Chapter II

Experiment

1. Materials

The following materials obtained from commercial source were used as received

1.1 Model Drug - Theophylline Anhydrous BP. (supplied by Pharmaceutical Trader Co, LTd. Thailand, Batch No. 8907052)

1.2 Additives - Ethylcellulose 10 cps. (Supplied by Rama Production, Thailand)

- PVP K30 (BASF, Geramany)
- Lactose anhydrous (Wyndale, New Zealand)
- Microcrystalline cellulose (Avicel PH101®, Asahi chemical industry, Japan)
- Dibasic calcium phosphate (Emcompress®, Abright & Wilson LTD, UK)
- Corn starch

1.3 Dissolution Medium

- Dihydrogen Potassium Phosphate, AR grade (Merck, Germany)
- Sodium Hydroxide, AR grade(Merck, Germany)
- Hydrochloric Acid (Merck, Germany)

1.4 Solvent

- Methylene Chloride (Merck, Germany)
- 95% Ethyl Alcohol

1.50 Disintegrant

- Sodium Starch Glycolate (Explotab^{R})

1.6 Lubricant

- Magnesium stearate (supplied by Pharmaceutical Sciences, Thailand)
- Talcum

2. Methods

2.1 Preparation of Granules

The granules containing 74.63% w/w of

theophylline were prepared by means of wet granulation. The fractions of drug and diluent were mixed in cube mixer (Erweka, Germany) for 10 minutes and wet granulated using aqueous 10% w/v polyvinylpyrrolidone solution as a binder in mortar. The granulation composition is presented in Table 4 The damp mass was pressed through a 18 mesh sieve, then they were oven-dried at 50°c for 8 hours. The 16/20 mesh fractions of dried granulation were classified using a sieve shaker fitted with U.S. Standard sieves and was shaken for 10 minutes in order to ensure no aggregates and fine particles prior to use. The granulation was stored in tightly-closed, dry containers.

Table 4 Compositions of Theophylline Granules for Coating

Theophylline anhydrous	300 mg.
diluent*	100 mg.
Polyvinylpyrrolidone	18.90 mg.

*Avicel PH 101®, Corn stach, Emcompress®, Lactose

2.2 Preparation of the Coated Granule

The 16/20 mesh fractions of granulation were coated by using fluidized bed coater (Glatt, Binzin-Haltingen, western germany). The special construction of the product container, container bottom and inner cylinder, the movement of the material can be controlled. The inner cylinder, into which the spray nozzle protrudes, transports the granules, quickly upwards and then the slide slowly back in the outer cylinder.The experimental setting for the coating process is shown in Figure 6

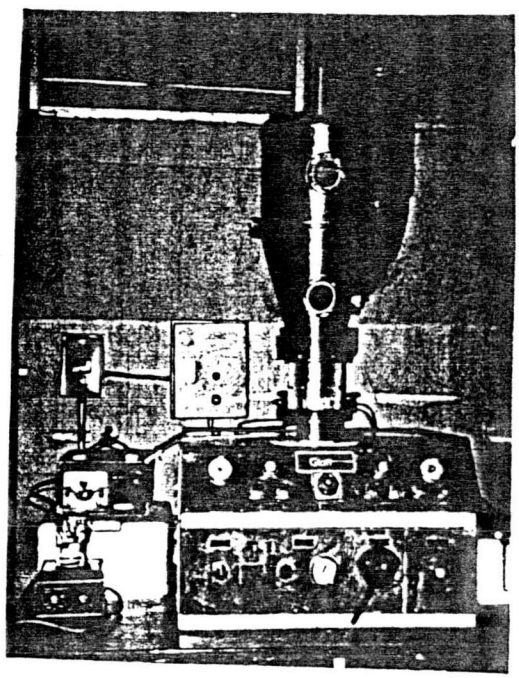

Figure 6 The Photograph of Fluidized Bed Coater (Glatt,Binzin-Haltingen,Western Germany)

50 g. of granules were coated with the coating solution contaning ethylcellulose and glyceryl monosterate in 95% ethyl alcohol + dichlorometane. The coating solution composition is presented in Table 5. The granules were fluidzed in chamber until the temperature in coating region of the apparatus reached 35°C then, spraying was operated. Coating solution was pumped through a peristaltic pump at a flow rate of 5 ml/min to the spray nozzle, which was operated at a spray pressure of 29 psi(2 atm). These conditions above were found to be optimal because there were no blockage of the spray nozzle, no aggregation of the granules and completion of coating, the granules were fludized for a further 5 minutes to remove solvent and drying.

