CHAPTER II

EXPERIMENT

1. Materials

The follwing materials obtained from commerical source were used as received

1.1 Model Drug
- Theophylline Anhydrous B.P.
  (supplied by Pharmaceutical Trader Co., Ltd. Thailand, Batch No. S920501)

1.2 Additives
- Ethylcellulose aqueous dispersion
  (Surelease®, Colorcon Limited, England, Batch No. 600119)
- Poly (ethylacrylate methylmethacrylate) aqueous dispersion
  (Eudragit® NE 30D,Rohm rama Production, Thailand, Lot. No. 1230312006)
- Gelatin
  (Srichand United Dispensary Co., Ltd. Thailand, Lot. No. GA10)
- Lactose Anhydrous
  (Wyndale, New Zealand, Lot. No. 2120002 122)
- Cab-O-Sil M-5

(Cobot, Belgium, Lot. No. S5330)

1.3 Dissolution Medium

- Dihydrogen Potassium Phosphate, AR grade
  (Merck, Germany, Lot. No. 227 A679473)
- Sodium Hydroxide, AR grade
  (Merck, Germany, Lot. No. 211190)
- Hydrochloric Acid
  (Merck, Germany, Lot. No. 403872)

1.4 Solvent of Internal Standard

- Ammonium Hydroxide, AR grade
  (Merck, Germany, Lot. No. 046 K15056832)

1.5 High Performance Liquid Chromatography Analysis

- 8-chlorotheophylline Lot. No. 52 H0745
  (Sigma Chemical Co., Ltd. USA)
- Sodium Acetate Trihydrate, AR grade
  (Carlo Erba, MILANO, Lot. No. 2691 F100)
- Glacial Acetic Acid
  (Merck, Germany, Lot. No. 227 K18049863)
- Acetonitrile, HPLC grade
  (J.T Baker Inc, USA, Lot. No. NJ 08865)
- Ammonium Hydroxide, AR grade
  (Merck, Germany, Lot. No. 046 K15056832)

2. Equipment

- Fluidized Bed Coater
  (Uni-Glatt Laboratory Units, Germany)

- Dissolution Tester
  (Hanson Research Corporation SR2, USA)
- pH meter
  (Hanna Instruments 8417, USA)
- Spectrophotometer
  (Milton Roy Spectronic 3000, USA)
- Peristaltic Tube Pumps
  (Verder Type VRX 88, Germany)
- Balance, Top Load
  (Sartorius 1264 MP, Germany)
- Sieve Shaker (Josef Deckehnann Aschaffenburg, Western Germany)
- Scanning Electron Microscope
  (Model JSM - T220 A, Jeol, Japan)
- Stirring Hot Plates
  (Thermolyne Corporation, USA)
- Oscillating granulator
  (Viuhang Corporation, Thailand)
- Hobart Mixer
  (Model EB 20F, Thailand)
- High Performance Liquid Chromatography
  (Millipore Corporation, Water Chromatography, USA)
- Surface area measurement (Micromeritics model 2300 FC, USA)

3. Methods

3.1 Preparation of Theophylline Granules

The granules containing 65.40% w/w of theophylline were prepared by means of wet granulation. The fractions of drug and diluent were mixed in Hobart mixer for 10 minutes and wet granulated by 12% w/v gelatin aqueous solution as a binder. The granulation composition is presented in Table 2. The damp mass was pressed through oscillating granulator and oven dried at 60°C for 4 hours. The dry granules were repressed through oscillating granulator. The 16/18, 18/20, 20/25, 25/30 mesh fractions of dried granulation were classified by a sieve shaker fitted with US standard sieves. The granulation was shaken for 10 minutes in order to ensure no aggregates prior to use. The granulation was stored in tightly-closed, dry containers.

