Chapter Two

Experimental

1. Materials

The following substances were obtained from commercial sources :

Powdered sugar (Saereewatt, Thailand), tapioca starch (Pharmaceutical Science, Thailand), magnesium stearate (Pharmaceuitcal Science, Thailand), talcum (Pharmaceutical Science, Thailand), diazepam (China), chlorpheniramine maleate (Pharmaceutical Science, Thailand), Starch-1500^{R} (Colorcon, USA), Emcompress^{R} (Edward Mendell, USA), Tabfine^{R} (Rama Production, Thailand), Emdex^{R} (Edward Mendell , USA), Tablettose^{R} (Rama Production, Thailand), polyvinylpyrrolidone (PVP, K-30, Germany), gelatin 150 bloom (Japan), methylcellulose type A 15 LV (Colorcon, USA), sulfuric acid (BHD, England), methanol (E. Merck, Germany), ethanol (E. Merck, Germany), hydrochloric acid (E. Merch, Germany), magnesium chloride (E. Merch, Germany), magnesium nitrate (E. Merch, Genmany), sodium chloride (E. Merch, Germany).

2. Methods

2.1 The Composition of the Granules

The mixtures of powdered sugar and tapioca

starch at various proportions in the ratio between 1:9 upto 9:1 were employed to prepare the granules. Polyvinyl pyrrolidone (PVP), gelatin, methylcellulose (MC), and tapioca starch were selected as the granulating agents.

2.2 Operations of Fluidized Bed Granulator

Laboratory unit fluidied bed granulator of 1 kg capacity was employed to prepare the granules (Uniglatt, West Germany). The batch size used in all granulations was 400 g. Powdered sugar and tapioca starch were passed through a seive number 20 to break up any agglomerates. Then, the powder mixtures were transfered into product container. The fluidizing air velocity and inlet air temperature were adjusted. When inlet air temperature reached the desired setting, the granulating solution was sprayed down onto the fluidized powders at the proper rate. The granulating liquid was fed to the binary nozzle by means of peristaltic pump and was atomized by using adjustable compressed air. Control of liquid rate was effected by mean of the variable driver. During granulation process the fine powder adhered to the filter bag was occasionally shaken down to prevent clogging of the fine powder to filter bag.

The granulation process was continued until the desired granule growth was attained. Upon completion of the spraying process, the drying cycle was initiated by

elevating inlet air temperature to 60°C for 10 minutes. The granules were then removed from the product container and dried at 60°C for 4 hours to remove the remaining trace of water.

As a starting point for these studies, preliminary test on the optinum conditions of fluidized bed granulator were conducted. In these preliminary investigations, 1:1 powdered sugar and tapioca starch mixtures were employed to prepared the granules with all binder materials by varying the following parameters : inlet air temperature, binder solution spraying rate, concentration of binder solution, concentration of binder in granule and nozzle pressure. Those conditions which produced good quality of granules were chosen as the criteria in preparation of all granulations.

2.3 Preparation of Binder Solutions

PVP solution was prepared by dissolved in water whereas gelatin solution was prepared by heating the slurry with constant stirring to 80°C after the gelatin powders were first thoroughly hydrating in water for 10 minutes. Methylcellulose solution was prepared by thoroughly dispersed in hot water about one-third of volume used and then filled with cold water to make clear solution. Tapioca starch solution was prepared by dispersion in water and then heated with constant stirring to 80°C till translucent solution was formed. During

spraying process in fluidized bed granulation, methylcellulose and tapioca starch solution must be warmed up to 80°C to prevent gelling of binder solution.

2.4 Evaluation on the Physical Properties of the Granules

2.4.1 Morphology Determination

Shape and surface texture of the granules were characterized using scanning electron photomicrograph (JSM-35CF, JEOL, Japan) in order to perceive the morphology and the formation of the granules prepared from powdered sugar and tapioca starch mixtures.

2.4.2 Particles Size Distribution Determination

Particle size distribution was determined by seive analysis. A fifty grams of granules was placed on the first seive of a nest of seives (Endecotts, England) arranged in order of decreasing aperture size. The nest of seives was clamped onto a seive shaker (Josef Deckelman, Germany) and then subjected to vibrations for ten minutes. The amount retained on each seive was ascertained as percent retained on seive size number.