The amount of coating solutions was varied to determine the effect of coating level by 50 g. of granules coated with 100 ml of coating solution were 10% coated granules, 150 ml for 15% coated granules and 200 ml for 20% coated granules.

Table 5 Composition of Coating Solution

Ethylcellulose 10 cps.	5 g
Glyceryl Monostearate	1 g
95% Etyl alcohol:Dichloromethane (1:1) qs to	100 g

2.3 Evaluation of Granules

2.3.1 Morphology of the Coated Granules

The coated granules were examined under a scanning electron microscope (SEM) (Models JSM T220A) for morphological evaluation. The granules were also cross-sectioned for observation of coated film. The samples were prepared by gold sputtering technique before SEM examinations.

2.3.2 Determination Theophylline Content in Coated Granule

The method for determining theophy lline content used in this study was modified from previous work,(Sa, Bandayopadhyay and Gupta, 1990) approximately 100 mg of sample was accurately weighed and dissolved in a 5 ml of chloroform. The 150 ml of 0.1 N HCl was added and the content was stirred for 60 minutes using magnetic stirrer. The mixture was then heated to eliminate chloroform. The mixture, after cool to room temperature, was adjusted to 200 ml in a volumetric flask. Finally , it was analyzed spectrophotometrically at 268.5 nm.

2.3.3 Studies of Drug Release from Uncoated and Coated Granules

A. Studies of Drug Release from Uncoated Granules

Drug release from uncoated granules containing various type of fillers were determined in 0.1 N Hcl and phosphate buffer pH 6.8 using apparatus I(the basket method) and apparatus II (the paddle method) according to USP XXI.

Nine hundred millilites of 0.1 N hydrochloric acid or phosphate buffer pH 6.8 were placed in a glass vessel specified in the USP dissolution test, the medium was equilibrated to 37±0.5 °C. A quantity of uncoated granules equivalent to 300 mg theophylline was placed in the dissolution medium . Five millilites of specimen were withdraw at the time interval 5, 10, 15, 20, 25 and 30 minutes. The same quantity of medium was added immediately after each sampling to maintain the medium at the same volume at all times.

The absorbance of the samples were determined spectro photometrically in a 1-cm cell at 268.5 nm for 0.1 N HCl and 270.3 nm for phosphate buffer pH 6.8

The amount of theophylline

released at any time interval was calculated from the calibration absorbance-concentration curve. A cumulation was made for the previously removed sample to determine the total amount of drug release. The procedure was carried out in triplicate for uncoated granules at each formulation

B. Studies of Drug Release from Coated Granules

Amount of drug release were determined by using USP XXI dissolution test (Hanson Research Corporation, Model SR-2 USA.) apparatus I (basket method) with PH change model, the pH of the medium was controlled by nine hundred milliliters of 0.1 N HCl for two hours, then the pH was increased to pH 6.8 by adding 4.4064 g of NaOH and 6.125 g. of KH_2PO_4 dissolved in a few ml. of 0.1N. HCl.

The sampling time were 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 hours. The amount of theophylline released at any time interval was determined spectro-photometrically as same as in uncoated granules.

2.3.4 Calibration Curve of Theophylline

Theophylline 200 mg was accurately weighed and dissolved in 0.1 N HCl or phosphate buffer pH

6.8. The solution was then adjusted to 2000 ml with 0.1 N HCl or phosphate buffer pH 6.8 and used as stock solution

The stock solution was individually pipetted 2, 3, 4, 5, 6 and 7 ml into a 50 ml volumetric flask and diluted to volume with 0.1 N Hcl or phosphate buffer pH 6.8. The final concentration of each solution was 4, 6, 8, 10, 12 and 14 μg/ml, respectively.

The absorbance of known drug concen tration was determined by a double beam spectro-photometer (The Bausch & Lomb, New York, USA.) in a 1-cm cell at 268.5 nm for 0.1 N HCl and at 270.3 nm for phosphate buffer pH 6.8. The 0.1 N HCl or phosphate buffer pH 6.8 was used as a blank solution. Each concentration was determined in duplicate.

The concentration versus absorbance of theophylline in 0.1 N HCl at 268.5 nm and in phosphate buffer pH 6.8 at 270.3 nm were presented in Table 15 and 16 (Appendix), showed a linear relationship. The standard curve of theophylline after regression analysis was illustrated in Figure 88A and 88B (Appendix).