Table 2 Composition of Theophylline Granules for Coating

Ingredient	% w/w
Theophylline anhydrous	65.40%
Lactose	31.41%
Gelatin	4.19%

3.2 Preparation of the Coated Granules

Theophylline granules were coated by using fluidized bed coater. The substrate is placed in the product container, which is typically an unbaffled, inverted, truncated cone with a fine retention screen and an air distribution plate at its base. The particles are accelerated from the product container part the

nozzle which sprays the coating aqueous dispersion.The experimental setting for the coating process is shown in Figure 7.

Top spray method of fluidized bed coating was used in preliminary investigation by trial and error for suitable coating conditions and the uniformity of coating film. The coating conditions were gradually adjusted by varying inlet air temperature, outlet air temperature, spraying air pressure, spraying rate to have uniformity of coating by visual observation of coated granules.

Granules of 350 g were coated with Surelease® or Eudragit® NE 30D. The composition of aqueous polymeric coating was presented in Table 3 and Table 4. The granules were fluidized in chamber until the temperature in coating region of the apparatus reached 60°C for coating with Surelease® and 40°C for coating with Eudragit® NE 30D then, spraying was operated. Aqueous polymeric dispersion was pumped through a peristaltic pump at a flow rate of 5 ml/min for Surelease® and 3 ml/min for Eudragit® NE 30D to the spray nozzle, which was operated at a spray pressure of 1 bar for the both aqueous polymeric coatings. These aforementioned conditions were found to be optimal because there were no blockage of the spray nozzle, no aggregation of the granules and completing of coating. The granules were fluidized for a further 10 minutes to remove residue water and drying. The optimal coating condition was presented in Table 5.

The amount of aqueous polymeric coating was varied to determine the effect of coating level and the effect of particle size variation of the granules on the release rate profile of theophylline granules.

Table 3 Composition of Ethylcellulose Aqueous Polymeric Coating

Surelease® (25% aqueous dispersion)	50.25	g (12.56 g dry substance)
Water	49.75	g
	100.00	g

The amount of Surelease® and Eudragit® NE 30D which were used for coating theophylline granules of various sizes were listed in Table 6 and Table 7, respectively. Each amount shown in Tables6 and 7 was used for coating a batch (350 g) of theophylline granules.

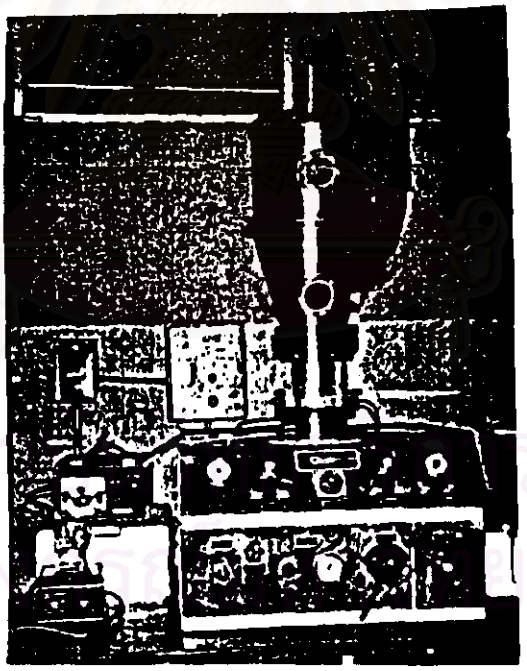


Figure 7 The Photograph of Fluidized Bed Coater
(Glatt, Binzin-Haltingen, Western Germany)

Table 4 Composition of Poly(ethylacrylate methyl methacrylate) Aqueous Polymeric Coating

Eudragit® NE 30D (30% aqueous dispersion)	25.07 g	(12.56 g dry substance)
Cab-O-Sil	2.26 g	
Water	72.67 g	
	100.00 g	

Table 5 The Coating Conditions by Top Spray Method

Coating Condition	Value	
	Surelease	Eudragit NE 30D
Inlet air temperature	60°C	40°C
Outlet air temperature	50°C	30°C
Spraying air pressure	1 bar	1 bar
Feed rate of coating polymer	5 ml/min	3 ml/min