2.4.3 Bulk Density Determination

Bulk density was determined using a

100-ml measuring cylinder tilted to an angle of 45 degree while the accurate weight (approximately thirty grams) of granules was poured. The volume occupied by the granules was read to the nearest 0.5 ml and the density was calculated as follow

$$\text{density} = \frac{\text{weight of granules}}{\text{volume occupied by the granules}}$$

The bulk density recorded was average of 3 determinations

2.4.4 Tap Density Determination

Following determination of bulk density, tap density was measured. The constant volume of granules was generally attained after 100 tamps at the height of 5 cm. The volume occupied by the granules was read to the nearest 0.5 ml and the density was calculated as determined in 2.4.3. The tap density recorded were average of 3 determinations. Percent compressibility of the granules was calculated from tap density and bulk density as follows.

$$\% \text{ compressibility} = \frac{100\ (\text{tap density} - \text{bulk density})}{\text{tap density}}$$

2.4.5 True Density Determination

True density was determined using a 50-ml pychnometric bottle (Wertheim, Germany). Five grams of the sample were accurately weighed and transfered into

the pychnometer. Enough absolute alcohol was added to fill up the pychnometer, and the granule-solvent mixture was shaken. The whole bottle was accurately weighed and the true density was calculated as follows.

$$\rho = W_2W_3/[50\ (W_2-W_4+W_3)\ ]$$

ρ = true density

W_2= solvent weight in a pychnometer

W_3= sample weight in a pychnometer

W_4= granules-solvent mixture weight in a pychnometer

True density determination was measured in triplicate and averaged.

2.4.6 **Flowability Determination**

"Flowgrams" of the granules without any additives were obtained by the use of flowmeter. The flowmeter consists of plastic tube which has 38.1 mm in diameter and 45 cm in height, stand on plastic plate which has a circular orifice of 11 mm in diameter. Granules were full-filled into the plastic tube and then the plastic column was pulled over the orifice of the plastic plate. The granules flew through the orifice into a beaker beneath the plastic plate. The granules were collected every 4 seconds intervals for 16 seconds. Amount of the granules flew through the orifice was plotted against times and flow rates were calculated from

the slope. The mean flow rates were the average of 3 determinations

2.4.7 Moisture Content Determination

The moisture content of the granules was determined by the moisture determination balance (Ohaus , Model No 6100-H, USA) operating at scale of 4 for 30 minutes. Ten grams of granules were used and reported values were the average of three determinations.

2.5 Evaluation of the Compressional Characteristics of the Granules

The study of the compressional characteristics of the granules was accomplished on a hydraulic laboratory press (Perkin-Elmer , Model C, USA). The 300 mg granules without addition of any adjuncts were manually compressed under a force ranged from 1000 upto 2500 pounds using a 9.3 mm in diameter, flat-faced, circular punch. The compression pressure was maintained for 3 seconds and then quickly released.

2.5.1 Compressional Force-Hardness Profile

The hardness of the compacts was determined immediately after compression by using a hardness tester (Schleuniger-2E, Switzerland) and expressed in kilopond (Kp). The hardness of the compacts was an average of four determinations. Compressional

force-hardness profile of the granules were obtained by plotting compressional forces against mean values of the hardness.

2.5.2 Compressional Force-Disintegration Time Profile

Disintegration times of compressed matrices at each compressional force were determined in distilled water using USP disintegration apparatus (Hanson Research, USA). Disintegration time of 6 tablets was individually observed and the averages were calculated. Compressional force-disintegration time curves were illustrated. Both compressional force-hardness profile and compressional force-disintegration time profile of granules which provided good physical quality of each binder were compared with other direct compressible diluents.

2.6 Evaluation of Tabletting Characteristics of the Granules

Tabletting characteristics of the granules were investigated on a single punch tabletting machine (Viuhang Engineering , NO 49 , Thailand), fitted with 9.3 mm, standard concave punch. The following tablet composition was used.