2.4 Preparation of tablets

Each batch of coated granules were mixed with Avicel pH 101®, magnesium stearate , talcum with and

without Explotab® (Tablet compositions were present in Table 6) then compressed into tablets by Carver Laboratory Press (PERKIN-ELMER, model C, Fred & Carver Inc., USA.) using 3/8 inch flat faced circular punch. The compression pressure was mantained for 10 seconds and quickly released. The pressure was varied from 500, 1000, 1500 pounds. All formulations of theophylline tablet for release profiles studies were presented in Table 7.

Table 6 Composition of each Tablet

Theophylline coated granule equivalent to an hydrous theophylline	300 mg
disintegrant*	25 mg
Talcum	4.2 mg
Magnesium stearate	2.3 mg
Avicel PH101®	100 mg

* Tablet with and without Explotab® as disintegrant were prepared and compressed at 500,1000,1500 lbs by Caver Laboratory Press.

2.4.1 Tablet Evalution

- Thickness

The thickness of tablet was

Table 7 Formulations of Theophylline Tablet For Release Profiles Study

	500 lbs		1000 lbs		1500 lbs	
Filler in granule %Ethylcellulose Coated	Explotab	No explotab	Explotab	No explotab	Explotab	Noexplotab
Avicel PH101®						
10%	$10\%A_1$	$10\%A_2$	$10\%A_3$	$10\%A_4$	$10\%A_5$	$10\%A_6$
15%	$15\%A_1$	$15\%A_2$	$15\%A_3$	$15\%A_4$	$15\%A_5$	$15\%A_6$
20%	$20\%A_1$	$20\%A_2$	$20\%A_3$	$20\%A_4$	$20\%A_5$	$20\%A_6$
Corn starch						
10%	$10\%C_1$	$10\%C_2$	$10\%C_3$	$10\%C_4$	$10\%C_5$	$10\%C_6$
15%	$15\%C_1$	$15\%C_2$	$15\%C_3$	$15\%C_4$	$15\%C_5$	$15\%C_6$
20%	$20\%C_1$	$20\%C_2$	$20\%C_3$	$20\%C_4$	$20\%C_5$	$20\%C_6$
Encompress®						
10%	$10\%E_1$	$10\%E_2$	$10\%E_3$	$10\%E_4$	$10\%E_5$	$10\%E_6$
15%	$15\%E_1$	$15\%E_2$	$15\%E_3$	$15\%E_4$	$15\%E_5$	$15\%E_6$
20%	$20\%E_1$	$20\%E_2$	$20\%E_3$	$20\%E_4$	$20\%E_5$	$20\%E_6$
Lactose						
10%	$10\%L_1$	$10\%L_2$	$10\%L_3$	$10\%L_4$	$10\%L_5$	$10\%L_6$
15%	$15\%L_1$	$15\%L_2$	$15\%L_3$	$15\%L_4$	$15\%L_5$	$15\%L_6$
20%	$20\%L_1$	$20\%L_2$	$20\%L_3$	$20\%L_4$	$20\%L_5$	$20\%L_6$

10%, 15%, 20% = Amount of Ethylcellulose Coated base on Weight of granule

A,C,E,L = Avicel PH101®, Corn Strach, Encompress®, Lactose

1,3,5 = Tablet Containing Explotab® and Compressed at 500, 1000, 1500 lbs

2,4,6 = Tablet without Explotab® and Compressed at 500, 1000, 1500 lbs

measured by using micrometer(Teclock Co.,Japan) and expressed in mm. The thickness was and average of ten determinations

- **Hardness**

The hardness was measured using the Schleuniger-2E hardness tester (Switzerland) and the mean and standard deviation were averaged from ten determintations.

- **Disintegration Time**

Disintergration time was determined according to USP XXI method the average of disintergration time was calculated from six determinations. The disintergration time was measured using the USP XXI apparatus (Hanson Research Corporation, Model QC-21, USA) with purified water at 37±2 °C as a disintergration fluid.

- **Dissolution Studies**

The dissolution method used for screening the formulations was USP paddles at 50 rpm in 900 ml of dissolution medium at 37°C. The dissolution medium consisted of 0.1 N HCl pH 1, which was adjusted after two housr to pH 6.8 using sodium hydroxide 4.4064 g

and 6.125 g of potassium dihydrogen phosphate.

Tablets prepared for release profile study (as shown in Table 7) were added to each dissolution vessel. Samples removed through filters for analysis at suitable time interval (0.25, 0.5, 0.7, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 hours). The samples were assayed spectro photometrically at 268.5 nm. for acid medium and 270.3 nm for buffer pH 6.8 medium

Each of the dissolution values reported was based on an average of three tablets of each formulation. The amount of theophylline release at anytime interval was calculated from the calibration absorbance concentration curve.