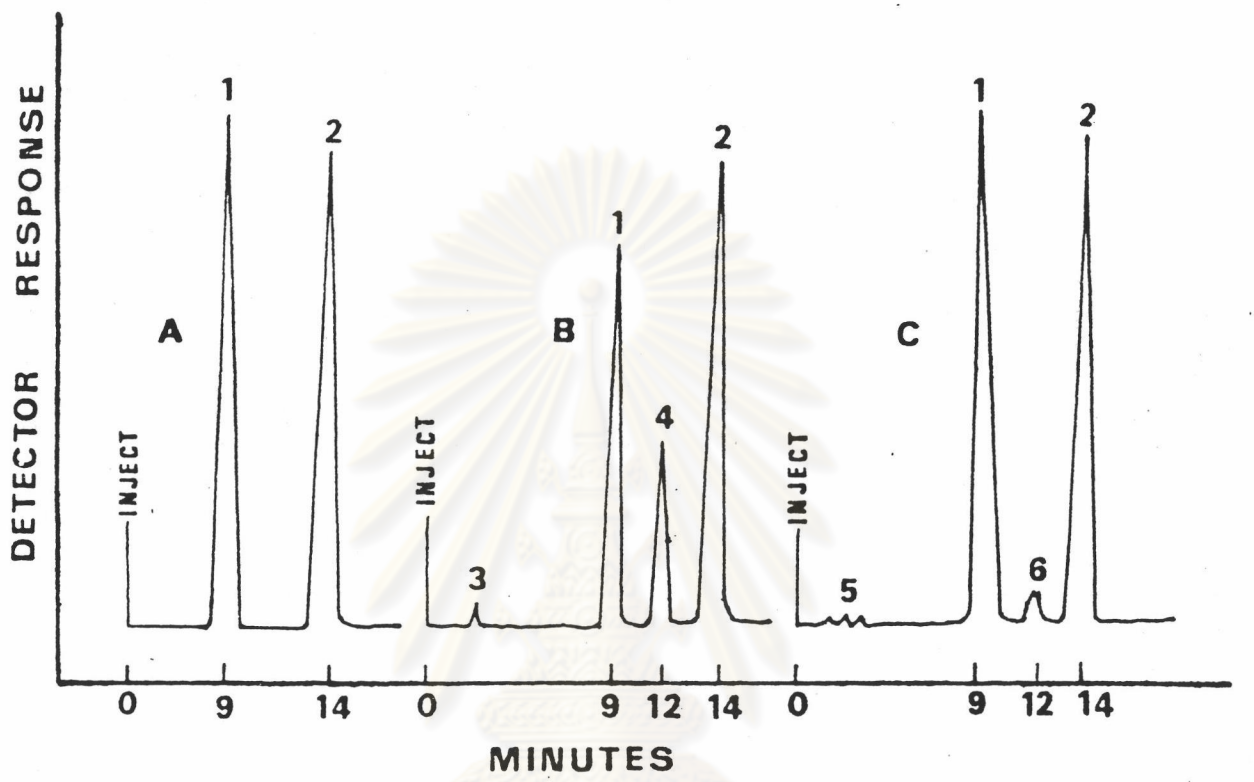


Figure 37 Sample chromatograms. Peak 1-2 from famotidine and sulfamerazine (the internal standard), respectively. Peak 3, 4, 5 and 6 were from the products of decomposition. Chromatogram A was from standard famotidine solution, B from lyophilized product (made in L-aspartic acid) and C from lyophilized product (produced in acetate buffer).

2.1 Lyophilized Powder

All the prepared lyophilized powder kept at 45 °C , 75 % RH for 3 months exhibited the percent content remains within the USP requirement. The commercial lyophilized product (brand A) also presented the percentage remaining of the drug within the USP requirement (Table 15).

Table 15 Concentration remains of lyophilized famotidine powder kept at room temperature or 45 °C, 75 % RH.