Table 6 The Amount of Surelease® for Coating a Batch of Theophylline Granules (350 g)

Size of Granule (mesh)	Amount of Surelease® ml,(g) / 350 g Granules			
16/18	50 (50.5)	100 (101)	150 (151.5)	200 (202)
18/20	100 (101)	150 (151.5)	200 (202)	250 (252.5)
20/25	150 (151.5)	200 (202)	250 (252.5)	300 (303)
25/30	200 (202)	250 (252.5)	300 (303)	350 (353.5)

Table 7 The Amount of Eudragit® NE 30D for Coating a Batch of Theophylline Granules (350 g)

Size of Granule (mesh)	Amount of Eudragit® NE 30D ml,(g) / 350 g Granules			
16/18	50 (51.75)	150 (155.25)	250 (258.75)	350 (362.25)
18/20	100 (103.5)	200 (258.75)	300 (310.5)	400 (414)
20/25	150 (155.25)	250 (258.75)	350 (362.25)	450 (465.75)
25/30	200 (207)	300 (310.5)	400 (414)	500 (517.5)

3.3 Evaluation of Uncoated and Coated Granules

3.3.1 Morphology

The morphology of the uncoated and coated granules were examined by using scanning electron microscopy (SEM). The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape and surface topography of the granules were determined. The granules were also cross-sectioned for observation of the coated film.

3.3.2 Particle Size Distribution

Particle size distribution was determined using sieve analysis method. The approximately 50 g of uncoated or coated granules were put on the top of a sieve series ranging from 1410, 1190, 1000, 900, 800, 710, 600, 500, 425, 250 μm (12, 14, 16, 18, 20, 25, 30, 35, 40 and 60 mesh). The nest of

sieves were placed on the sieve shaker for 15 minutes. The results averaged from two determinations were reported as percentage of weight of granules retained on each sieve size. The geometric mean diameter was determined.

3.3.3 Bulk, Tapped Density and Carr's Compressibility Index

The bulk and tapped density were determined by pouring 30 g of the uncoated or coated granules into a 100 ml graduated cylinder. The bulk volume was recorded and bulk density was calculated. Tapped density was performed by dropping graduated cylinder on a hard surface from height 5 cm until a constant volume was obtained. Then, tapped volume was divided by weight to attain tapped density. Both densities were averaged from three determinations. The Carr's compressibility was calculated from the following equation.

$$\% \text{ Carr's compressibility} = \frac{(T - B) \times 100}{T} \qquad (1)$$

T and B are tapped and bulk density, respectively.

3.3.4 Moisture Determination

The moisture content of granules was determined by using Ohaus moisture determination balance. About 7 g of granules were spread uniformly in a thin layer and accurately weighed on a pan. Then it was exposed to an infrared lamp at 5 cm from the pan and intensity of 5 watts until constant weight was obtained. The percent moisture content was calculated based on the following equation.

$$\% \text{ Moisture content} = \frac{(\text{wet weight} - \text{dry weight}) \times 100}{\text{wet weight}} \quad (2)$$

3.3.5 Determination of Angle of Repose

Angle of repose was determined by cylinder method. Appropriate amount of granules were carefully filled in the cylinder, which was placed on the graph paper, until it was filled at the top of cylinder, then slowly lifted the cylinder in the vertical way, producing round heap. Angle of repose was calculated from the following equation.

$$\text{Angle of repose} = \tan^{-1} H/R$$

H and R are the height and radius of heap respectively.

3.3.6 Determination of Flow Rate

Accurate weight of about 30 g of granules were filled in a glass funnel with 8 mm internal stem diameter fixed on a clamp. The time was recorded when the granules started to flow until finished. The flow rate was expressed in g/second.

3.3.7 Measurement of Surface Area

The surface area of theophylline granules was measured by Surface Area equipment. The principle of this apparatus was nitrogen gas absorption on surface area of test material. Thereby, the resultant record would show in term m^2 per gram of theophylline granules. The method used for determination was based on the guideness of the American Society for Testing Material method D4567-86.

