Chapter II

Experimental

Materials

The following materials were obtained from commercial sources.

1. **Model Drug**
   - Famotidine (ParEKH chemicals, Switzerland)

2. **Carriers**
   - PVP 12 PF (BASF, Germany)
   - PVP 17 PF (BASF, Germany)
   - PEG 6000 (supplied by Pharmaceutical Traders, Thailand)
   - D- Mannitol (Fluka, Germany)
   - D- Sorbitol (Fluka, Germany)
   - D- Glucose (Fluka, Germany)
   - Xylitol (RS., Germany)
   - Glycine (Merck, Germany)
   - Citric acid anhydrous (Srichand United Dispensary Co., Ltd., Thailand)

3. **Tablet Additives**
   - Dibasic calcium phosphate (Emcompress®, Abright & Wilson Ltd, USA.)
   - Sodium starch glycolate (Explotab®)

- Microcrystalline cellulose (Avicel PH 101 [®], Asahi chemical
industry , Japan)
- Magnesium stearate (Supplied by Pharmaceutical Sciences,
Thailand)
- Talcum (Haicheng , China)

4. Others

- Sulphamerazine (China national chemicals , China)
- N-N Dimethyl formamide (Fluka, Germany)
- L-aspartic acid (Merck, Germany)
- Sodium acetate anhydrous (Carlo Erba, Germany)
- Methanol, analytical grade (Baker, USA.)
- Acetonitrile, analytical grade (Baker, USA.)
- Acetic acid (Merck , Germany)

All materials were used without further purification except tablet additives which were dried at 60 ° C for three hours before use.

Methods

1. Preparation of Famotidine Solid Dispersions

1.1 Melting Method. Famotidine and carriers were accurately weighed in the ratios as shown in Table 2 . Each preparation of drug and carrier was physically mixed in glass motors and transferred to evaporators. These physical mixtures were heated directly with constant stirring on a hot plate untill they were melted.

Table 2 Weight of drug and carrier used in preparing physical
mixtures and solid dispersions .

Drug carrier ratio*	Weight of (mg)	
	Famotidine	Carrier
Famotidine:Mannitol (1:1)	50	50
Famotidine:Mannitol (1:2)	50	100
Famotidine:Mannitol (1:5)	50	250
Famotidine:Mannitol (1:10)	50	500
Famotidine:Mannitol (1:20)	50	1000
Famotidine:Mannitol (1:30)	50	1500
Famotidine:Mannitol (1:40)	50	2000
Famotidine:Sorbitol (1:1)	50	50
Famotidine:Sorbitol (1:2)	50	100
Famotidine:Sorbitol (1:5)	50	250
Famotidine:Sorbitol (1:10)	50	500
Famotidine:Sorbitol (1:20)	50	1000
Famotidine:Sorbitol (1:30)	50	1500
Famotidine:Sorbitol (1:40)	50	2000
Famotidine:Xylitol (1:1)	50	50
Famotidine:Xylitol (1:2)	50	100
Famotidine:Xylitol (1:5)	50	250
Famotidine:Xylitol (1:10)	50	500
Famotidine:Xylitol (1:20)	50	1000
Famotidine:Xylitol (1:30)	50	1500
Famotidine:Xylitol (1:40)	50	2000

* tripicate

The obtained masses were kept in a desiccator untill constant weight was obtained. The dry dispersions were pulverized in a glass motar with a pestle and passed through a 80-mesh sieve. The dispersions were kept in the desiccator for further studies.

      1.2 <u>Solvent Method.</u> Famotidine and carriers were accurately weighed in amount as shown in Table 2 . The famotidine was then dissolved in sufficient volume (based on its solubility) of glacial acetic acid and the carrier was added and mixed thoroughly. This solution was transferred to a folded piece of foil and the solvent was subsequently removed under vacuum in a desiccator. When evaporation was complete, the resulting coprecipitate was scraped from the foil using a microspatula and kept in a desiccator for at least 24 hours untill constant weight was obtained. The dispersions were ground and sieved through the 80-mesh sieve and kept in the desiccator for further studies.

Assay for the Content of Famotidine

All carriers did not interfere with the determination at the wavelength used.

1. Standard Curve for Famotidine in Phosphate Buffer.

About 150 mg. of famotidine was accurately weighed and dissolved in 100 ml. of 0.1 M phosphate buffer pH 4.5 . Appropriate dilutions were made to obtain standard solutions of known concentrations. The absorbances of these solutions were determined

in a 1cm.cell at the wavelength of **265 nm** with uv spectrophotometer, using the same medium as a blank. Absorbances obtained versus known concentrations were fitted to a straight line using linear regression analysis .

