CHAPTER II

MATERIALS AND METHODS

1. Materials

The following substances were obtained from commercial sources.

1.1 Model drug - Dipyrone (Sulpyrin J.P., Chemexco, West Germany)

1.2 Additives

1.2.1 Diluent
- Corn Starch (C.P., England)
- Lactose B.P. (Vehgel, Holland)

1.2.2 Binding agent
- Corn Starch (C.P., England)
- Gelatin 250 bloom (Australia)
- Polyvinylpyrrolidone (PVP K-30, GAF Corporation, U.S.A.)

1.2.3 Disintegrating agent
- Corn Starch (C.P., England)
- Sodium Alginate B.P.C. (Wright Layman & Umney Ltd., England)
- Sodium Carboxymethylcellulose (Nymcel®, FMC Corporation, Japan)
- Sodium Starch Glycolate (Carboxymethyl Starch, Explotab®, E. Mendell Inc., U.S.A.)

1.2.4 Lubricating agent
- Magnesium Stearate B.P. (Durham Chemical Ltd., England)

- Talc B.P. (Shin Industial, Korea)

1.3 Dissolution medium - Hydrochloric acid, analytical grade (Reidal-De Haën AG Seelze-Hannover, Germany)

2. Methods

Dipyrone powder was identified and analysed to pass the requirement of the Pharmacopoeia of Japan[28] before used.

2.1 Preparation of Tablets for Dissolution Studies

The formulations of experimental dipyrone tablets in this study are shown in Tables 1 and 2.

All the materials were passed through 60-mesh sieve to break up any agglomerate and dried at 60° for 6 hours before used. The tablets were prepared according to the following procedures :-

2.1.1 Preparation of Granules

The amount of dipyrone and diluent employed in the formulation were weighed and mixed thoroughly in a V-shape Dry Mixer Machine (Kan Seng Lee Machinery (1960) Ltd., Part., Thailand) for 15 minutes. After dry mixing, the mixture was transfered to a Planetary Mixer (Model E B20F, Crypto-Peerless Ltd., England). The mixture was kneaded into a suitable damp mass with binding agent for 10 minutes (amount of binding agent listed in the Tables 1 and 2 were the amount of dry mass of binding agent). The damp mass was passed through 12-mesh sieve and dried in a Hot Air Oven (Lytzen Oven, Copenhagen, Lyngby-Denmark) at 60° for 6 hours. After drying the dried granules were again passed through 12-mesh sieve by using an Oscilla-

Table 1 Formulation of Experimental Dipyrone Tablets Containing Starch as Diluent.

Ingredients mg/tab	Formula														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dipyrone	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
Corn Starch	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Corn Starch Paste	14.00	14.00	14.00	14.00	14.00	-	-	-	-	-	-	-	-	-	-
Polyvinylpyrrolidone	-	-	-	-	-	11.00	11.00	11.00	11.00	11.00	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-	-	11.00	11.00	11.00	11.00	11.00
Corn Starch	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-	-	-
Sodium Alginate	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-	-
Sodium Carboxymethylcellulose	-	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-
Sodium Starch Glycolate	-	-	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00
Talc	17.22	17.97	17.97	17.97	17.97	17.13	17.88	17.88	17.88	17.88	17.13	17.88	17.88	17.88	17.88
Magnesium Stearate	5.47	5.99	5.99	5.99	5.99	5.71	5.96	5.96	5.96	5.96	5.71	5.96	5.96	5.96	5.96

Table 2 Formulation of Experimental Dipyrone Tablets Containing Lactose as Diluent.

Ingredients	Formula														
mg/tab	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Dipyrone	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
Lactose	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Corn Starch Paste	12.00	12.00	12.00	12.00	12.00	-	-	-	-	-	-	-	-	-	-
Polyvinylpyrrolidone	-	-	-	-	-	9.50	9.50	9.50	9.50	9.50	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-	-	9.50	9.50	9.50	9.50	9.50
Corn Starch	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-	-	-
Sodium Alginate	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-	-
Sodium Carboxymethylcellulose	-	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00	-
Sodium Starch Glycolate	-	-	-	-	25.00	-	-	-	-	25.00	-	-	-	-	25.00
Talc	17.16	17.91	17.91	17.91	17.91	17.08	17.84	17.84	17.84	17.84	17.08	17.84	17.84	17.84	17.84
Magnesium Stearate	5.32	5.97	5.97	5.97	5.97	5.90	5.94	5.94	5.94	5.94	5.70	5.94	5.94	5.94	5.94

ting Granulator (Kan Seng Lee Machinery (1960) Ltd., Part., Thailand).

The binding agents were, corn starch paste in the concentration 10% W/W in water, gelatin solution 10% W/W in water (prepared by dissolving the required quantity in warm water and used during its still warm solution), polyvinylpyrrolidone solution 10% W/W in water (prepared by dissolving the required quantity in water), and used respectively.

