CHAPTER II

MATERIALS AND METHODS

1. Materials

The following substances were obtained from commercial sources.

1.1 Model drugs
- Griseofulvin (China National Chemicals Import and Export Co., Shanghai, China)
- Prednisolone (Upjohn Co., USA)
- Furosemide (Pharmaceutical and Chemical Work Ltd., Budapest, Hungary)

1.2 Additives

1.2.1 Diluents
- Lactose direct compress (Meggle Milch Industrie GMBH & Co. KG)
- Dicalcium phosphate dihydrate (MAPA Aussenhandels Gesellschaft. m.b.H., Germany)

1.2.2 Binding agent
- Gelatin powder (Tsuka Chem. Co., Ltd., Japan)

1.2.3 Disintegrating agents
- Ac-Di-Sol^{R} (FMC Corporation, Philadelphia, USA)
- Avicel^{R} PH 101 (FMC Corporation, Philadelphia, USA)
- Polyplasdone^{R} XL (GAF Corporation, USA)

- Explotab^{R} (AVEBE Chemical, Holland)

1.2.4 Lubricating agents

- Magnesium stearate (Durham Chem, Ltd., England)
- Cab-O-sil (Cabot Co., Germany)

1.3 Dissolution medium

0.02 % polysorbate 80 in 1:100 HCl in aqueous solution.

- Polysorbate 80 (Chemical House, Thailand)
- Hydrochloric acid (analytical grade, E. Merck, Darmstadt, Germany)

2. Methods

Griseofulvin[56], prednisolone[57], and furosemide[58] were identified and analysed to pass requirement.

2.1 Preparation of Tablets

The formulations of experimental griseofulvin tablets, prednisolone tablets, and furosemide tablets were demonstrated in table 1, 2, and 3, respectively.

2.1.1 Wet Granulation Method

Preparation of Granules

The drug and diluents employed in each formulation were passed individually through a 40 mesh screen to break agglomerate and dried at 50^{o}C for one hour before used. The certain amount of drug, diluents and one-half of the amount of disintegrant used in the formulation were weighed and mixed thoroughly by motar and pestle for five minutes. The mixture was kneaded into damp mass with binding agent for five minutes. The binding agent was 10 % $^{w}/_{w}$ gelatin solution in water, prepared by dispersion method and dissolving gelatin powder in warm water.

Table 1 The Formulations of Experimental Griseofulvin Tablets

Ingredients	Amount of the ingredients (mg) in the formulation number						
	# 1	# 2	# 3	# 4	# 5	# 6	#7
Griseofulvin micropowder	125.00	125.00	125.00	125.00	125.00	125.00	125.00
Lactose direct compress	87.50	87.50	87.50	87.50	87.50	87.50	87.50
Dicalcium phosphate dihydrate	87.50	87.50	87.50	87.50	87.50	87.50	87.50
Ac-Di-Sol^{R}	-	0.5 %	1.0 %	2.0 %	3.0 %	4.0 %	5.0 %
Gelatin, dry basis	3.08	3.08	3.08	3.08	3.08	3.08	3.08
Magnesium stearate	3.18	3.18	3.18	3.18	3.18	3.18	3.18

Note : Wet granulation used 10 % gelatin solution as binding agent

Table 2. The Formulations of Experimental Prednisolone Tablets.

Ingredients	Amount of ingredients (mg) in the formulation number						
	# 8	# 9	# 10	# 11	# 12	# 13	# 14
Prednisolone powder	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lactose direct compress	125.00	125.00	125.00	125.00	125.00	125.00	125.00
Ac-Di-Sol^{R}	-	0.5 %	1.0 %	2.0 %	3.0 %	4.0 %	5.0 %
Gelatin, dry basis	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Magnesium stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cab-O-sil	0.20	0.20	0.20	0.20	0.20	0.20	0.20

Note : Wet granulation used 10 % gelatin solution as binding agent.

Table 3. The Formulations of Experimental Furosemide Tablets

Ingridients	Amount of the ingredients (mg) in the formulation number						
	# 15	# 16	# 17	# 18	# 19	# 20	# 21
Furosemide powder	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Dicalcium phosphate dihydrate	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ac-Di-Sol^{R}	-	0.5 %	1.0 %	2.0 %	3.0 %	4.0 %	5.0 %
Gelatin, dry basis	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Magnesium stearate	1.49	1.49	1.49	1.49	1.49	1.49	1.49

Note : Wet granulation used 10 % gelatin solution as binding agent.

The amount of binding agent used in the preparation was listed in table 1, 2, and 3. The damp mass was then passed through a 12 mesh screen for griseofulvin and a 16 mesh screen for prednisolone and furosemide. The granules were tray dried in a drying oven "Memmert TV 400 UL 998001" for six hours at 50°C. The dried granules were again passed through a 16 mesh screen for griseofulvin and a 20 mesh screen for prednisolone and furosemide.

The granules, the other half of disintegrant, and lubricants were mixed thoroughly by manual bottle tumbling method.

2.1.2 Dry Granulation Method

Drug and diluents employed in each formulations were prepared as in the wet granulation method, except that the binder was used in dry powder. The amount of dry binder used was equal to the corresponding formulations in wet granulation method. The mixture was slugged and then the slugs were passed through a 16 mesh screen for griseofulvin and a 20 mesh screen for prednisolone and furosemide by an oscillating granulator "KSL". The granules, the other half of disintegrant, and lubricant were then mixed thoroughly by manual bottle tumbling method.