Preparation	% Remain* at month			
	0	1	2	3
DP L RT	100.00	98.57	99.22	98.55
DP A RT	100.00	98.94	97.44	95.95
DP L 45	100.00	100.02	98.28	95.28
DP A 45	100.00	97.99	96.10	92.90
DP Br. A 45	100.00	99.15	97.19	95.64

* average of two values (n=2)

Table 16 Data of famotidine content (produced in L-aspartic acid) at room temperature (27.5°C), 45°C, 55°C and 65°C

at	Room Temp (25-30 °c)		45 ° c		55 ° c		65 ° c	
day	% A/A_0 •	ln A/A_0	% A/A_0 •	ln A/A_0	% A/A_0 •	ln A/A_0	% A/A_0 •	ln A/A_0
0	100.00	4.6051	100.00	4.6051	100.00	4.6051	100.00	4.6051
1	99.95	4.6047	97.19	4.5766	94.72	4.5510	96.26	4.5671
2	99.70	4.6021	94.64	4.5500	90.72	4.5078	82.74	4.4158
3	98.40	4.5891	90.89	4.5096	86.01	4.4545	70.72	4.2587
4	95.98	4.5641	84.90	4.4415	78.95	4.3689	62.31	4.1321
6	97.92	4.5842	79.91	4.3802	72.93	4.2895	44.93	3.8051
8	92.44	4.5266	70.26	4.2523	59.72	4.0897	34.61	3.5442
10	94.55	4.5492	63.25	4.1472	53.03	3.9709	19.77	2.9844
15	93.05	4.5331	-	-	-	-	-	-
25	72.37	4.2818	-	-	-	-	-	-

Note A/A_0 : The ratio of famotidine content remained at any time (A) to the initial content (A_0)
a : average of two values (n=2)

Table 17 Data of famotidine content (produced in acetate buffer) at room temperature (27.5°C), 45°C, 55°C and 65°C

at	Room Temp (25-30 °c)		45 ° c		55 ° c		65 ° c	
day	% A/A_0 a	ln A/A_0	% A/A_0 a	ln A/A_0	% A/A_0 a	ln A/A_0	% A/A_0 a	ln A/A_0
0	100.00	4.6051	100.00	4.6051	100.00	4.6051	100.00	4.6051
1	102.04	4.6254	102.57	4.6306	101.46	4.6107	102.59	4.6308
2	102.06	4.6256	101.87	4.6237	101.39	4.6190	100.70	4.6122
3	101.69	4.6219	100.59	4.6111	100.55	4.6107	100.32	4.6084
4	101.32	4.6183	100.35	4.6087	100.54	4.6106	99.47	4.5999
6	100.35	4.6087	99.97	4.6049	99.57	4.5991	97.95	4.5845
8	99.54	4.6006	99.83	4.6035	99.39	4.6009	96.51	4.5697
10	99.38	4.5990	98.91	4.5942	99.52	4.5968	95.57	4.5599
15	99.31	4.5983	98.13	4.5638	94.99	4.5537	92.78	4.5303
25	98.59	4.5910	94.99	4.5537	90.14	4.5014	86.36	4.4586
35	98.12	4.5862	89.89	4.4986	86.41	4.4591	64.29	4.1635
45	94.56	4.5492	79.52	4.3759	70.90	4.2612	-	-
60	90.87	4.5095	75.15	4.3196	-	-	-	-

Note A/A_0 : The ratio of famotidine content remained at any time (A) to the initial content (A_0)
a : average of two values (n=2)

Table 18 Correlation coefficient (r^2) of the rate constant (k) of reconstituted famotidine solution produced in L-aspartic acid treated as zero, first and second order reaction.

Temp (°C)	order of reaction		
	zero	first	second
45	0.9276	0.9235	0.9278
55	0.9970	0.9934	0.9978
65	0.9922	0.9860	0.9896

Table 19 Correlation coefficient (r^2) of the rate constant (k) of reconstituted famotidine solution produced in acetate buffer treated as zero, first and second order reaction.

Temp (°C)	order of reaction		
	zero	first	second
45	0.9528	0.9485	0.9328
55	0.9294	0.9002	0.8741
65	0.9098	0.8757	0.8382

2.2 Reconstituted Solution

The accelerated thermodegradation process were performed at 65°C, 55°C, 45°C and at apparent room temperature (25-30 °c). The lyophilized powder was reconstituted with 5 ml of water and kept as these conditions. Duplicate of the reconstituted products from each condition were analyzed at the suitable interval of time by the HPLC method.

Table 16 and 17 showed the data of famotidine content remains which produced in L-aspartic acid and acetate buffer, respectively. The correlation coefficient (r^2) calculated from each linear of 65° C , 55° C, 45° C and room temperature (27.5° C) when treated as zero, first and second order was exhibited in Table 18 and 19. The degradation rate constant of the reconstituted famotidine solution treated as zero and first order reaction was shown in Table 20 and 21, respectively.

