CHAPTER II

EXPERIMENTAL

2.1 Materials

The following materials obtained from commercial source were used in this experiment.

- Model Drug: - Aspirin BP 1988 (China) [devided and pakaged by Srichan Sahaosote Co,Ltd., lot.& Cont. No. AK 04/883]
  - Commercial Aspirin Tablet 325 mg. (Aspaco® B.P.O.) [produced by Burapha Osote, Lot. & Cont. No. 66032, Mfg. Date 8/04/00, Thailand]
- Excipients : - Chitin [Unicord Co,Ltd., Thailand]
  - Corn Starch [devided and pakaged by O.V. Chemical, Thailand]
  - Dibasic Calcium Phosphate [Emcompress®, Lot. No. T27B, USA]
- Lubricant : - Magnesium Stearate [devided and pakaged by O.V. Chemical, Thailand]
- Dissolution Medium : - Sodium Acetate Trihydrate [AnalaR® BDH Lot. No. 301TA395404]
  - Acetic Acid [AnalaR® BDH Lot. No. 7304420N]

2.2 Equipments

- Laboratory Test Sieve No. 60 Mesh (250 μm)[Retsch, Ser. No. 5603631, Germany]
- Laboratory Test Sieve No. 70 Mesh (212 μm) [Retsch, Ser. No. 613858, Germany]
- Laboratory Test Sieve No. 80 Mesh (180 μm) [Retsch, Ser. No. 611354, Germany]
- Laboratory Test Sieve No. 120 Mesh (125 μm) [Retsch, Ser. No. 595725, Germany]
- Laboratory Test Sieve No. 170 Mesh (90 μm) [Retsch, Ser. No. 621128, Germany]
- Laboratory Test Sieve No. 325 Mesh (45 μm) [Retsch, Ser. No. 620079, Germany]
- Sieve Shaker [Retsch, Model AS200 digit, Ser. No.90203005, Germany]

- Hot Air Oven [KAN 1960 Model HA-20 No.405, Thailand]
- Ball Mill [Haldenwanger, Germany]
- Stampf Volumeter [Model 2003 Ser. No.18263435]
- Disintegration Apparatus [Pharma Test, Model PTZ1 No. 1-3869/A, Switzerland]
- Carver Laboratory Press [Model C, Ser. No. 25576-082, USA]
- Erweka Abrasion Tester [Model 1AP No.24438, Germany]
- BP Dissolution Apparatus II [Hanson Research, Model No. 64-100-160, USA]
- Hardness Tester [Pharma Test, Model PTB-311 Ser. No. I-4390/B]
- Micrometer [Fowler]
- JMP® Software [Version 3.6.1.2, USA]
- SAS® Software [Version 6.12, USA]
- Hanseaten Single Stroke [Model EI No. 359, Germany]
- Analytical Balance [Sartorius, Model 1702, Ser. No. 35070130, Germany]
- pH meter [Mettler delta 345, Model 345, Ser. No. M2650]
- Spectrophotometer [Spectronic Genesys TM 5, Ser. No. 3V15222011, USA]
- Cube Mixer [Erweka-Apparatebau, Model KU1 No. 3142560, Germany]

2.3 Particle Size Distribution

Particle size distribution was determined by sieve analysis. The approximately 10 g of powder was put on the top sieve series ranging from 212, 180, 125, 90 to 45 μm, respectively. The nest of sieves were placed on the sieve shaker [Retsch, Model AS200 digit, Ser. No.90203005, Germany] for 10 minutes. The results were reported as percent of weight retained on each sieve size.

2.4 Preparation of Powder Mixtures

In the Augment Simplex Centroid Design, there are 10 formulations of the mixture that fixed the concentration of excipients at 24.5 %, magnesium stearate at 0.5 % and the active drug, aspirin, at 75.0 %. The mixture that used as excipients are consists of chitin, dibasic calcium phosphate and corn starch. From the mixture design,

the weight of each formulation was 30 g that can be calculated and showed in Table 2.1.