3.3.8 Determination theophylline Content in Uncoated and Coated Granules

The method for determining theophylline content used in this study was modified from USP XXIII.

The HPLC method was used to determine the amount of the drug as follow :

Buffer solution

Sodium acetate trihydrate of 2.72 g was transfered to a 2000 ml volumetric flask, add about 200 ml of water, and shake until dissolved completely.Add 10 ml of glacial acetic acid, dilute with water to volume, and mix.

Mobile phase

Acetonitrile of 250 ml was transfered to a 2000 ml volumetric flask, dilute with buffer solution to volume, and mix. Degas, and filter before using. Make adjustments if necessary

Standard calibration curve of theophylline

Theophylline anhydrous of 100 mg was accurately weighed and dissolved in mobile phase. The solution was adjusted to 100 ml with mobile phase and used as a stock solution at the concentration about 1 mg/ml.

As internal standard, 8-chlorotheophylline 35 mg was accuratly weighed and dissolved with 6N ammonium hydroxide. The solution was adjusted to 100 ml with mobile phase and used as internal standard stock solution with concentration about 0.35 mg/ml.

Theophylline stock solution was individually pipetted 1,3,5,7 and 9 ml into 50 ml volumetric flask and pipetted 10 ml internal standard stock solution filled in all flasks and adjusted with mobile phase to volume and mixed. The final concentration of each solution was 20, 60, 100, 140 and 180 μg/ml, respectively and the concentration of internal standard was about 70 μg/ml in all flasks.

The ratio between peak area of theophylline anhydrous and internal standard was determined by HPLC method at absorbance of 272 nm. Each concentration was determined in duplicate. The calibration curve and representative chromatogram were shown in Figures 83 and 84 (Appendix).

Assay of Theophylline Anhydrous in Uncoated and Coated Granules

The approximately 3.20 g of sample was accurately weighed and dissolved in 100 ml of water +50 ml of 6N ammonium hydroxide then adjusted to 500 ml with water and heated on hot plate, with ocassional stirring, just to boiling remove from the hot plate, and sonicate for about 1 minute while still hot. Cool to room temperature, dilute with water to volume, mix, and filtered. Pipet 4 ml of filtrate and transfer to 50 ml volumetric flask. Add 10 ml of Internal standard stock solution, dilute with Mobile phase to volume and mix. The concentration of theophylline anhydrous was determined by HPLC method at absorbance 272 nm and calculated with standard calibration curve in Figure 83 (Appendix).

3.3.9 Study of Drug Release from Uncoated and Coated Granules

The release of drug from capsule containing uncoated and coated granules were examined

A. Study of Drug Release from Uncoated Granules

Drug release from uncoated granules were determined according to USP XXIII in phosphate buffer pH 6.6 using apparatus I (the basket method).

Thousand milliliter of phosphate buffer pH 6.6 were placed in a glass vessel specified in the USP dissolution test, the medium was equilibrated to 37 $\pm$ 0.5°C. A quantity of uncoated granules equivalent to 200 mg theophylline was placed in the dissolution medium. Ten milliliter of specimen were withdrawn at the time intervals 5, 10, 15, 20, 25, 30, 40, 50, 60 minutes. The same quantity of medium was added immediately after each sampling to maintain the medium at the same volume at all times.

The absorbances of the samples were determined by spectro photometrically in a 1-cm cell at 272 nm for phosphate buffer pH 6.6.

The amount of theophylline released at any time interval was calculated from the calibration absorbance - concentration curve. A cumulation was made from 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

Drug release from coated granules was determined in phosphate buffer pH 6.6 using apparatus I (the basket method) according to USP XXIII.

The sampling times were 1, 2, 4, 5, 8, 10, 14, 20 and 24 hours. The amount of theophylline released at any time interval was determined spectrophotometrically as in 3.3.9a.