The percentage remaining of the famotidine reconstituted solution plotted as zero order was presented in Figure 38 and 39. Concentration remains profiles of the first order reaction were plotted in Figure 40 and 41.

The Arrhenius plot of the reconstituted famotidine solution treated as zero order kinetic was shown in Figure 40 and 41. The correlation coefficient (r^2) of the famotidine solution produced in L-aspartic acid and acetate buffer was 0.9935 and 0.9694, respectively.

Table 20 Comparison of the extrapolated and apparent rate constant famotidine reconstituted solution at room temperature treated as zero order reaction.

prepared in	Extrapolate (day)	Apparent (day)
L-aspartic acid	3.2957	0.1577
acetate buffer	3.2836	0.0861

Table 21 Comparison of the extrapolated and apparent rate constant famotidine reconstituted solution at room temperature treated as first order reaction.

prepared in	Extrapolate (day)	Apparent (day)
L-aspartic acid	5.5161×10^{-3}	11.59×10^{-3}
acetate buffer	1.1162×10^{-3}	0.9567×10^{-3}

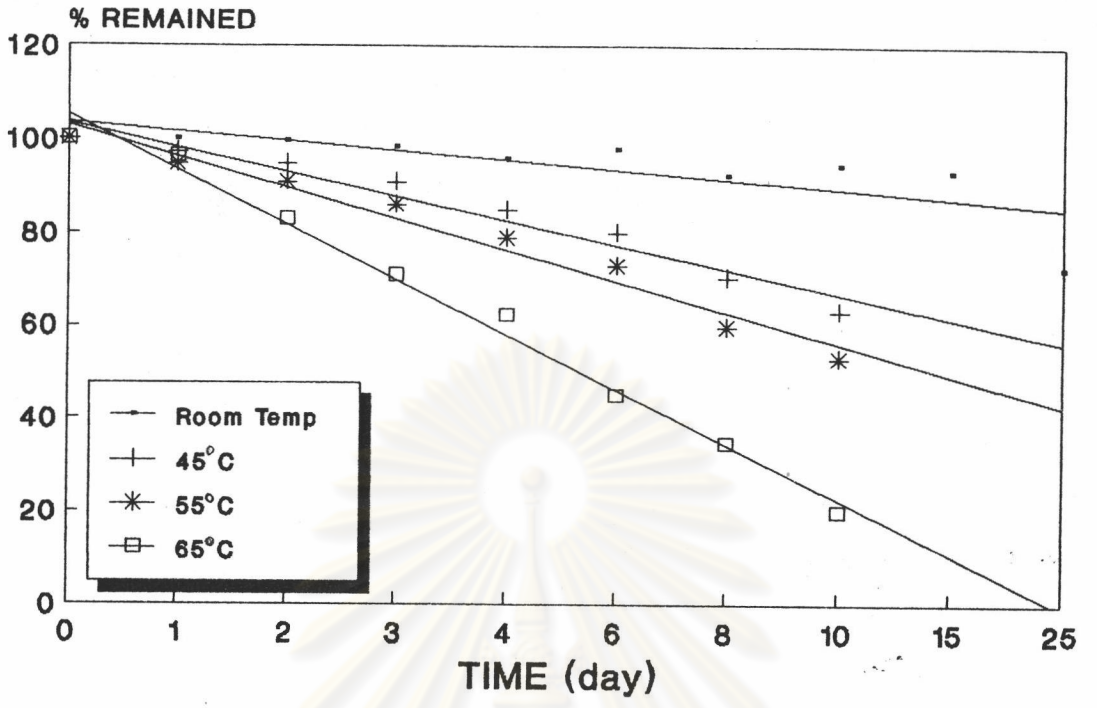


Figure 38 Remaining concentration (zero order) of famotidine reconstituted solution produced in L-aspartic acid.