2. <u>Standard Curve for Famotidine by HPLC.</u>

The HPLC method for famotidine was modified from the published method of Parasrampuria and Gupta (1989). The HPLC was performed by using an equipment of Water Associated reverse phase column (μ-bondapack c 18 , 10 μ, 300x39 mm. i.d.). Condition was as follow :

Mobile Phase	:	2.5 % methanol, 2 % acetronitrile and 0.1 % acetic acid in 0.01 M KH_2PO_4 aqueous buffer solution.
Standard Soultion	:	Famotidine in dimethyformamide
Internal Standard	:	Sulfamerazine in methanol
Flow Rate	:	2.5 ml/min
Retention Time	:	Famotidine 9 min, Sulfamerazine 13 min
Injection Volume	:	20 μl
Peak Threshold	:	200
Chart Speed	:	0.25 cm/min
Wave Length	:	267 nm
Pump	:	Water Model 510
Detector	:	Water Associate Model 440
Integrator	:	Water 740 Data Model

3. Solubility Determination

Solubility studies were carried out on famotidine, famotidine-carrier physical mixtures and solid dispersions by a known excess of the sample to 10 ml. water in 20 ml. screw-capped test tube mounted on a top to bottom shakers at the temperature of 37 ± 1 ° C. After the equilibration (4 h.), the solution was filtered through 0.22 μm filter (Swinex-25 , Millipore Filter Corp., Bedford, MA U.S.A). Drug concentration in each of the sample was determined spectrophotometrically at 267 nm after appropriate dilution with 0.1 M phosphate buffer pH 4.5 . Solubility determination were carried out in duplicate.

4. Determination of Physicochemical Properties of Solid Dispersions

The following physicochemical properties of the selected solid dispersions and physical mixtures were determined and compared with the pure famotidine and carriers.

a. Infrared (IR) Absorption Studies.

IR spectrum of the samples were recorded. They were in the form of KBr disks, which had been stored in a desiccator at ambient temperature before analysis (model 1760x Perkin Elmer, USA.).

b. Differential Thermal Analysis (DTA)

DTA thermograms were obtained on a thermal analyser, with a heating rate 10 $^\circ$ C / minute, sensivity $\pm$ 50 mcV., and open cell in static air (Shimadzu, Japan)

c. Scanning Electron Microscopy (SEM)

Electron photomicrographs of powder samples were taken with scanning electron microscope (JSM-T 220 A, JEOL, Japan). The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape, and surface topography of the powder samples were observed.

d. Powder X-Ray Diffraction Studies.

The crystallinity of the samples were examined by x-ray diffractometry (JDX-8030, JEOL, Japan). They were firmly packed into the cavity of a thin rectangular metal plate using two glass slides which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, before taken the prepared sample to expose the x-ray in the x-ray diffraction chamber. The x-ray diffraction patterns were recorded at the rate of 80 $^\circ$ per minute from 5 $^\circ$ to 105 $^\circ$ in the term of 2 θ angle.

5. Preparation of Famotidine Tablets

From the soluility studies of all solid dispersions, carrier that could most highly increase the solubility of famotidine was selected for further investigation on preparation of tablet products.

The composition of all formulation for a 250 mg. tablet was as follows :

	% w/w
Famotidine	8
Mannitol	80
Emcompress [®]	4
Avicel PH 101 [®]	4
Explotab [®]	1
Magnesium stearate	1
Talcum	2

Physical Mixture : Famotidine and mannitol were physically mixed before the other tablet additives were added.

Solid Dispersions : Famotidine and mannitol were solidified by the fusion method , then the other tablet additives were added.

All tablet ingredients were compressed to tablet by a caver press (model C, Perkin Elmer, USA.), using 4 mm. diameter-punch.

5.1 Evaluation of Tablets

The following physical properties of tablets were examined.

5.1.1 Weight Variation

Twenty tablets of each sample were individually weighed , using an analytical balance (Satorious , Germany). The average and standard variation were examined.

5.1.2 Tablet Thickness

The thickness of tablets were examined by using a micrometer (Teclock Corp., Japan) and expressed in millimeter. The thickness value was an average of six determinations.

5.1.3 Tablet Hardness

The hardness of compressed tablets were determined by using a hardness tester (Schleuniger-2E, Switzerland). The hardness recorded was the average of six determinations.

5.1.4 Disintegration Time

The disintegration time was measured by using the apparatus according to USP XXII (Hanson-Research , USA.) with deionized water as disintegration fluid. The test was performed without disks. This value was measured in seconds. The mean of six determinations of each sample was presented.

5.1.5 Dissolution Studies

The famotidine tablets prepared from the selected famotidine solid dispersion were observed for dissolution behavior.