Table 2.1 The Amount of Component in Each Formulation

Formulation	Aspirin (%)	Mg St (%)	Excipient		
			CT (%)	DCP (%)	CS (%)
1	75.0	0.5	24.5	-	-
2	75.0	0.5	-	24.5	-
3	75.0	0.5	-	-	24.5
4	75.0	0.5	12.25	12.25	-
5	75.0	0.5	-	12.25	12.25
6	75.0	0.5	12.25	-	12.25
7	75.0	0.5	4.0817	4.0817	16.3342
8	75.0	0.5	16.3342	4.0817	4.0817
9	75.0	0.5	4.0817	16.3342	4.0817
10	75.0	0.5	8.1659	8.1659	8.1659

Mg St: Magnesium stearate CS : Corn starch

CT : Chitin DCP : Dibasic calcium phosphate

At first, each components; aspirin, chitin (CT), corn starch (CS), and magnesium stearate (Mg St) were sieved by using laboratory sieve No. 60 mesh. Then, chitin (CT) and corn starch (CS) were dried by using hot air oven at 60 ° C for 3 hours. Finally, each component was weighed (the amounts of each formulation can be seen in Table 2.1) and was mixed in mortar & pestle by using geometric dilution technique for 10 minutes.

2.5 Evaluation of Powder Flow

The flowability of each formulation powder mixture was evaluated by two methods: angle of repose and percent compressibility.

2.5.1 Angle of Repose

The paper was weighed by analytical balance [Sartorius, Model 1702, Ser. No. 35070130, Germany]. The width and the length of paper were measured by ruler for calculate the area of paper. The weight per square centimeter of paper was obtained.

The funnel was used to determined the angle of repose by setting at the height from end of funnel to ground about 10 centimeters. The powder mixture was poured through the funnel. Then, the cone-like pile of powder mixture was obtained. The height of cone-like pile was measured by ruler. The radial of paper of cone-like pile was calculated the weight of cutted circular paper. Then, the tangent of angle of repose was calculated by Equation 2.1.

$$\text{Tan } \theta = \frac{H}{R} \quad \text{.....(2.1)}$$

Where: H = the height of cone-like pile + 1/4 inch

R = the radial of circle, that calculated from the area and weight of cone-like pile

2.5.2 Percent Compressibility

The powder mixture was weighed, then introduced without compacting into the graduated cylinder. The powder mixture was tapped by using stampf volumeter [Model 2003 Ser. No.18263435] for three times, then the volume (Bulk volume) was read. The tapped volume was obtained by tapping for 1000 times. Then the percent compressibility can be calculated by Equation 2.2.

$$\% \text{ Compressibility} = \frac{(\text{Tapped density} - \text{Bulk density}) \times 100}{\text{Tapped density}} \quad \text{.....(2.2)}$$

2.6 Preparation of Direct Compression Tablets

The powder mixture was approximately weighed of 433.33 mg, so the amounts of aspirin was about 325 mg. Then, the powder was compressed at compressional

pressure of one ton by Carver Laboratory Press [Model C, Ser. No. 25576-082, USA] using 10.3 mm flat faced bevel edge, circular punch. The compression pressure was maintained for 7 seconds.

The 1.5 tons tablet was obtained from the same condition but the compressional pressure was used of 1.5 tons.

2.7 Evaluation of Tablet Properties

The direct compression tablets were used to determine the tablet properties by following procedures.

2.7.1 Tablets Hardness

The hardness was examined by using hardness tester [Pharma Test, Model PTB-311 Ser. No. I-4390/B]. The mean and standard deviation were calculated from three determinations.

2.7.2 Percent Friability

The friability of tablets was determined by using Erweka Abrasion Tester [Model 1AP No.24438, Germany]. First, the 20 tablets were weighed by Analytical Balance [Sartorius, Model 1702, Ser. No. 35070130, Germany], the W_0 was obtained (the powder dust was eliminated before weighing). Second, turn on the tester for 4 minutes (it circulates for 25 rpm). Then, the tablets were taken off and the dusts were removed. Finallly, the weight of tablet (W_1) was measured. The tablet friability was calculated by Equation 2.3.

$$\% \text{ Friability} = \frac{(W_0 - W_1) \times 100}{W_0} \qquad \text{.....(2.3)}$$

The friability of tablets was not more than 1%, that according to BP 1998.

2.7.3 Disintegration Time

The disintegration time was measured by using USP XXIV disintegration apparatus [Pharma Test , Model PTZ1 No. 1-3869/A] with purified water at 37 °C as disintegration medium. The six tablets were used to examined.

2.7.4 Dissolution Studies

2.7.4.1 Preparation of Acetate Buffer

First, sodium acetate trihydrate was weighed of 2.99 g. Then, purified water was added to dissolve it. The 1.66 ml of acetic acid was added into the solution. Finally, adjust the medium to 1000 ml with purified water and the pH was measured by using pH meter [Mettler delta 345, Model 345, Ser. No. M2650] and adjusted to 4.5.

