

CHAPTER II

EXPERIMENT

1. Materials

The following materials obtained from commercial sources were used as received.

1.1 Model Drug

- Theophylline Anhydrous B.P.
(supplied by B.L. Hua & Co., Ltd. Thailand, Lot. No. 951203)

1.2 Additives

- Poly (ethylacrylate methylmethacrylate) aqueous dispersion
(Eudragit[®] NE 30D, Rohm GmbH, Germany, Lot. No. 1240512042
{ lot A } and 1260112014 { lot B })
- Ammonio Methacrylate Copolymer, Type A aqueous dispersion
(Eudragit[®] RL 30D, Rohm GmbH, Germany, Lot. No. 0450316165)
- Hydroxypropyl Methylcellulose
(Methocel E 15 LV, The Dow Chemical Company, USA, Batch
No. MM94110921 E)
- Gelatin
(Srichand United Dispensary Co., Ltd. Thailand, Lot. No. GA10)
- Lactose Anhydrous
(Wyndale, New Zealand, Lot. No. 2120002122)



- Cab-O-Sil M-5
(Cobot, Belgium, Lot. No. S5330)

1.3 Dissolution Medium

- Potassium Dihydrogen Phosphate
(Merck, Germany, Lot. No. A852573)
- Sodium Hydroxide, AR grade
(Merck, Germany, Lot. No. 211190)
- Hydrochloric Acid
(Merck, Germany, Lot. No. 403872)

1.4 High Performance Liquid Chromatography Analysis

- 8-chlorotheophylline
(Sigma Chemical Co., Ltd. USA, Lot. No. 52 H0745)
- Acetonitrile, HPLC grade
(J.T.Baker Inc., USA, Lot. No. K08827)
- Ammonium Hydroxide, AR grade
(Merck, Germany, Lot. No. K221311082 531)
- Glacial Acetic Acid
(Merck, Germany, Lot. No. 227 K18049863)
- Sodium Acetate Trihydrate, AR grade
(Carlo Erba, Italy, Lot. No. 2691 F100)

2. Equipment

- Analytical balance (Sartorius 1264 MP, Germany)
- Dissolution apparatus (Model SR-2, Hansan Research, USA)
- Fluidized bed coater (Uni-Glatt Laboratory Units, Germany)
- Fourier transform infrared spectrometer (Model 1760 X, Perkin Elmer, USA)

- High performance liquid chromatography (Millipore Corporation, Water Chromatography, USA)
- Hobart mixer (Model EB 20F, Thailand)
- Oscillating granulator (Viuhang Corporation, Thailand)
- Peristaltic tube pumps (Verder Type VRX 88, Germany)
- pH meter (Hanna Instruments 8417, USA)
- Scanning electron microscope (Model JSM-5410LV, Japan)
- Stirring hot plates (Thermolyne Corporation, USA)
- Surface area measurement (Flowsorb 2300 ,USA)
- Tensometer (Model 4301, Instron Corp., USA)
- Ultraviolet/visible spectrophotometer (Shimadzu UV-160A, Japan)

3. Methods

3.1 Preparation of Theophylline Granules

Granules containing 65.42 % w/w of theophylline were prepared by means of wet granulation. Fractions of drug and diluent were mixed in Hobart mixer for 10 minutes and wet granulated using 12 % w/w of aqueous gelatin solution as a binder. The granulation composition is presented in Table 2. The damp mass was passed through a 20 mesh sieve , then oven-dried at 50 °C for 4 hours. The 18/20 mesh fraction of dried granules was classified using a sieve shaker fitted with U.S. Standard sieves. It was shaken for 10 minutes in order to ensure no aggregates and fine particles prior to use. The granules were stored in tightly-closed , dry containers.

Table 2 Composition of theophylline granules for coating.

Theophylline anhydrous	65.42 %
Lactose	30.37 %
Gelatin	4.21 %

3.2 Preparation of the Controlled-release Theophylline Granules

3.2.1 Preliminary Investigation for Suitable Coating Condition and Coating Dispersion

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, atomizing air pressure and spraying rate to have uniformity of coating by visual observation of coated granules.

The compositions of coating dispersion which applied on the granules in preliminary investigation are presented in Table 3. During this study, Cab-O-Sil which was used to reduce the stickiness of drying coated granules was varied to obtain the optimum level for coating. The amount of Cab-O-Sil was varied in concentration of 25 % , 30 % , 35 % and 40 % w/w of polymer calculated on dry polymer basis (1.875 % , 2.250 % , 2.625 % and 3.000 % w/w of coating formulations), respectively.

Table 3 Compositions of coating dispersion in preliminary investigation.