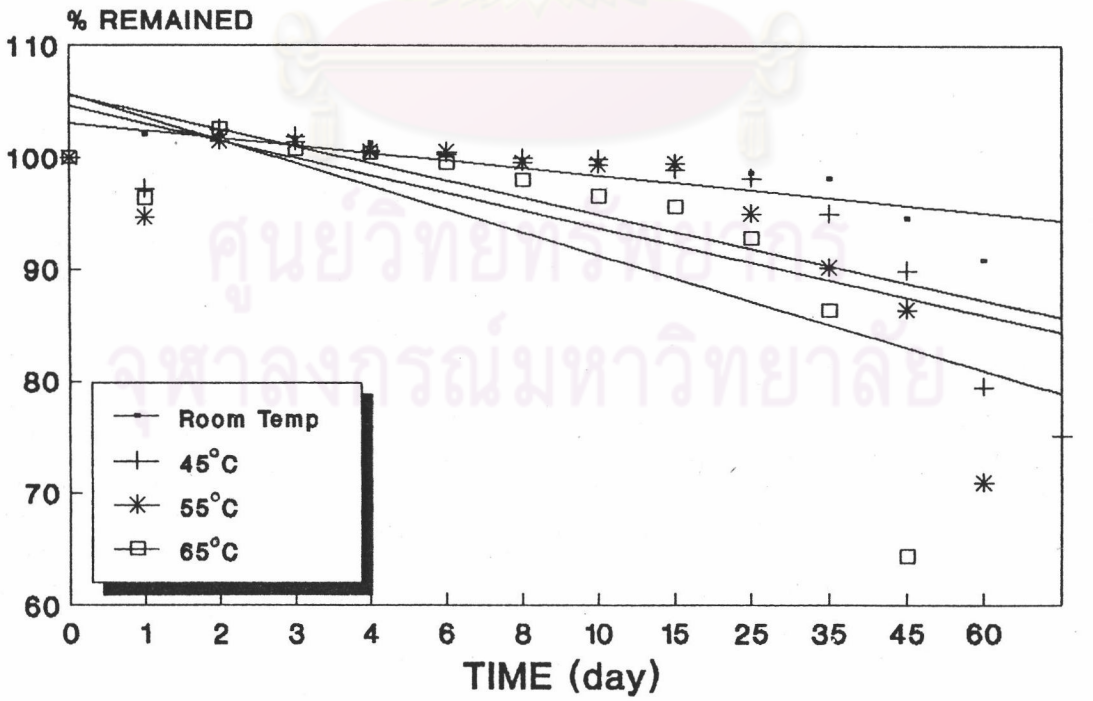


Figure 39 Remaining concentration (zero order) of famotidine reconstituted solution produced in acetate buffer.

