CHAPTER II

MATERIALS AND METHODS

A. Materials

The following substances were obtained from commercial sources

1. Model drug

- Aspirin powder (Lot No. 4F 743, Monsanto Company, U.S.A.)

2. Additives

- Methylcellulose (Methocel A. 15LV Premium U.S.P. grade Lot MM 83102221 A
  Methocel A. 4C Premium U.S.P. grade Lot MM 83051611 A
  Methocel A. 15C Premium U.S.P. grade Lot MM 83013001 A
  Methocel A. 4M Premium U.S.P. grade Lot MM 83101602 A
  The Dow Chemical Company, U.S.A.)
- Polyvinylpyrrolidone (P.V.P. K-30, Batch No 65-2955 Pharmaceutical Science Ltd., Bangkok,Thailand.)
- Stearic acid (Batch No. 2A-1193, Pharmaceutical Science Ltd., Bangkok, Thailand.)
- Silinium tetrachloride (Aerosil 200, Batch No. 831127 Pharmaceutical Science Ltd., Bangkok, Thailand.)
- Absolute alcohol (Lot.No. 3262, Garantieanalyse/guarantee analysis, Germany.)

3. Dissolution media

- Hydrochloric acid (Lot No. 2622792, E. Merck Darmstadt, Germany.)

- Sodium chloride (Batch No. 6008782, B.D.H. Chemical Ltd., Poole, England)
- Sodium hydroxide (Batch No. 480507, Farmitalia Carlo Erba, Italy.)
- Potassium dihydrogen phosphate (Lot No. 49229, May & Baker Ltd., Dagenham, England)

B. Equipment

- Analytical balance (Sartorius, Model 2442, Germany.)
- Harrard trip balance (Ohaus Scale Corporation, U.S.A.)
- Morta and Pestle
- Seive No. 10,12,60 and 80 (Endecotts Ltd., England.)
- Hardness tester (Schleuniger, Model 2E/205, Dr. K. Schleuniger Co., Switzerland.)
- Micrometer (Teclock corporation, Japan.)
- Single punch (Viuhang Engineering, No 49, Thailand.)
- Disintegration apparatus (Manesty T.D. 65T170, Manesty Machines Ltd., England.)
- Dissolution apparatus U.S.P. type I (Hanson research corporation, Model 500-230, U.S.A.)
- Spectrophotometer (Spectronic 2000, Bausch & Lomb, U.S.A.)
- Scanning Electronmicroscope (JEOL, JSM-35CF, JEOL company, Japan.)

C. Preparation of Aspirin Tablets

1. Formulations

Aspirin tablets were formulated according to the tablets.

Table 5 : Composition of P.V.P. K-30 in aspirin tablets.

Ingredients	Formulation (mg/tab)				
	#1	#2	#3	#4	#5
Aspirin powder	650.0	650.0	650.0	650.0	650.0
P.V.P. K-30	6.5(1%)	19.5(3%)	32.5(5%)	45.5(7%)	65.0(10%)
Stearic acid	2.5%	2.5%	2.5%	2.5%	2.5%
Aerosil 200	0.5%	0.5%	0.5%	0.5%	0.5%

Table 6 : Composition of Methocel A - 15 LV and P.V.P. K-30 in aspirin tablets.

Ingredients	Formulation (mg/tab)			
	Control	#6	#7	#8
Aspirin powder	650.0	650.0	650.0	650.0
Methocel A-15LV	-	32.5(5%)	65.0(10%)	97.5(15%)
P.V.P. K-30	32.5	32.5	32.5	32.5
Stearic acid	2.5%	2.5%	2.5%	2.5%
Aerosil 200	0.5%	0.5%	0.5%	0.5%

Table 7 : Composition of Methocel A - 4C and P.V.P. K-30 in aspirin tablets.

Ingredients	Formulation (mg/tab)			
	Control	#9	#10	#11
Aspirin powder	650.0	650.0	650.0	650.0
Methocel A-4C	-	32.5(5%)	65.0(10%)	97.5(15%)
P.V.P. K-30	32.5	32.5	32.5	32.5
Stearic acid	2.5%	2.5%	2.5%	2.5%
Aerosil 200	0.5%	0.5%	0.5%	0.5%

Table 8 : Composition of Methocel A - 15C and P.V.P. K-30 in aspirin tablets.

Ingredients	Formulation (mg/tab)			
	Control	#12	#13	#14
Aspirin powder	650.0	650.0	650.0	650.0
Methocel A-15C	-	32.5(5%)	65.0(10%)	97.5(15%)
P.V.P. K-30	32.5	32.5	32.5	32.5
Stearic acid	2.5%	2.5%	2.5%	2.5%
Aerosil 200	0.5%	0.5%	0.5%	0.5%

Table 9 : Composition of Methocel A - 4M and P.V.P. K-30 in aspirin tablets.

