Chapter II

Experimental

1. Materials

All materials obtained from commercial sources were used as received.

1.1 Active Drug -Theophylline anhydrous, BP (Lot. No. 8907052, China ,supplied by Pharmaceutical Traders Co., Ltd, Thailand)

1.2 Additives -Surelease(R) (Formula No. E-7-7050, Lot. No. 600007, Colorcon Ltd, USA)
-Cab-O-Sil (Cabot, Belgium)
-Lactose hydrous (Wyndale, New Zealand)
-Magnesium stearate (supplied by Pharmaceutical Sciences, Thailand)

1.3 Dissolution Media
-Potassium dihydrogen phosphate, AR grade (E.Merck, Germany)
-Sodium hydroxide, AR grade (E.Merck, Germany)

-Hydrochloric acid, AR grade
(E.Merck, Germany)

1.4 Solvents -25% Ammonium hydroxide, AR grade
(E.Merck, Germany)
-Chloroform, AR grade
(E.Merck, Germany)

2. Preparation of Co-spray Dried Powders

2.1 Formulation of Co-spray Dried Solution

The compositions of spray dried solution employed for investigation on the effect of processing variables were presented in Table 1.

Table 1 Formulation of Co-spray Dried Solution

Ingredients	%W/W of Solid
Theophylline	69.7
Surelease(R) *	4.3
Lactose	25.0
Cab-O-Sil	1.0
2% Ammonia Solution q.s to	1,500 ml.

* In 4.3 g. of Surelease(R) (dry weight) contained three percentage ethylcellulose.

2.2 Preparation of Co-spray Dried Solution

Theophylline was weighed and dissolved in 2% ammonia solution. Then Surelease(R) , Cab-O-sil and lactose were added, respectively. After the solution was mixed homogeneously, it was adjusted to 1,500 ml. with 2% ammonia solution. The solution contained 200 g. total solid.

2.3 Co-Spray Drying Process

The spray drying apparatus used was a laboratory type (NIRO ATOMIZER Mobile Minor Unit, Denmark), having drying chamber of 80 cm. in diameter, 60 cm. in cylindrical height and conical base. The cone angle was 60°. The solution were atomized into a drying chamber by rotating centrifugal wheel atomizer.

The processing variables of spray drying technique such as inlet air temperature, atomizing air pressure, feed rate and concentration of solution were varied according to the Table 2.

Table 2 Parameters of Spray Drying Process Variables

Inlet Air Temperature : 120° , 130° , 150 ° , 170°C
Feed Rate : 18, 24, 27, 30 ml/min
Atomizing Air Pressure : 2, 3, 4, 6 bar
Concentration of Solution : 10, 13.33, 20, 25 %

Only one factor was varied while the other spray drying conditions were kept constant as presented in Table 3. For example, when the inlet air temperature was changed at various levels, the feed rate, atomizing air pressure and concentration of solution would kept at 24 ml/min, 4 bar and 13.33% respectively.

Table 3 Spray Drying Conditions During Process Variable Studied

Inlet Air Temperature : 130°C
Feed Rate : 24 ml/min
Atomizing Air Pressure : 4 bar
Concentration of Solution : 13.33 %

3. Evaluation of Physical Properties of Co-spray Dried Powder

3.1 Powder Morphology

Morphology of powder samples were determined with scanning electron microscopy (JSM-T220A, JEOL, Japan). The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape and surface topography of the co-spray dried powders were observed.

3.2 Particle Size Distribution

Particle size distribution was determined using sieve analysis. The approximately 25 g. of powder was put on the top of a sieve series (Endecotts Ltd, London, England) ranging from 250, 125, 106, 75 to 45 μm. , respectively. The nest of sieve was placed on the sieve shaker (Josef Deckelman,Germany) for 20 minutes. The results averaged from two determinations were reported as percentage of weight retained on each sieve size. The geometric mean diameter was taken from graph.

3.3 Bulk, Tapped Density and Compressibility

The bulk and tapped density were determined from the weight of about 30 gm.(record accurate weight), carefully charged into a 100 ml. graduated cylinder and the bulk volume was recorded. Division of weight by bulk volume showed bulk density. Tapped density was performed by dropping graduated cylinder on a hard wood surface from a height of 5 cm. until a constant volume was obtained. Division of weight by this volume showed tapped density. Both densities were average from three determinations. The compressibility was calculated from the following equation.

$$\text{Percent Compressibility} = \frac{(T-B)}{T} * 100$$

T and B are tapped and bulk density, respectively.

3.4 Angle of Repose

Angle of repose was determined by cylinder method. Appropriate amount of powder was carefully filled in the cylinder (height=5.2 cm., radius=2.4 cm.) placed on the graph paper. When powder was filled at the top of cylinder, slowly lifted the cylinder in the vertical direction, producing round heap. The results averaged from three determinations were reported. Angle of repose was calculated from the following equation.

$$\alpha = \mathrm{Tan}^{-1} \frac{H}{R}$$

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

3.5 Moisture Determination

The moisture content of powder was determinated using the Mettler LP16 integrated with Mettler PM 100 (Mettler Instrument AG, Switzerland). About 1 g. of sample was exposed to an IR Lamp until constant weight was obtained. The percent moisture content was calculated automatically. Results were obtained from the average of three determinations.