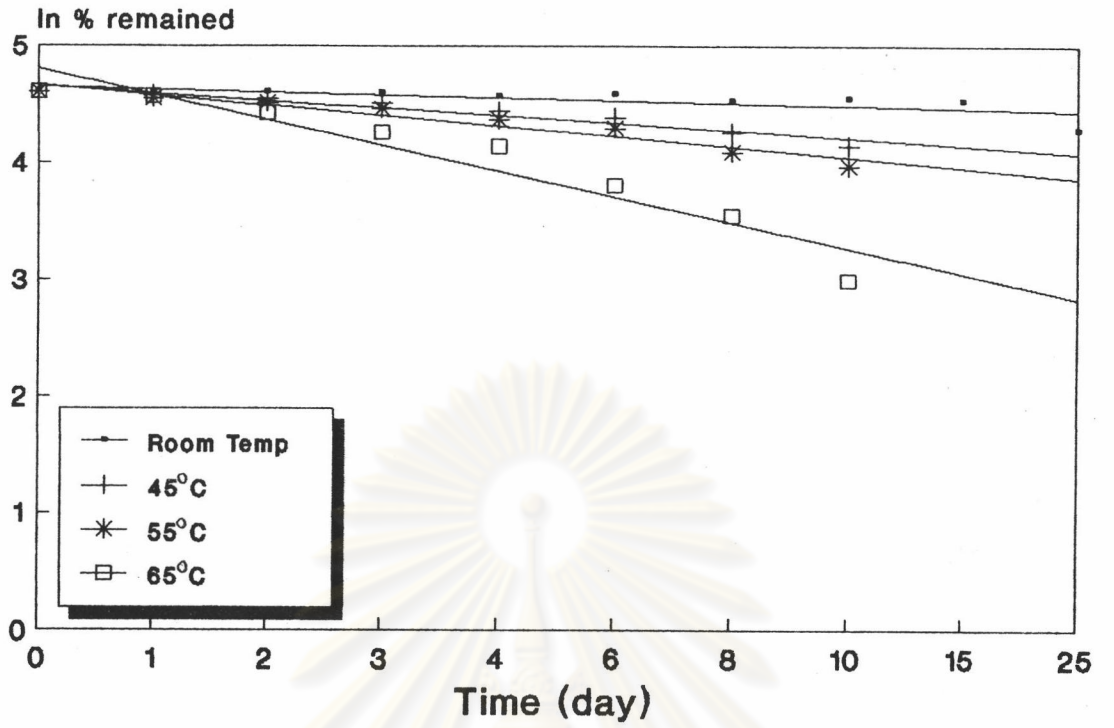


Figure 40 Remaining concentration (first order) of famotidine reconstituted solution produced in L-aspartic acid.

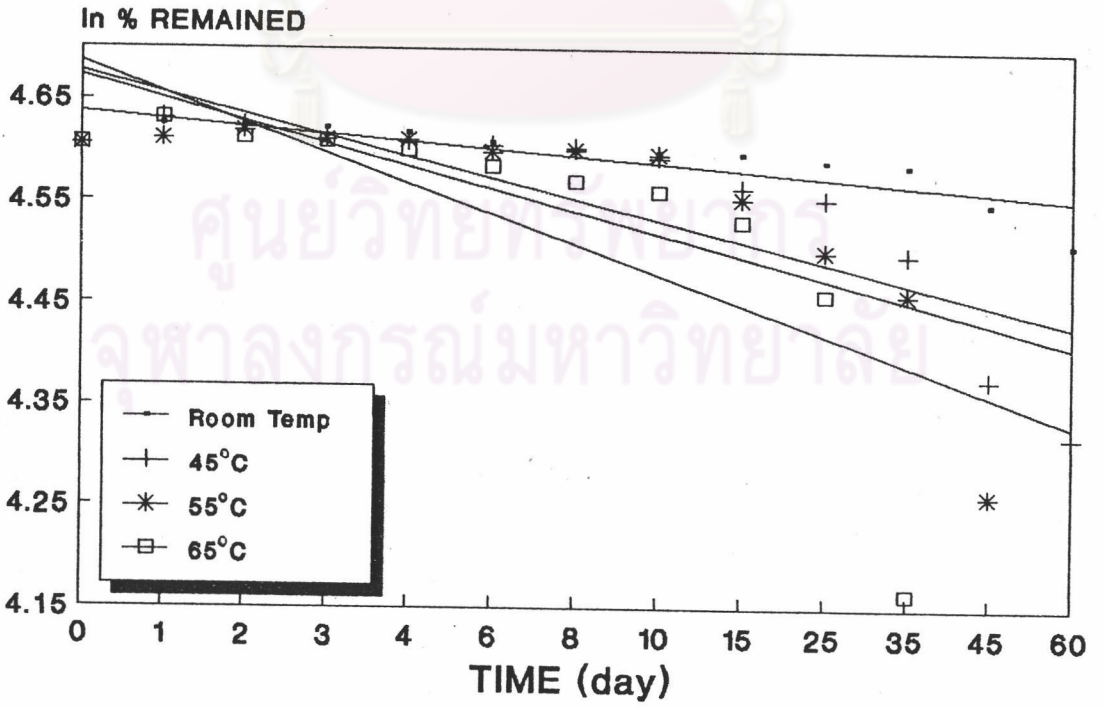


Figure 41 Remaining concentration (first order) of famotidine reconstituted solution produced in acetate buffer.

Similarily, the Arrhenius plot of the reconstituted famotidine solution treated as first order reaction was presented in Figure 42 and 43. The correlation coefficient (r^2) of the famotidine solution produced in L- aspartic acid and acetate buffer was 0.9064 and 0.9634, respectively.

By plotting the rate constant versus 1/T, the activation energy (Ea) can be obtained from the slope. The extrapolated value of the rate constant at room temperature was also calculated from the linear regression line. The activation energy was shown in Table 22.

The shelf-life of the reconstituted famotidine solution was calculated by the Arrhenius equation. Comparison of the extrapolated and apparent shelf-life treated as zero and first order was exhibited in Table 23 and 24, respectively.

The statistic values of the concentration remains of the lyophilized product was presented in Table 25. The variance ratio (F), the reaction rate constant (k) and its standard error of the products made in L-aspartic acid and acetate buffer at various temperature conditions were listed. The calculation of the data was demonstrated in Appendix 2.