C. Standard Calibration Curve of Theophylline

Theophylline of 200 mg was accurately weighed and dissolved in phosphate buffer pH 6.6. The solution was then adjusted to 2000 ml with phosphate buffer pH 6.6 and used as stock solution.

The stock solution was individually pipetted 2, 3, 4, 5, 6 ml into a 50 ml volumetric flask and diluted to volume with phosphate buffer pH 6.6. The final concentration of each solution was 4, 6, 8, 10, 12 μg/ml, respectively.

The absorbance of known drug concentration was determined by a double beam spectro-photometer (Milton Roy Spectronic 3000) in a 1-cm cell at 272 nm for phosphate buffer pH 6.6. The phosphate buffer pH 6.6 was used as a blank solution. Each concentration was determined in triplicate.

The concentration versus absorbance of theophylline in phosphate buffer pH 6.6 at 272 nm presented in Table 40 (Appendix) showed a

linear relationship. The standard curve of theophylline after regression analysis is illustrated in Figure 82 (Appendix).

3.4 Preparation of Surelease® or Eudragit® NE 30D Films

A. Surelease® was diluted with deionized water in the ratio of 1:1 (polymer : water) added dibutyl phthalate (5%, 10%, 15%, 20% and 25% w/w of polymer) was added in diluting polymer and cast on a glass plate. After casting, the diluting polymer was allowed to dry at room temperature for over night.

B. Eudragit® NE 30D. was diluted with deionized water in the ratio of 1:3 (polymer : water) added cab-o-sil of 30% w/w of polymer was added in diluting polymer and cast on a glass plate After casting, the diluting polymer was allowed to dry at room temperature for over night.

3.4.1 Determination of Water Sorption

Each aqueous polymeric film was cut into 4.3 cm diameter circular and dried to constant weight in desicator at room temperature. Then it was immersed in deionized water at 37°C in incubator for 24 hours. The resultant wet film was blotted with filter paper to remove excess surface water and weighed again. Water sorption could calculate from the following formula

$$\frac{\text{(wet weight of film - dried weight of film) x 100}}{\text{dried weight of film}} \qquad (3)$$

In this experiment, water sorption was performed in triplicate.

3.4.2 Determination of Ultimate Tensile Strength and Percent Elongation at Break.

The apparatus for measuring ultimate tensile strength and percent elongation at break of test film was a tensile tester. Film specimen was cut out using a standard template (Figure 8). The thickness of each specimen was the average value of four separate measurements taken along the middle 4 cm section of the specimens using thickness tester. The cross-section area of the test film was calculated by multiplying the mean thickness with gauge width (6.25 cm). The test film was clamped by an upper and lower grip then, tensile tester was operated.

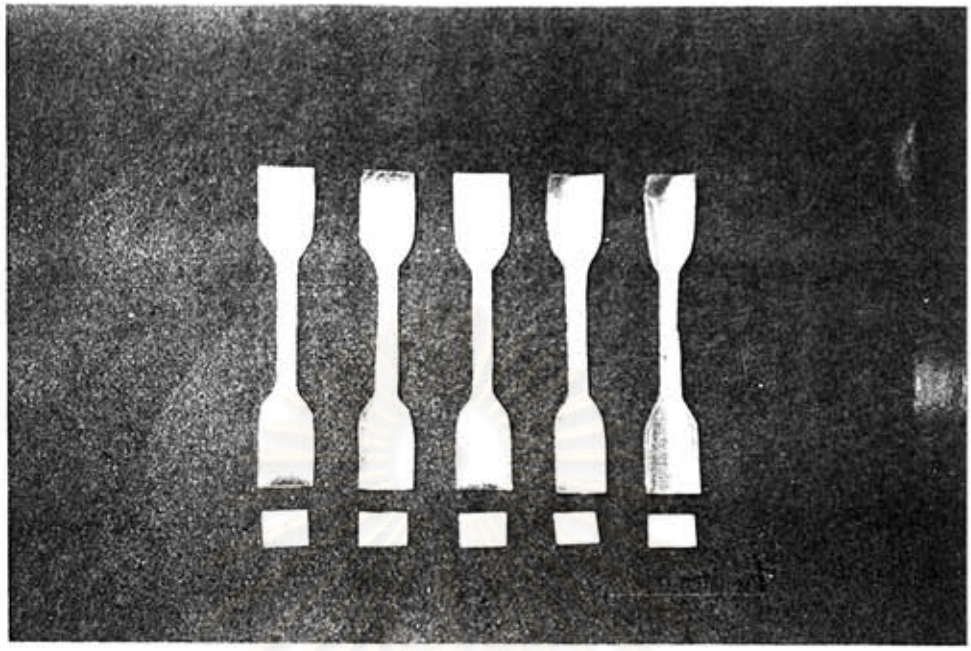
In this experiment, three specimens were subjected to the test for each film formulation. After the test film was broken, the using force was recorded by a digital system. For the measurement of percent elongation at break, the gauge length was marked at 2.0 cm in the middle section of the test film which was calibrated to zero percent elongation. Then percent elongation at break was measured visually by using ruler scale and recorded from the different in the length at the breaking point of the specimen.