2.1.3 Compression of Tablets

Tablets were compressed on a Stokes' single punch tablet machine, model A, series A 89644 Lot. No. A 86935, USA, using slightly concave, $\frac{12}{32}$ inch punches for griseofulvin and $\frac{8}{32}$ inch punches for prednisolone and furosemide. The hardness of tablets were adjustted to about 5-7 kp and 9-11 kp for griseofulvin tablets, 1-2 kp and 3-5 kp for prednisolone tablets, and 4-6 kp and 8-11 kp for

furosemide tablets.

2.1.4 Preparation of Tablets Containing Different Disintegrants.

The formulations of tablets containing various disintegrants were demonstrated in table 4. Avicel^{R} PH 101, Polyplacdone^{R} XL, and Explotab^{R} were used as tablet disintegrants at the concentration of 1 %. The amount of diluents and lubricants were constantly maintained in the formulation for each drug. The tablets were prepared by the same procedures as in wet granulation and dry granulation.

2.1.5 Preparation of Tablets by Different Method of Incorporating Disintegrant

One % of Ac-Di-Sol^{R} was used as disintegrant in the formulation. The amount of diluents and lubricants were constantly maintained in the formulation for each drug. The tablets were prepared by the same procedure as wet granulation. The disintegrant was incorporated by three different methods : intragranular, extragranular, and 50 % intragranular plus 50 % extragranular.

2.2 Evaluation of Tablets

2.2.1 Weight Variation of Tablets

Individual weight of 20 tablets was determined on an analytical balance, Sartorius, type 2442, Lot. No.194677. The average weight, standard deviation, and coefficient of variation were calculated.

Table 4. Experimental Formulations of Three Drugs With Different Disintegrants

Ingredients	Amount of the ingredients (mg) in the formulation number								
	# 22	# 23	# 24	# 25	# 26	# 27	# 28	# 29	# 30
Griseofulvin micropowder	125.00	125.00	125.00	-	-	-	-	-	-
Prednisolone powdor	-	-	-	5.00	5.00	5.00	-	-	-
Furosemide powder	-	-	-	-	-	-	40.00	40.00	40.00
Lactose direct compress	87.50	87.50	87.50	125.00	125.00	125.00	-	-	-
Dicalcium phosphate dihydrate	87.50	87.50	87.50	-	-	-	100.00	100.00	100.00
Gelatin, dry basis	3.08	3.08	3.08	0.51	0.51	0.51	1.85	1.85	1.85
Avicel^{R} PH 101	1.0 %	-	-	1.0 %	-	-	1.0 %	-	-
Polyplasdone^{R}XL	-	1.0 %	-	-	1.0 %	-	-	1.0 %	-
Explotab^{R}	-	-	1.0 %	-	-	1.0 %	-	-	1.0 %
Magnesium stearate	3.18	3.18	3.18	0.50	0.50	0.50	1.49	1.49	1.49
Cab - O - sil	-	-	-	0.20	0.20	0.20	-	-	-

Note : Wet granulation used 10 % gelatin solution as binding agent

2.2.2 Hardness of Tablets

Ten tablets were randomly selected and subjected to a hardness tester, Schleuniger-2E, model 2E / 205. The mean, standard deviation and coefficient of variation were calculated.

2.2.3 Friability of Tablets

Twenty tablets were randomly selected and subjected to an Erweka friabilator, type TAP. No 27635 for five minutes. The total weight of 20 tablets were determined on an analytical balance before and after test. The percentage of friability was calculated.

2.2.4 Disintegration Time of Tablets

The disintegration times of tablets were determined in 1 : 100 HCl in aqueous solution using Manesty tablet disintegration tester, TD 63T170 (USP XX method). The mean of six determinations for each batch was calculated.

2.2.5 Dissolution Study

The dissolution studies were conducted by USP dissolution type I method[59]. A 900 ml of 0.02 % polysorbate 80 in 1:100 HCl in aqueous solution was used as dissolution medium, which was maintained at 37° C. Previous studies showed that this medium was more closely resemble the surface tension of G I fluids than distilled water[60]. The present of surfactant prevented aggregates of pure drug from floating on the surface of dissolution medium. The basket, containing one tablet, was rotated at the speed of 100 rpm. Five milliliters samples were withdrawn by a syringe at various time interval and then filtered by a 0.45 millimicron membrane filter. The absorbances of samples were determined by ultraviolet spectrophotometer,

Bausch and Lomb, spectronic^{R} 2000 at the maximum wavelength of the drug being studied, 292.8 NM for griseofulvin, 246.3 NM for prednisolone, and 232.3 NM for furosemide and the contents were calculated from the absorbance concentration relationships in table 5, 6 and 7, respectively.

To maintain a constant volume of dissolution medium, a five millititers of fresh medium was replaced after removal of each sample. The reported data were averaged of at least duplicate dissolution runs.

Table 5. The Absorbance of Standard Solution of Griseofulvin in 1:100 HCl in Aqueous Solution + 0.02 % polysorbate 80 at 292.8 NM.

Concentration, mcg/ml	Absorbance
2.4	0.159
4.8	0.320
7.2	0.481
9.6	0.640
12.0	0.789

Table 6. The Absorbance of Standard Solution of Prednisolone in 1:100 HCl in Aqueous Solution + 0.02 % polysorbate 80 at 246.3 NM.

Concentration, mcg/ml	Absorbance
2.4	0.113
4.8	0.221
9.6	0.442
14.4	0.654
19.2	0.865

Table 7. The Absorbance of Standard Solution of Furosemide in 1:100 HCl in Aqueous Solution + 0.02 % polysorbate 80 at 232.3 NM.

Concentration, mcg/ml	Absorbance
4.8	0.435
6.0	0.538
7.2	0.641
8.4	0.756
9.6	0.859