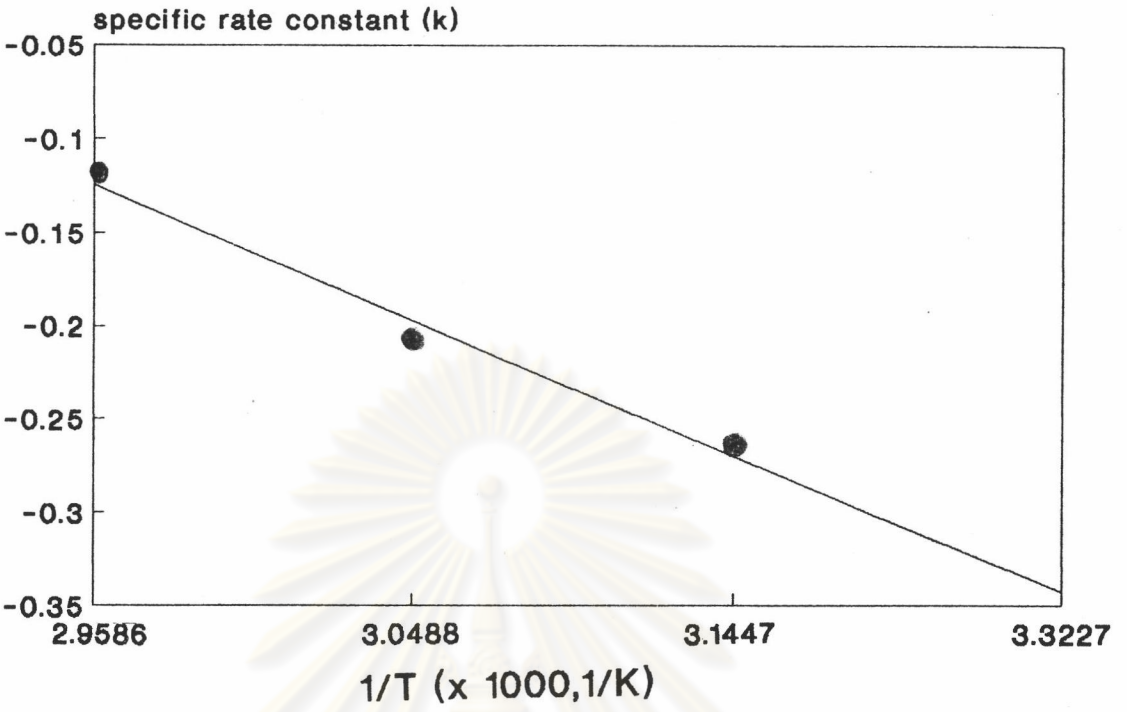


Figure 42 Arrhenius plot (zero order) of famotidine reconstituted solution produced in L-aspartic acid.