Ingredients	Formulation (mg/tab)			
	Control	#15	#16	#17
Aspirin powder	650.0	650.0	650.0	650.0
Methocel A-4M	-	32.5(5%)	65.0(10%)	97.5(15%)
P.V.P. K-30	32.5	32.5	32.5	32.5
Stearic acid	2.5%	2.5%	2.5%	2.5%
Aerosil 200	0.5%	0.5%	0.5%	0.5%

2. Procedure

Aspirin powder, methylcellulose (Methocel A) and polyvinyl-pyrrolidone (P.V.P. K-30) were individually passed through a 60 mesh sieve to break up any agglomerate before used. The appropriate quantities of aspirin powder, Methocel A, and P.V.P. K-30 in each formulation (Table 5-9) were weighed and mixed thoroughly by manual bottle method. The mixture was remixed and granulated with absolute alcohol in mortar. The wet granulation was screened manually through a 10 mesh seive, placed on trays and dried in an oven set at 37°C for 3 hours. The dried granulation was screened manually through a 12 mesh sieve. The granulation was lubricated with stearic acid (2.5% w/w) and aerosil 200 (0.5% w/w) which were passed through a 100 mesh seive before used, and then mixed thoroughly by manually bottle tumbling method for 10 minutes.

The tablets were compressed on a single punch press using 14 mm. diameter flat-faced, beveled edge punches to a hardness of approximately 13-15 K.P.

D. In-Vitro Studies

1. Scanning Electronmicroscope study

The samples were treated for scaning method using Scaning Electronmicroscope.

Solid form : the powder was fixed on stub with silver paste and coated with gold using Fine Coat Ion Sputter (JFC-1100) about 10 minutes. Coated powder was scanned using Scanning Electronmicroscope and photographed.

Liquid form : the sample solution was smeared on stub, allowed to dry and coated with gold like solid form. Coated sample was scanned using Scanning Electronmicroscope and photographed.

2. Weight variation

Twenty aspirin tablets of each formulation were individually weighed using an analytical balance.

3. Hardness

Ten aspirin tablets of each formulation were individually tested using a hardness tester.

4. Thickness

Ten aspirin tablets of each formulation were individually measured using a micrometer.

5. Disintegration

Six aspirin tablets of each formulation were tested using a disintegration apparatus at 37°C, using water as disintegration medium.

6. Dissolution studies

Dissolution studies were conducted by half-change method (47).

6.1 Preparation of dissolution media

6.1.1 Gastric fluid, simulated T.S. (37)

2.0 g of sodium chloride was dissolved in 7.0 ml of hydrochloric acid and sufficient water to make 1,000 ml. This test solution had a pH of about 1.2.

6.1.2 Intestinal fluid, simulated T.S. (37)

6.8 g of monobasic potassium phosphate was dissolved in 250 ml of water, mixed, and 190 ml of 0.2 N sodium hydroxide and 400 ml of water was added. The resulting solution was adjusted with 0.2 N sodium hydroxide to a pH of 7.5 $\pm$ 0.1, and diluted with water to 1,000 ml.

6.2 Determination of dissolution rate

Dissolution rates of the drug from aspirin tablets containing various grades of Methylcellulose (Methocel.A) were studied according to the following procedure (47):

Nine hundred milliliters of simulated gastric fluid was placed in the glass vessel specified in the U.S.P. test and permitted to equilibrate to 37°C. A tablet was placed in sample basket and then immersed in dissolution medium at the center of the vessel, the distance of 2.0 cm between the bottom of the vessel. At the moment of contact between sample basket and the dissolution medium, the motor and the timer were started simultaneously. The basket was rotated at the speed of 100 rpm.

The dissolution experiment was conducted over a period of 3 hours. At the time interval of 5,15,30 minutes, 1,2 and 3 hours, a 10 ml of sample was withdrawn by sampling pipette. The same quantity of simulated gastric fluid was added immediately after each sampling to keep the volume of dissolution medium constant during the course of the test.

Simulated gastric fluid was replaced with simulated intestinal fluid, then the experiment was continued for another of 5 hours. Every 1 hour interval, a 10 ml of sample was withdrawn by sampling pipette. The same quantity of simulated intestinal fluid was added immediately after each sampling to keep the volume of dissolution medium constant during the course of the test.

7. Determination of free salicylic acid by spectrophotometric method (51)

7.1 Sample assay

A 5.0 ml sample from the first time interval, or a 2.0 ml sample from the following time interval was transfered to a 50.0 ml volumetric flask containing 3.0 ml of distilled water. A 0.5 ml quantity of 50% w/v solution of sodium hydroxide was added to each flask and mixed thoroughly. After 15 minutes, 1.5 ml of concentrated hydrochloric acid was added and the volume was brought to the mark with 0.1 N HCl. The solution was read on a spectrophometer for salicylic acid at 302 nm, using an appropriate blank. The amount of aspirin dissolved at any time interval was calculated by comparison with a standard reference obtained by treating known solutions as the samples from calibration curve.

7.2 Calibration curve of aspirin

Exact amount of aspirin was dissolved in simulated gastric fluid and simulated intestinal fluid, then treated with the same procedure as in the sample assay. The standard solutions were read on a spectrophotometer at 302 nm.