3.6 Determination of Theophylline Content of Co-spray Dried Powder

The method for determining theophylline content used in this study was modified from Sa, Bandyopadhyay, and Gupta (1990). The approximately 100 mg. of sample was accurately weighed and dissolved in a 5 ml. chlorofrom. The 150 ml. of 0.1N. HCl was added and stirred for 60 minutes using magnetic stirrer. The mixture was then heated to eliminate chlorofrom completely. After it was cooled to room temperature, adjusted with 0.1N. HCl to 200 ml. in volumetric flask. Filter the solution, discard the first 10 ml. of filtrate and transfer 1.0 ml. to 50 ml. volumetric flask. Add 0.1N. HCl to volume and mix. Measure the absorbance of resulting solution at 268.5 nm. using double beam spectrophotometer (Spectronic 2000, Bausch & Lomb,USA). The 0.1N.HCl was employed as a blank. The content was calculated from standard curve.

4. Preparation of Matrix

The powder was compressed into matrix at compressional pressure of 500 pounds by Carver Laboratory Press (Perkin-Elmer, Model C, Fred & Carver Inc, USA) using a 10 mm. diameter round flat faced punch. The compression pressure was maintained for 10 seconds and quickly released (before each tablet was compressed, die would be lubricated with magnesium stearate). The compositions of each matrix were shown in Table 4.

Table 4 Composition of Each Matrix

Ingredients	Amount per Matrix (mg.)
Theophylline	300 mg.
Ethylcellulose	3 %
Lactose	25 %
Cab-O-Sil	1 %

5. Matrix Evaluation

5.1 Thickness

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

5.2 Hardness

The hardness of matrix was determined using the Schleuniger-2E hardness tester (Dr. K. Schleuniger Co., Switzerland) . The mean and standard deviation of ten determinations were calculated and expressed in kilopound (Kp).

5.3 Disintegration Time

Disintegration time was determined according to USP XXII method (Disintegration apparatus : Hanson Research Corporation, Model QC-21, USA). The average of disintegration time was evaluated in water at 37 ± 2°C with disk.

5.4 Dissolution Studies

As oral controlled release tablets were supposed to pass the entire upper gastrointestinal tract, it would be ideal when the release of drug was constant over a wide range of pH values (from 1 to about 7). Therefore, an in vitro test for controlled release tablets should at least cover this pH range. For this reason, pH change method was used (Jonkman, Berg and De Zeeuw, 1983).

In the dissolution model with pH change, the pH of the medium was kept by 0.1N. HCl for two hours. Then the pH was increased to 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. All fluids were boiled to deaerated before use.

Nine hundred milliliters of medium were placed in a glass vessel specified in the USP dissolution test and equilibrated at 37 $\pm$ 0.5°C. One tablet was immersed in the vessel and the paddle, specified in the compendium, was placed at the center of the vessel and at 2.5 cm. above the bottom of the vessel. The dissolution apparatus (Hanson Research Corporation, Model SR-2, USA) was operated at the speed of 50 rpm.. Six tablets were evaluated.

Five milliliters of specimen were withdrawn at the time interval of 15, 30, 45 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours. The same quantity of medium was added

immediately after each sampling to keep the volume of the medium constant during the experiment.

Each sample was diluted to suitable concentration. The absorbance was determined spectrophotometrically in a 1-cm. cell at 268.5 nm. for 0.1N. HCl and 270.3 nm. for phosphate buffer pH 6.8.

The amount of theophylline release at any time interval was calculated from the calibration absorbance-concentration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug release.

5.5 Calibration Curve of Theophylline

Theophylline 200 mg. was accurately weighed and dissolved in 0.1N. HCl or phosphates buffer pH 6.8 . The solution was then adjusted to 2000 ml. with 0.1N HCl or phosphate buffer pH 6.8 and used as stock solution.

The stock solution was individually pipetted 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml. into 50 ml. volumetric flask and diluted to volume with 0.1N. HCl or phosphate buffer pH 6.8. The final concentration of each solution was 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 µg./ml. respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm.

cell at 268.5 nm. for 0.1N.HCl and 270.3 nm. for phosphate buffer pH 6.8. The 0.1N.HCl and phosphate buffer was used as blank solution. Each concentration was determined in duplicate.

The calibration curve of theophylline was illustrated in Figure 63 and 64 (in Appendix), respectively.

6. Study on the Effect of Tabletting Process on the Properties of Matrices

After the study in 2.3- 5.4 was completed , the suitable spray drying conditions were selected and employed to prepare co-spray dried powder for further study.

The effects of tabletting process on the properties of matrices, especially their release characteristics, were investigated into three categories:

(a) The effect of adding magnesium stearate on the release characteristics of the matrices were carried out. The spray dried powders were mixed with magnesium stearate which passed through 80 mesh sieve at 0.75 and 1.5 % concentration levels. Then it was compressed into matrices at a compressional pressure of 500 lbs using hydraulic press. The dissolution of the matrices were measured as previously described.

(b) The spray dried powders were compressed into matrices at three compressional pressure of 500 lbs, 1000 lbs, 1500 lbs by Carver Laboratory Press. And the relationships of compression force and release characteristics were investigated.

(c) The spray dried powders were mixed with 0.75 % of magnesium stearate before tabletting with instrumented single punch machine. A 11 mm. diameter concave rounded punch was used. The matrix was produced at a compressional force of 300 lbs, 500 lbs and 700 lbs. The release behaviors of the matrices produced were evaluated.

7. Study on the Reproducibility of Drug Release Pattern of Matrices Prepared by Spray Drying Technique

Reproducibility of the drug release pattern for three consecutive batches was investigated. The co-spray dried powders were prepared in the same condition as described in 6 . The matrices were prepared by adding 0.75% of magnesium stearate before compression on instrumented single punch tabletted machine at the force of 500 lbs. Dissolution rate of matrices of each batch was tested.

Scale-up batch of 0.5 kg. of co-spray dried powder was produced and tabetting into matrices with the same procedure. Dissolution rate of those matrices was evaluated.