Component (% w/w)	Formulation			
	1	2	3	4
Eudragit® NE 30D	25.0	25.0	25.0	25.0
Cab-O-Sil	1.875	2.250	2.625	3.000
Water qs.	100.0	100.0	100.0	100.0

3.2.2 Preparation of Coated Granules using Different Spraying Method

Granules of 300 g were coated with Eudragit® NE 30D lot A and lot B by using top spray fluidized bed coater, setting the coating condition according to Table 4 which was based on the conditions previously found in the preliminary investigation. These conditions were found to be optimal because there were no blockage of the spray nozzle, no aggregation of the granules and the coating was completed. A suitable composition of aqueous polymeric coating is presented in Table 5. During the coating operations, the aqueous dispersions were stirred continuously to prevent sedimentation of the insoluble particles. The granules were fluidized in chamber until the temperature in coating region of the apparatus reached 40 °C then, spraying was operated. Coating dispersions were pumped through a peristaltic pump at a flow rate of 4 ml/min. and the pneumatic spraying pressure was 1 bar. Finally, the coated granules were dried in the same apparatus during 10 minutes at the same inlet temperature to remove the residue water.

Table 4 The coating condition using top spray method.

Coating conditions	Value
Inlet air temperature	40 °C
Outlet air temperature	30 °C
Spraying air pressure	1 bar
Feed rate of coating dispersion	4 ml/min.

Table 5 Composition of aqueous polymeric coating dispersion.

Component	% w/w
Eudragit® NE 30D	25.00
Cab-O-Sil	2.25
Water qs.	100.00

To study effect of spraying method, the bottom spray method was also used to coat theophylline granules. Granules of 300 g were coated with Eudragit[®] NE 30D lot B in the bottom product container with a cylinder about half the diameter of the base inside located (Wurster column). The coating procedure and condition were similar to that previously described in top spray coating method.

The amount of aqueous polymeric coating was varied to determine the effect of coating level on the release profile of theophylline granules. The coating level of each batch was determined by calculating on the basis of Eudragit[®] NE 30D content in aqueous polymeric coating used.

3.2.3 Preparation of Coated Granules Using Different Atomizing Air Pressure

Effect of atomizing air pressure on the release characteristics and physical properties of coated granules was investigated by coating granules with Eudragit[®] NE 30D lot A at the same coating level (about 10 % of core granules) using top spray method. The coating condition was similar to that described in Table 4 except the spraying air pressure was varied from 1 to 2 and 3 bar.

3.2.4 Preparation of Coated Granules Using Polymer Blends

The permeabilities and release rates of granules coated with Eudragit[®] NE 30D were increased by adding the higher permeable or hydrophilic substances. In this study, hydroxypropylmethylcellulose (HPMC), a water-soluble cellulose derivative, and Eudragit[®] RL 30D, a higher permeable film formed acrylic polymer, were selected to mixing with Eudragit[®] NE 30D lot B and used as the coating materials.

The blends of polymers were prepared as follow :

Eudragit[®] NE 30D/HPMC : (3:2), (3:1.5), (3:1), (3:0.5), (12.5:1),
(12.5:0.5), (100:1)

Eudragit[®] NE 30D/RL 30D : (50:50), (80:20), (90:10), (95:5)

Preparation of polymer blends of Eudragit[®] NE 30D/HPMC

Cab-O-Sil was prior dispersed in water. Before mixing, HPMC was prepared into aqueous solution and added. Then, Eudragit[®] NE 30D was slowly poured into the dispersion, and mixed homogeneously before starting the coating process.

Preparation of polymer blends of Eudragit[®] NE 30D/RL 30D

Cab-O-Sil was prior dispersed in water. Dispersions containing Eudragit[®] NE 30D/RL 30D required the addition of Tween 80 as a stabilizer to the emulsifier-free Eudragit RL dispersions. Before mixing, the Eudragit[®] NE 30D was first brought to pH 5-6 by the addition of small amounts of diluted acid. The mixing was achieved by slowly pouring one dispersion under moderate stirring into the other (Lehmann and Dreher , 1988). Then all components of coating dispersion were blended and mixed homogeneously before starting the coating process.

Top spray method of fluidized bed was used to coat the granules with the polymer blends. The coating level in each formulation was 10 % of core granules. The coating condition was similar to that presented in Table 4 except the inlet and outlet temperatures for coating with Eudragit[®] NE 30D/HPMC were 50 °C and 30 °C, respectively.

4. Evaluation of Uncoated and Coated Granules

4.1 Morphology

The uncoated and coated granules were examined under a scanning electron microscope (SEM) for morphological evaluation. Shape and surface topography of granules were determined. The granules were also cross-sectioned for observation of the coated film. The samples were prepared by gold sputtering technique before SEM examinations.