The granules, disintegrant, talc and magnesium stearate were mixed thoroughly by manual bottle tumbling method. The amount of disintegrant was used at the concentration of 5% of dipyrone in the granules. The amount of talc and magnesium stearate were maintained constant at the concentration of 3% and 1% of granules, respectively.

2.1.2 Compression

Tablets were compressed on a Single Punch Tablet Machine (Kan Seng Lee Machinery (1960) Ltd., Part., Thailand) using a bisected flat-face 1/2-inch punch. The hardness of tablets were adjusted to about 5-6 kg. and 9-10 kg. for each formulation, respectively.

2.2 Factorially Designed Experiment

2.2.1 At an intial stage, the dissolution times ($t_{90\%}$) of tablets were used as indices to comparatively indicate the different effects of diluents, binding agents, and disintegrating agents by statistical methods. At later stage, single optimum additive of each type were chosen by using statistical and economical evaluation.

The following additives were selected to be used in the

factorially designed experiment, i.e. :- corn starch as diluent, polyvinylpyrrolidone as binding agent and sodium carboxymethylcellulose as disintegrating agent. The factorially designed experiment was carried out to study the effects produced by the diluent, binding agent, and disintegrating agent singly and in combinations. The various formulations include two levels of corn starch (5% and 15% of the amount of dipyrone), two levels of polyvinylpyrrolidone (10% and 20% W/W solution) and two levels of sodium carboxymethylcellulose (1% and 5% of the amount of dipyrone). Detailed formulations are shown in Table 3.

The tablets were prepared by the same procedure as above. The hardness of tablets were adjusted to about 5-6 kg.

2.2.2 The dipyrone tablets prepared from formular E were selected for further study of the effect produced by the interaction of talc and magnesium stearate by factorially designed experiment. The formulations employed include two levels of talc (3% and 5%) and two levels of magnesium stearate (0.3% and 1.0%), the granules were blended with high and low level of lubricants as shown in Table 4. The compressed tablets possessed hardness of about 5-6 kg.

2.3 Preparation of Tablets Containing Various Concentrations of Diluent, Binding Agent and Disintegrating Agent

Detailed formulations of tablets are shown in Tables 5 and 6. Corn starch was used as diluent at the concentration of 5%, 10% and 15%, respectively. Polyvinylpyrrolidone solution was used as binding agent at the concentration of 10% and 20% W/W. Sodium carboxymethyl-

Table 3 Formulation of Experimental Dipyrone Tablets for Factorially Designed Experiment.

Ingredients mg/tab	Formula							
	A	B	C	D	E	F	G	H
Dipyrone	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
Corn Starch	25.00	25.00	25.00	25.00	75.00	75.00	75.00	75.00
Polyvinylpyrrolidone	7.00	7.00	21.50	21.50	10.00	10.00	22.00	22.00
Sodium Carboxymethylcellulose	5.00	25.00	5.00	25.00	5.00	25.00	5.00	25.00
Talc	16.11	16.71	16.50	17.15	17.70	18.30	18.05	18.65
Magnesium stearate	5.37	5.57	5.50	5.70	5.90	6.10	6.02	6.22

Table 4 Combination of Talc and Magnesium Stearate Mixed with Granules.

Concentration, % of granules	
Talc	Magnesium stearate
3.0	0.3
5.0	0.3
3.0	1.0
5.0	1.0

Table 5 Formulation of Experimental Dipyrone Tablets.

Ingredients mg/tab	Formula								
	I	II	III	IV	V	VI	VII	VIII	IX
Dipyrone	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
Corn Starch	25.00	25.00	25.00	50.00	50.00	50.00	75.00	75.00	75.00
Polyvinylpyrrolidone (10% W/W)	7.00	7.00	7.00	8.70	8.70	8.70	10.00	10.00	10.00
Sodium Carboxymethylcellulose	5.00	15.00	25.00	5.00	15.00	25.00	5.00	15.00	25.00
Talc	16.11	16.41	16.71	16.91	17.21	17.51	17.70	18.00	18.30
Magnesium Stearate	5.37	5.47	5.57	5.64	5.74	5.84	5.90	6.00	6.10

Table 6 Formulation of Experimental Dipyrone Tablets.

Ingredients mg/tab	Formula								
	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
Dipyrone	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
Corn Starch	25.00	25.00	25.00	50.00	50.00	50.00	75.00	75.00	75.00
Polyvinylpyrrolidone (20% W/W)	21.50	21.50	21.50	20.00	20.00	20.00	22.00	22.00	22.00
Sodium Carboxymethylcellulose	5.00	15.00	25.00	5.00	15.00	25.00	5.00	15.00	25.00
Talc	16.54	16.84	17.14	17.25	17.55	17.85	18.06	18.36	18.66
Magnesium Stearate	5.51	5.62	5.72	5.75	5.85	5.95	6.02	6.12	6.22

cellulose was used as disintegrating agent at the concentration of 1%, 3% and 5%, respectively. The amounts of talc and magnesium stearate were constantly maintained at 3% and 1% of granules, respectively, in all formulations.