Powdered sugar-tapioca starch granules	96 % W/W
Magnesium stearate	1 % W/W
Talcum	3 % W/W

Granules were mixed with 80 mesh-seived lubricants for 2 minutes in cube mixer (Erweka AR 400 , type UG, West Germany) and then compressed into 300 mg tablets to a targeted hardness about 4 kiloponds. After compression, the following physical properties of tablets were assessed

2.6.1 **Weight Uniformity**

Weight of twenty tablets were individually determined using an analytical balance (Sartorious , A-200S, Germany). The average weight in mg, standard deviation, and coefficient of variation were calculated.

2.6.2 **Thickness**

The thickness of ten teblets was individually measured in mm using a micrometer (Teclock Corp., 0.01 mm, Japan). The average thickness, standard deviation, and coefficient of variation were obtained.

2.6.3 **Hardness**

Hardnes values of ten tablets were determined immediately after compression by the method has been described in section 2.5.1. In this case, the

hardness recorded were the average of 10 determinations.

2.6.4 **Disintegration Time**

A disintegration test apparatus and procedure complying to the specifications of USP was employed using distilled water maintained at 37°C as the disintegration medium. Disintegration time of 6 tablets was individually observed.

2.6.5 **Friability**

Twenty tablets were dedusted with a soft brush to removed all adhering particles and accurately weighed. The tablets were placed in a Roche Friabilator (Erweka, Germany) rotated for 15 minutes at 25 r.p.m. The tablets were dedusted and reweighed. The weight loss after the test was used to calculate percent friability. Three determinations were completed for each formulation and the mean percent friability was reported.

2.7 **Application of the Granules in Preparing the Tablets of Microdose Drug Substances.**

On the basis of experiment data studies in section 2.4 , 2.5 , and 2.6 only one granule formulation prepared from each type of binder which provided the good quality granule and good tabletting characteristics was selected to produce tablets containing drug substances. Diazepam and chlorpheniramine maleate were chosen as model

drugs to make the tablets according to the following formulations

Diazepam tablet formulation :

Diazepam	5.0 mg
Sugar-starch granules	110.2 mg
Magnesiun stearate	1.2 mg
Talcum	3.6 mg

Chlorpheniramine maleate tablet formulation :

Chlorpheniramine maleate	4.0 mg
Sugar-starch graunles	111.2 mg
Magnesium stearate	1.2 mg
Talcum	3.6 mg

Diazepam or chlorpheniramine maleate powders were milled with mortar and pestle, passed through seive # 100, then mixed with granules by geometric dilution in a plastic bag. The mixtures of drug-granules were transfered into a cube mixer and mixed for 15 minutes. The 80-mesh lubricants were added and mixed for another 2 minutes. The granules were compressed into 120 mg tablets by a double punch tabletting machine (Viuhang Engineering , No 49, Thailand) fitted with 6.35 mm, flat-faced punch and compressed to a targeted hardness about 6 kiloponds. The physical properties of finished tablets were determined. These included : weight variation, thickness, hardness, disintegration time, friability, content

uniformity and dissolution. The methods of evaluations of weight variation, thickness, hardness, disintegration time, and friability were the same as described in section 2.6

2.7.1 **Content Uniformity of Diazepam Tablets (26)**

Sample of ten tablets were assayed individually. One tablet was placed in a test tube added with 5.0 ml of water and allowed to disintegrate for 20 to 30 minutes with occasionally swirling. Five ml of methanol was added and mixed. This solution was diluted quantitatively and stepwise with a 1 in 360 solution of sulfuric acid in dehydrated alcohol to obtained a solution containing about 10 µg of diazepam per ml and filtered. The standard solution of diazepam was prepared in the same medium to obtain a solution having a known concentration of about 10 µg per ml. Concomitantly determine the absorbances of both solution in 1-cm cells at the maximum absorbance wavelength of 285 nm using a spectrophotometer (Spectronic 2000, Bausch & Lomb, USA). The alcoholic sulfuric acid was used as the blank. Quality, in mg, of diazepam in a tabet was calculated by the formula.