2.7.4.2 Dissolution of Tablets

Five hundred milliliters of acetate buffer was placed into each vessel, then the temperature of dissolution medium was warm up to 37 ± 0.5 °C. One tablet was filled in each vessel. The paddle was rotated at 50 rpm. The release profile of tablet was determined from six tablets.

The five milliliters of sample was withdrawn and pass 0.45 μm filter at the time interval of 2, 5, 8, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. Acetate buffer was replaced into the vessel in equal volume to maintain the constant volume of dissolution medium. The absorbance of sampling solution was determined by using spectrophotometer [Spectronic Genesys TM 5, USA, Ser. No. 3V15222011] at 265 nm. Some collections may be diluted with dissolution medium to obtain the appropriate absorption.

The specification of the standard BP stated that, for each unit tested, not less than 70 % of the active ingredient or ingredients dissolves within 45 minutes. If one unit fails, a retest may be carried out using the same number of units, all unit in the retest must comply.

2.7.4.3 Preparation of Aspirin Calibration Curve

To calculate the concentration of aspirin that release from tablet, the calibration curve of aspirin was obtained by following procedures.

Aspirin was weighed of 100.5 mg, then added with acetate buffer to dissolve and adjust to 100 ml in volumetric flask. The concentration of aspirin stock solution was 1.005 mg/ml.

The stock solution was pipetted of 0.35, 0.5, 1.0, 1.5, 2.0 and 2.5 ml into each 10 ml volumetric flask, then adjusted volume to 10 ml with acetate buffer pH 4.5. The concentration of aspirin solution was 35.18, 50.25, 100.50, 150.75, 201.00 and 251.25 μg/ml, respectively. The absorbance of serial solution were determined by using spectrophotometer [Spectronic Genesys TM 5, USA, Ser. No. 3V15222011] at wavelength 265 nm and use acetate buffer as the blank. Then, calibration curve of aspirin was obtained.

2.7.5 Selection the Optimal Response Surface Area

The data of powder flow and tablet properties were used to evaluate the model and R-square by using SAS® software program [Version 6.12, USA]. The model search was started with a full model equation:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3 \quad .....(2.4)$$

The non-significant terms of the model were excluded. Only significant terms (α = 0.05) were used in the fitted model. The contours of response model were plotted by using JMP® software [Version 3.6.1.2, USA]. Then, the range of optimal value of each property was selected. All selecting response surface areas were superimposed and the optimal range of all properties was obtained.

2.7.6 Scale Up the Direct Compression Tablets

To scale up the direct compression tablet, the amount of each formulation (X1, X2) was weighed of 200 g, that consists of the model drug, aspirin 75 %, magnesium

stearate 0.5 %, and the excipients at 24.5 %. In 24.5 % of excipients, there are three components; chitin (CT), dibasic calcium phosphate (DCP), and corn starch (CS). The weight of each component was calculated from the proportion of three components in the selected response surface. The amounts of each component of formulations X1 and X2 can be seen in Table 2.2.

Table 2.2 The Amounts of Each Component in the Scale Up Formulation

Formulation	Aspirin (g)	Mg St (g)	Excipient		
			CT (g)	DCP(g)	CS (g)
X1	150.0	1.0	31.85	14.70	2.45
X2	150.0	1.0	19.60	24.50	4.90

Mg St: Magnesium stearate CS : Corn starch

CT : Chitin DCP : Dibasic calcium phosphate

The amounts of each formulation was weighed (in Table 2.2). Then, aspirin and excipients; CT, DCP and CS were mixed in the cube mixer [Erweka-Apparatebau, Model KU1 No. 3142560, Germany] for 10 minutes. After that, the lubricant, Mg St, was added and mixed for 2 minutes.

The flowability of powder mixture, angle of repose and percent compressibility, was measured by the same procedures as previous described.

In the scale up, the direct compression tablets were produced by using Hanseaten Single Stroke [Model EI No. 359, Germany], using 10.3 mm flat faced bevel edge, circular punch.

The tablet properties were determined as the same procedures as previous described. Tablet thickness and hardness were obtained from ten determinations, weight variation and tablet friability were examined from 20 determinations. Other properties, disintegration time and dissolution, were determined from six determinations.

2.7.7 Comparison between the Scale Up Direct Compression Aspirin Tablets and Commercial Tablet

The selected optimize formulation (scale up formulation) were used to determine and compare with the commercial aspirin tablets in tablet properties by using the same procedure as previous described.