The tablets were prepared by the same procedure as above. The hardness of tablets were adjusted and kept at about 5-6 kg.

2.4 Evaluation of Tablets

2.4.1 Weight Variation of Tablets

Individual weight of 20 tablets were determined on an Analytical Balance (Beckers Sons Analytical Balance Type R 0.1, Brummen, Holland). The average weight, standard deviation, and the coefficient of variation were determined.

2.4.2 Hardness of Tablets

Ten tablets were randomly selected and subjected to Monsanto Tablet Hardness Tester. The mean, standard deviation, and coefficient of variation of hardness were determined.

2.4.3 Disintegration Time of Tablets

The disintegration time of the tablets was determined in 0.1 N HCl at $37^{\circ} \pm 0.5^{\circ}C$ in a Tablet Disintegration Tester (Erweka All-Purpose Disintegration Tester Type ZT 2, Erweka-Apparatebau GMBH, West Germany) by the United State Pharmacopoeia Method[3]. The tablets were considered completely disintegrated when all the particles pass through the wire mesh. The average of six tablets determination was reported in each case.

2.4.4 Friability of Tablets

Twenty tablets were subjected to the Friabilator (Roche Type, Germany) for 5 minutes. The total weight of the tablets were determined on an Analytical Balance both before and after the test. The friability was determined by the equation :

$$\% \text{ Friability} = \frac{\text{Weight lose}}{\text{original weight}} \cdot 100$$

2.4.5 Percent Labeled Amount of Tablets

The tablets were assayed by the method of Varcel[29] as follow :

Weigh and finely powdered 20 Dipyrone Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg. of dipyrone, to a 100-ml. volumetric flask. Add about 80 ml. of water and shake the mixture until the dipyrone has dissolved. Dilute to volume with water, and mix well. Filter, rejecting the first 15 ml. of filtrate. Pipet 10 ml. of the filtrate into a 100-ml. volumetric flask, dilute to volume with water and mix well. Pipet 5 ml. of the solution into a 100-ml. volumetric flask, add 10.0 ml. of 1 N HCl and dilute to volume with water. Determine the absorbance of the solution at the wavelength at 260 nm., with a Spectrophotometer (The Bausch & Lomb Spectronic® 710 Spectrophotometer, Rochester, New York, U.S.A.), using 0.1 N HCl as the blank. The concentration of the solution was determined by comparison with the absorbance of a standard solution of dipyrone and the quantity of dipyrone per tablet was determined.

2.4.6 Dissolution Time of Tablets

Dissolution time of tablet was determined using a Dissolution Test Apparatus Type DT (Erweka-Apparatebau GMBH, Frankfurt, West Germany).

Procedure :- 500 ml. of 0.1 N HCl was placed in the vessel and permitted to equilibrate to $37^{\circ} \pm 0.5^{\circ}C$. Place a tablet in the basket and immerce the basket into the vessel. At the moment of contact between tablet and the dissolution medium, the motor and the timer were started simultaneously. The basket was rotated at the speed of 100 rpm.

At various suitable time intervals, 1 ml. of the sample solution was withdrawn by a sampling pipette. The same quantity of 0.1 N HCl was added immediately after each sampling to keep the volume of dissolution medium constant during the course of the test. The dissolution experiment was conducted until the time required for 90% of dipyrone to dissolve could be read from the dissolution profile. The amount of dipyrone in each sample was analyzed spectrophotometrically as described later.

The dissolution profile was obtained by plotting the percent of dipyrone dissolved against time. Time required for 90% of dipyrone to dissolve was read from the dissolution profile. The average of four determinations was reported for each formulation.

Analytical Method :- The standard concentration-absorbance curve of dipyrone was constructed by preparing the standard solution at the concentration of 0.50 , 1.00, 1.50, 2.00 , 2.50, 3.00

and 4.00 mg.% in 0.1 N HCl. The absorbance was measured at 260 nm. by the Bausch & Lomb Spectronic® 710 Spectrophotometer. The data are listed in Table 7. The slope of regression line is 0.25 per mg.%

The samples withdrawn were diluted with 0.1 N HCl to 25 ml., the absorbances were measured spectrophotometrically at 260 nm. by using 0.1 N HCl as blank. The concentration of the samples were determined by comparison with a standard curve and the amount of dipyrone dissolved at the specified time interval were calculated. A cumulative correction was made the previously removed sample in determined the total amount dissolved. Percent dipyrone dissolved was calculated based on the quantity of dipyrone per tablet from the assay process.

Table 7 The Absorbance of Standard Solution of Dipyrone in 0.1 N HCl at 260 nm.

Concentration, mg%	Absorbance
0.5	.133
1.0	.254
1.5	.381
2.0	.499
2.5	.617
3.0	.738
4.0	.978