$$\text{Diazepam content} = \frac{T.C.Au}{D.As}$$

T = label quantity

D = concentration, in µg per ml, of diazepam in the test solution

C = concentration, in µg per ml, of diazepam standard solution

Au = absorbance of the test solution

As = absorbance of the standard solution

2.7.2 **Content Uniformity of Chlorpheniramine Maleate Tablet (27)**

Sample of ten tablets were assayed individually. One tablet was placed in a mortar added with approximately 5 ml of water. The tablet was grinded for 10 minutes and transfered to a 100-ml volumetric flask. The volumetric flask was adjusted with water to 100 ml, mixed and filtered. The standard solution of chlorpheniramine maleate was prepared with the same medium to obtain solution having a known concentration about 40 µg per ml. Concomitantly determine the absorbances of both solutions in 1-cm cell at the maximum absorbance wavelength of 262 nm using spectrophotometer. Quantity, in mg, of chlorpheniramine maleate in a tablet was calculated by the formula.

$$\text{Chlorpheniramine maleate content} = \frac{T.C.Au}{D.As}$$

T = label quantity

D = concentration, in µg per ml, of diazepam in the test solution

C = concentration, in µg per ml, of diazepam standard solution

Au = absorbance of the test solution

As = absorbance of the standard solution

2.7.3 **Dissolution Rate Determination of Diazepam Tablet (28)**

The dissolution profile of diazepam tablets was determined according to the USP specification. The dissolution medium was 900 ml 0.1 N HCl maintained at 37 ± 0.5°C. The basket containing the test tablet was rotated at 100 r.p.m. Aliquots of the dissolution medium were withdrawn at predetermined time intervals. Each volume of sample withdrawn was replaced with an equivalent volume of dissolution medium. The amount of drug dissolved were assayed spectrophotometrically. The absorbance of samples measured at the wavelength of 242 nm and the amount of drug was calculated from the calibration curve of diazepam (Appendix A). Dissolution profiles were attained from the average of three determinations.

2.7.4 Dissolution Rate Determination of Chlorpheniramine Maleate Tablet. (29)

The dissolution profiles of the chlorpheniramine maleate tablets were determined according to the USP specifcation. Five hundred millitres of water maintained at 37 ± 0.5°C was used as the dissolution medium. The rotating speed of paddle was setted at 50 r.p.m. Aliquots of the dissolution medium were withdrawn at predetermined time intervals. Each volume of sample withdrawn was replaced with an equivalent volume of dissolution medium. The concentration of drug dissolved in the sample were determined spectrophotometrically by measuring the absorbance at the wavelength of 262 nm. The amount of chlorpheniramine maleate was calculated from the standard curve (Appendix B). Dissolution profiles were obtained from the average of three determinations.

2.8 Aging Studies of the Tablets

Following the physical evaluations of diazepam and chlorpheniramine maleate tablets after preparation as described in section 2.7. Those tablets taken from the same production batch were stored over a three month period for subsequent physical stability test. The tablets were kept in closed glass bottles at room condition and in opened plastic container at three relative humidity condition of 32, 51 and 75 percent. The various relative humidity storages were established by

keeping the samples in close dessicators containing the saturated salt solutions with the presence of excess solid salts of $MgCl_2$, $Mg(NO_3)_2$, and NaCl which could give the relative humidity at 32, 51 and 75 percent, respectively. The samples of storage tablets were taken for physical property tests at the end of 1, 2, and 3 month period. Tablets were evaluated by weight, hardness, thickness, friability, disintegration time, and dissolution behavior. The test methods and procedures were the same as described in section 2.7

2.9 **Comparative Studies of the Tablets Prepared from the Granules under Storage**

The investigation on physical property changes of the granules under storage was also conducted. The granules of the same production batches employed to prepare the tablets as described in section 2.7 were stored at room condition in closed glass bottles. The samples of granules were taken for preparation of diazepam and chlorpheniramine maleate tablets at the end of first, second, and third month. Methods of tablet manufacture and physical evaluations were the same as described in section 2.7. Study in this part would provide the information on the property of granules that could be used to produce the tablets of the same qualities of those made from freshy prepared granules.