Dissolution tests of famotidine, famotidine-carrier physical mixture, solid dispersions and three brands of commercial tablet products were carried out in 900 ml. of 0.1 M phosphate buffer pH 4.5 equilibrated at $37 \pm 0.5\,^\circ$ C, using the USP. XXII dissolution apparatus type II (paddle) at the rate of 50 ± 1rpm(Hanson Research Corp.,USA.). Famotidine powder 20 mg., physical mixture, solid dispersions (pressed as tablet) and the commercial tablet products containing equivalent amount of the pure drug, were introduced in each vessel. Five mililiter of samples were collected, at the interval of 3, 5, 7, 9, 11, 15, 20, 25 and 30 minutes, then filtered through glass filter and analyzed for drug content by the spectrophotometer as described in 1(page 44). The volume withdrawn each time were then replaced by equivalent amount of temperature equilibrated 0.1 M phosphate buffer pH 4.5 to maintain a constant volume of dissolution medium during the course of the test. The amount of famotidine dissolved was calculated from the calibrated concentration curve as described earier. The mean of six determinations of each sample was presented. The dissolution profile was obtained by plotting the percent of famotidine dissolved against time.

6. Preparation of Lyophilized Products

The composition of one vial of freeze-dried product was as follows :

Famotidine	20	mg
Carrier	20 or 40	mg
vehicle	qs	

In this study, drug carrier ratio, type of carrier and buffer system were varied in order to select the best formulation to produce the famotidine lyophilized powder.

Drug Carrier Ratio

Two drug carrier ratios , 1:1 and 1:2 were investigated in comparison. The ratio that provided better physical appearance and more easily dissolved products was selected for further study.

Type of Carrier

Carriers used were PVP 12 PF, PEG 6000, glycine, mannitol, sorbitol, xylitol, glucose and citric acid anhydrous.

Vehicle System

The following vehicle systems were used :
- acetate buffer pH 5.5 ,   6 ml
- acetate buffer pH 3.7 ,   2 ml
- phosphate buffer pH 4.5 , 5 ml
- L- aspartic acid pH 3.7 ,   5 ml
- L- aspartic acid pH 3.3 ,   2 ml

Preliminary study was performed in   order to select the suitable vehicle system,   drug carrier ratio and type of carrier. In preparing solution for freeze dried process, 1:1 or 1:2 drug - carrier were dissolved in the least amount of vehicle that can obtain clear solution. Then, the preparation was under gone by the following procedure.

The filled vials were stoppered with siliconed , notched butyl rubber closers in the raised position (model 228060 F, Labconco, USA.). The shelf temperature, the product temperature, the chamber pressure, and the electric resistant of the product were monitored during the freezed drying cycle. During the freezing stage, product temperature was kept lower than the eutectic temperature as determined by measurement of electric resistivity and product temperature. The product temperature was about $-38\ ^\circ$ C, when the shelves of the lyophilzer were kept at $-40\ ^\circ$ C for 2 hours to ensure freezing of the liquid beyond the effects that precooling might induced. After solidification of the sample was confirmed, a vacuum

was drawn. The samples were lyophilized for about 30 hr. At the conclusion of the drying cycle, with the product temperature at about 25 $^\circ$ C, the evacuated chamber was vented with dry nitrogen, and the vials closed with the stoppers inside the lyophilizer. After stoppering, the chamber was open and the vials were sealed with crimped aluminum caps.

The selection of suitable preparation of the freeze dried product was based on the appearance of the obtained cake, reconsition time, pH of solution after diluted with 5 ml of water and the duration of time used in the whole lyophilization process.

After appropriate systems were selected , the chosen formulations were prepared and the solution was sterilized by filtering through the 0.22 μm membrane filter using vacuum filtration ,about 400 filled vials were obtained. Then, the vials were placed in the lyophilizer as the procedure mentioned above untill dry cake was observed. The prepared lyophilized products were subsequently checked for their physicochemical properties.

6.1 Evaluation of Lyophilized Products

6.1.1 Reconstitution Time

Lyophilized products were dissolved in 5 ml. water. Time used when the product was completely dissolved was recorded. The reconstitution time was observed in duplicate every month for three months.

6.1.2 pH and Osmolality Determination

The acidity changes and the osmolality (model 030-D, P. Intertrade, Germany) of the solution were checked every month for three months.

6.1.3 Stability Study

a. The lyophilized powder products were kept in the room temperature . Percent of famotidine content remains was determined every month for three months.

b. The products of famotidine lyophilized powder were kept at 45 ° C ,75 % RH. The drug concentration left was analyzed every month for three months.The experimental data were compared with those of the commercial products.

c. The lyophilized products were dissolved in 5 ml water and Incubated under accelerated temperature; 45 ° C, 55 ° C and 65 ° C. Samples were taken at the appropiated time and the content remains was determined.

Each a , b and c procedure was analyzed by the HPLC method which described earlier (page 42).

The data were treated as zero order , first order and second order reaction, and the concentration-time profile was plotted. The correlation coefficient was calculated, and the order of reactions was statistically analyzed. The shelf-life was calculated by the method of Arrhenius equation .