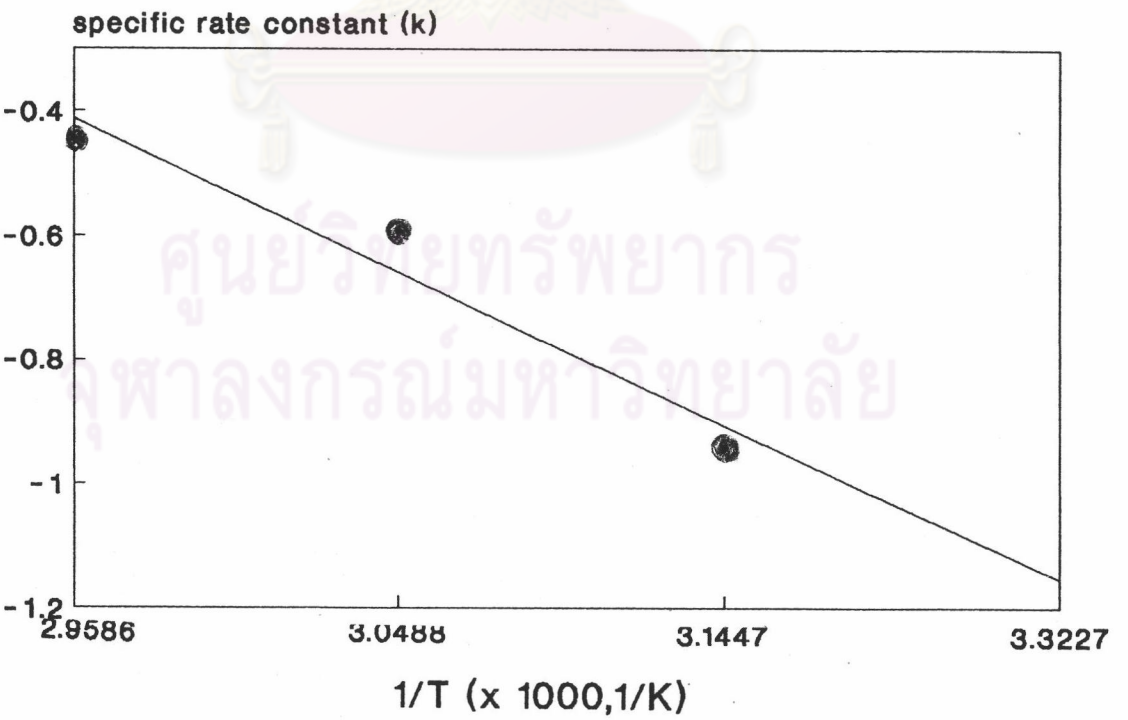


Figure 43 Arrhenius plot (zero order) of famotidine reconstituted solution produced in acetate buffer.

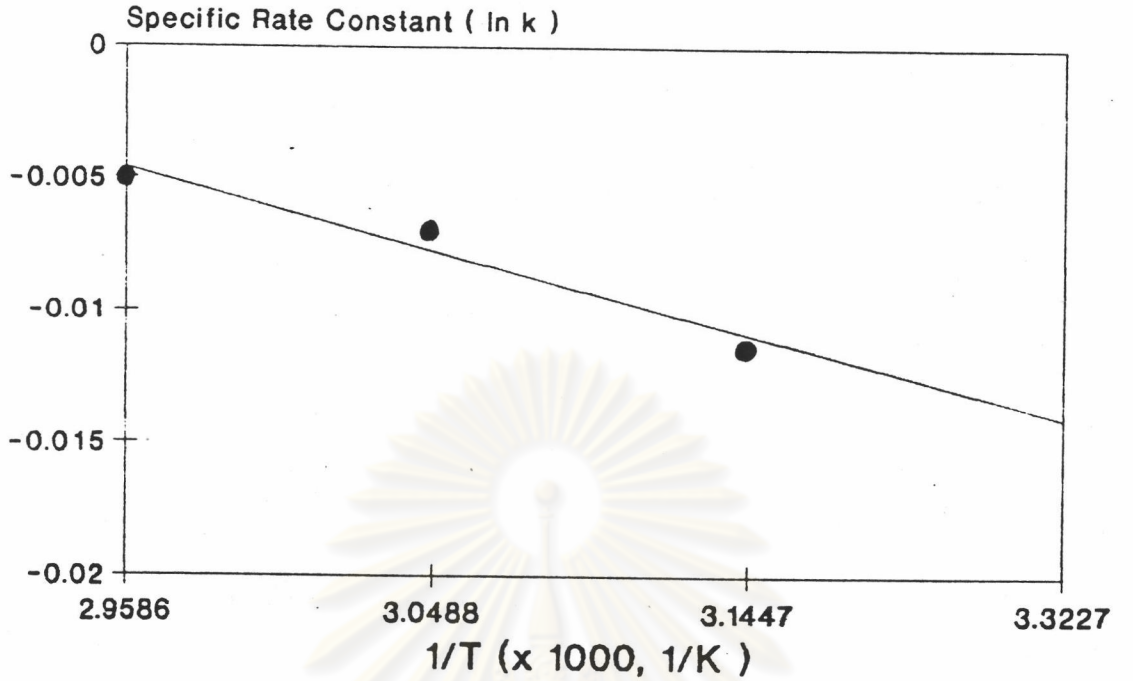


Figure 44 Arrhenius plot (first order) of famotidine reconstituted solution produced in L-aspartic acid.