The ultimate tensile strength and percent elongation at break were calculated from the following formulas.

$$\text{Ultimate tensile strength} : \frac{\text{breaking load}}{\text{cross-section area of the test specimen}} \quad (4)$$

$$\text{Percent elongation at break} : \frac{(\text{different in the length at breaking point}) \times 100}{\text{original length of the test specimen}} \quad (5)$$

A



B

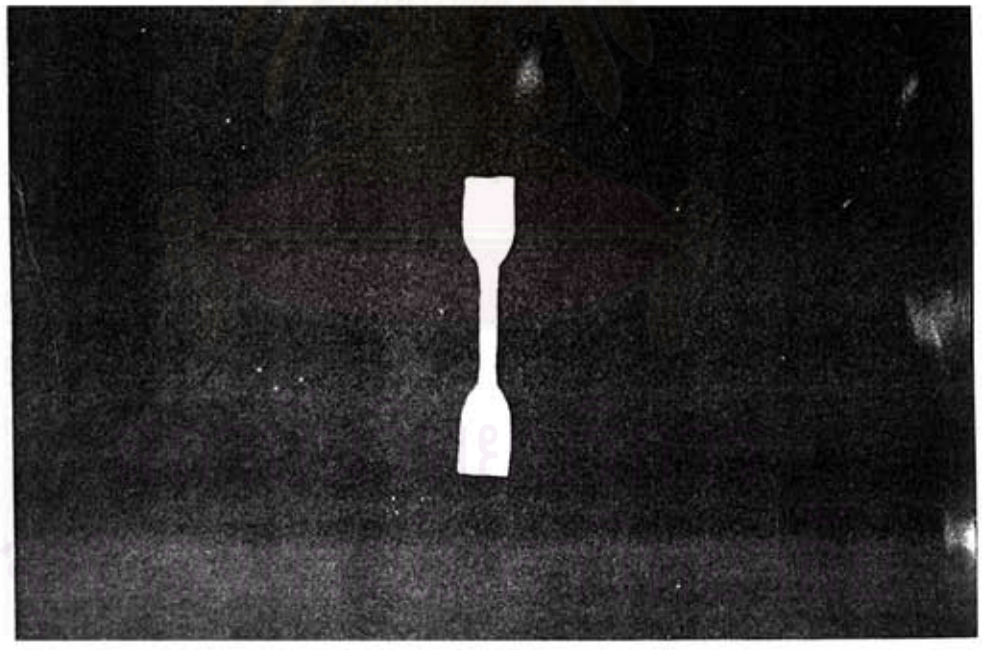


Figure 8 The photograph of standard template of aqueous polymeric films

A. standard template of Surelease® films (5%, 10%, 15%, 20% and 25% of dibutyl phthalate)

B. standard template of Eudragit® NE 30D film