4.2 Bulk, Tapped Density and Carr's Compressibility Index

The bulk and tapped densities 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.

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

where T and B are tapped and bulk density, respectively.

4.3 Moisture Determination

The moisture content of granules was determined by using moisture determination balance. About 7 g of granules were spread uniformly in a thin layer of aluminium plate and accurately weighed on a pan. Then, it was exposed to an infrared lamp at intensity of 4 watts until constant weight was obtained. The

percentage of moisture content was calculated based on the following equation. The result were obtained from the average of three determinations.

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

4.4 Determination of Angle of Repose

Each angle of repose was determined by the cylinder method. An appropriate amount of powder was carefully filled into a cylinder which was placed on the graph paper until it was filled at the top of the cylinder. Then the cylinder was slowly lifted in the vertical direction, thus producing around heap of powder. The result was averaged from three determinations. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} \frac{H}{R} \dots\dots\dots(3)$$

where α , H and R are the angle of repose, height and radius of the heap respectively.

4.5 Determination of Flow Rate

Accurate weight of about 30 g of granules was 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 averaged from three determinations was reported in term of g/second.

4.6 Measurement of Surface Area

A method for determination of surface area of theophylline granules was based on the guidance of the American Society for Testing Material method

D4567-86. The principle of surface area measurement was nitrogen gas adsorption on the surface area of testing material. Thereby, the resultant record would show in term of m^2 per gram of theophylline granules.

4.7 Determination of Theophylline Content in Uncoated and Coated Granules

The isocratic reversed-phase high performance liquid chromatographic (HPLC) method for determining theophylline content used in this study was modified from USP XXIII as follow :

The buffer solution was prepared by transferring 2.72 g of sodium acetate trihydrate to a 2000 ml volumetric flask. About 200 ml of reversed osmosis treated water was added. The mixture was shaken until dissolved completely. Then, glacial acetic acid of 10.0 ml was added. Finally, the solution was adjusted up with water to volume , and mixed.

As mobile phase, acetonitrile HPLC grade of 250.0 ml was transferred to 2000 ml volumetric flask , diluted with buffer solution to volume , and mixed. This solution was filtered through a 0.45 μm membrane filter and degassed by sonication for 15 minutes before using.

Calibration Curve of Theophylline

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

To prepared the internal standard solution, 8-chlorotheophylline of 35 mg was accurately weighed and transferred to a 100 ml volumetric flask. About 10 ml of 6 N

ammonium hydroxide was added. The mixture was shaken until dissolved completely, adjusted to 100 ml with mobile phase and used as internal standard stock solution with concentration about 0.35 mg/ml.

The theophylline stock solution volume of 1.0 , 3.0 , 5.0 , 7.0 and 9.0 ml was individually into 50 ml volumetric flask and 10.0 ml of internal standard stock solution filled was pipetted to all flasks and adjusted with mobile phase to their volumes. The final concentration of each solution was 20.0 , 60.0 , 100.0 , 140.0 and 180.0 $\mu\text{g/ml}$, respectively and the concentration of internal standard was about 70.0 $\mu\text{g/ml}$ in all flasks.

The ratio between peak area of theophylline anhydrous and internal standard was determined by HPLC method at an absorbance of 272 nm. Each concentration was determined in duplicate. The calibration curve and representative chromatogram are shown in Figures 74 and 75 (Appendix A), respectively.

Assay of Theophylline Anhydrous in Uncoated and Coated Granules

Approximately 3.20 g of sample was accurately weighed and transferred to 500 ml volumetric flask. About 100 ml of water and 50 ml of 6 N ammonium hydroxide solution was added. The mixture was adjusted to volume with water and heated on hot plate with occasional stirring just to boiling. Then, it was removed from the hot plate and sonicated for about 1 minute while it was still hot. Subsequently, it was cooled to room temperature , diluted with water to volume, mixed and filtered. A volume of 2.0 ml filtrate was pipetted into a 50 ml volumetric flask. The internal standard stock solution of 10.0 ml was added. Finally , the solution was diluted with mobile phase to volume and mixed. The concentration of theophylline anhydrous was determined by HPLC method at an absorbance of 272 nm and calculated using standard calibration curve in Figure 74 (Appendix A).

Chromatographic Conditions

The chromatographic system in this study consisted of

Chromatographic column	:	Spherisorb-ODS, particle size 5 μ m with dimension of 250 x 4.6 mm
Flow rate	:	1.0 ml/min.
Injection volume	:	20.0 μ l
Detector	:	UV detector was set at 272 nm
Chart speed	:	0.25 cm/min.