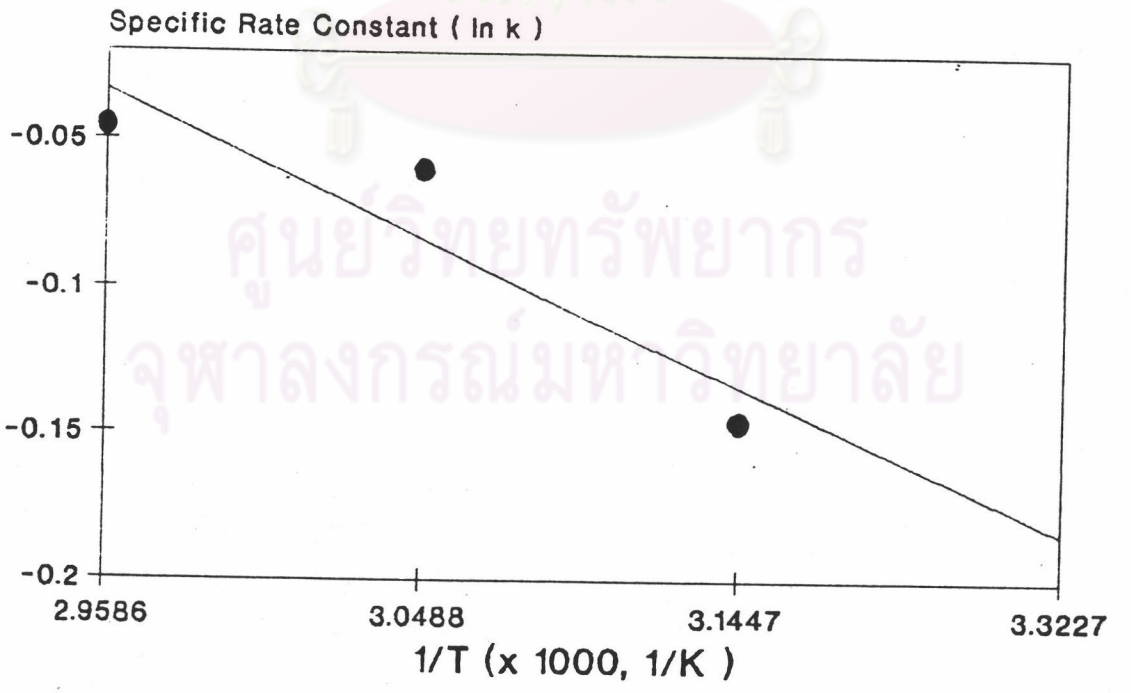


Figure 45 Arrhenius plot (first order) of famotidine reconstituted solution produced in acetate buffer.

Table 22 Activation energy of famotidine reconstituted solution.

prepared in	zero order(kcal/mol)	first order(kcal/mol)
L-aspartic acid	2.54	16.89
acetate buffer	6.52	26.80

Table 23 Comparison of the extrapolated and apparent shelf-life of famotidine reconstituted solution at room temperature treated as zero order reaction.

prepared in	Extrapolate (day)	Apparent (day)
L-aspartic acid	3.01-3.05	62.95-63.88
acetate buffer	3.04-3.05	95.52-148.12

Table 24 Comparison of the extrapolated and apparent shelf-life of famotidine reconstituted solution at room temperature treated as first order reaction.

prepared in	Extrapolate (day)	Apparent (day)
L-aspartic acid	18.97-19.12	6.34-9.60
acetate buffer	93.97-94.17	95.45-129.99

Table 25 The statistic values (r^2, K , F , sk) of lyophilized famotidine.

Lyopholized famotidine (produced in L-aspartic acid)

Temp (° c) / statistic value	27.5	45	55	65
r^2	0.9323	0.9276	0.9970	0.9922
F	53.04	986.71	1027.22	381.02
K (day^{-1})	$4.77x10^{-3}$	$1.75x10^{-2}$	$2.21x10^{-2}$	$3.76x10^{-2}$
sk	$6.55x10^{-4}$	$5.58x10^{-4}$	$6.91x10^{-4}$	$1.92x10^{-3}$

Lyophilized famotidine (produced in acetate buffer)

Temp (° c) / statistic value	27.5	45	55	65
r^2	0.9035	0.9528	0.9294	0.9098
F	1903.07	142.42	1.01	126.84
K (day^{-1})	$6.50x10^{-3}$	$2.35x10^{-2}$	$3.42x10^{-3}$	$5.76x10^{-3}$
sk	$1.49x10^{-4}$	$1.97x10^{-4}$	$3.40x10^{-3}$	$5.11x10^{-4}$

: use spss/pc for calculation these statistic values.