4.8 Dissolution Studies of Uncoated and Coated Granules

4.8.1 Dissolution Studies of Uncoated Granules

Dissolution of theophylline from uncoated granules was studied by using USP XXIII basket method with thousand milliliters of phosphate buffer pH 6.6 as a dissolution medium maintained at 37 ± 0.5 °C. The basket was adjusted to rotate at 100 rpm. A quantity of uncoated granules equivalent to 200 mg of theophylline anhydrous was placed in the dissolution medium. Ten milliliters aliquots of the dissolution medium were pipetted out at the predetermined interval of 5, 10, 15, 20, 30, 40, 50 and 60 minutes. The equivalent volume of fresh dissolution medium was immediately replaced at each time interval of withdrawal.

Each sample was diluted to suitable concentration which gave the absorbance between 0.2-0.8. The absorbance was determined spectrophotometrically at 272 nm.

Each of the dissolution values reported was based on an average of three determinations of each formulations. The amount of theophylline released at any time interval was calculated from the calibration absorbance-concentration curve.

4.8.2 Dissolution Studies of Coated Granules

The dissolution studied of coated granules were performed according to USP XXIII basket method. The procedure was similar to that described in dissolution studies of uncoated granules except the sampling were 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours.

4.8.3 Calibration Curve of Theophylline

Theophylline anhydrous of 200 mg was accurately weighed and dissolved in phosphate buffer pH 6.6. The solution was then adjusted to 2000 ml in a volumetric flask with the same medium and used as a stock solution to obtain the solution of known concentrations between 0-14 µg/ml calibration.

The UV absorbance of each concentration was determined by using a single beam spectrophotometer in a 1-cm cell at 272 nm. 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 24 (Appendix A) showed a linear relationship. The standard curve of theophylline after regression analysis is illustrated in Figure 73 (Appendix A).

4.9 Preparation of Aqueous Polymeric Films

4.9.1 Preparation of Eudragit® NE 30D Films

Eudragit® NE 30D film was prepared by dilution of Eudragit® NE 30D with deionized water in a ratio of 1:3 (polymer : water). Cab-O-Sil of 30 % w/w of

polymer calculated on dry polymer basis was added. Then, the diluting polymer was cast on a glass plate and allowed to dry at room temperature overnight.

4.9.2 Preparation of Eudragit® NE 30D/HPMC Films

Eudragit® NE 30D/HPMC films were prepared in ratios of 3:2, 3:1.5, 3:1, 3:0.5, 12.5:1, 12.5:0.5 and 100:1, respectively. HPMC was dispersed in deionized water in a ratio of 1:3 (polymer : water). Eudragit® NE 30D and Cab-O-Sil of 30 % w/w of polymer calculated on dry polymer basis were added. Then, the diluting polymer was cast on a glass plate and allowed to dry at room temperature overnight.

4.10 Determination of Water Sorption

Each aqueous polymeric film was circularly cut into 4.3 cm diameter and dried to constant weight in a desiccator at room temperature. Then it was immersed in deionized water at 37 °C in an incubator for 24 hours. The resultant wet film was blotted with filter paper to remove excess water and weighed. Water sorption of polymeric films was calculated from the following equation and reported from the average of three determinations.

$$\% \text{ water sorption} = \frac{(\text{wet weight of film} - \text{dried weight of film}) \times 100}{\text{dried weight of film}} \dots(4)$$

4.11 Determination of Ultimate Tensile Strength and Percent Elongation at Break

The tensile tester was used as an apparatus for measuring ultimate tensile strength and percent elongation at break of test films. A film specimen was cut out using a standard template as shown in Figure 8. The thickness of each specimen was

the average value of four separate measurements taken along the middle 4.0 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 (4.0 mm). The test film was clamped by an upper and lower grip then tensile tester was operated.

In this study, three specimens were subjected to the test for each film formulation. After the test film was broken, the forces were 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 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 equations.

$$\text{Ultimate tensile strength} = \frac{\text{breaking load}}{\text{cross-section area of the test specimen}} \dots\dots\dots(5)$$

$$\text{Percent elongation at break} = \frac{(\text{different in the length at breaking point}) \times 100}{\text{original length of test specimen}} \dots\dots(6)$$

4.12 Infrared Absorption Study

The Eudragit®NE 30D lot A and B, HPMC including blend of Eudragit®NE 30D / HPMC in ratio of 1:1 were used to study the interaction between Eudragit®NE 30D and HPMC in the polymeric film using infrared (IR) spectrophotometer in a range of 4000 - 750 cm⁻¹ of wavenumber.



A



B

Figure 8 The photographs of standard template of aqueous polymeric films.

A. Standard template of Eudragit®NE 30D

B. Standard template of Eudragit®NE 30D/HPMC films

(3:3, 3:1.5, 3:1, 3:0.5, 12.5:1, 12.5:0.5 and 100:1)