CHAPTER II

MATERIALS AND METHODS

1. Materials

The following materials were obtained from commercial sources

1.1 Model drug

Diazepam (Batch No. 810414, CHINA)

1.2 Additives

1.2.1 Diluents

a) Spray Dried Lactose (BASF, Germany)

b) Mannitol (Pharmaceutical Science Co., Ltd., Thailand.)

c) Sucrose (Singhaburi Sugar Co., Ltd., Thailand)

d) Dibasic Calcium Phosphate (Pharmaceutical Science Co, Ltd., Thailand.)

e) Microcrystalline Cellulose (Avicel^{R} PH 101, FMC Corporation, U.S.A.)

1.2.2 Lubricants

Magnesium stearate B.P. (Duraham Chemical Ltd., England.)

1.2.3 Glidants

Talc B.P. (Il Shin Industrial Co., Ltd., Korea)

1.3 Capsules No. 4 (Parke Davis)

2. Equipment

The following equipment were used

2.1 Capsule Filling Machine (KSL, Bangkok Thailand.)

2.2 US. Standard Sieve No. 10, 20, 40 (Endecotts Ltd., London, England.)

2.3 Ball Mill (The Pascall Engineering Co., Ltd., England.)

2.4 Analytical Balance (Satorius Type 2442, Switzerland.)

2.5 Balances (Ohaus, Florham Park. N.J., U.S.A.)

2.6 Hot Air Oven (Lytzen Oven, Switzerland.)

2.7 Manesty Disintegration Test Unit (Manesty Machines Ltd., Liverpool, 24. England.)

2.8 Dissolution Tester (Hanson Research Corp. Model 500-230 with dissolution drive control, U.S.A.)

2.9 Corn Wall Syringe (B-D, U.S.A.)

2.10 Membrance Filter 0.45 um Porosity (Gelman, U.S.A.)

2.11 Spectrophotometer (Spectronic-2000, Baush & Lomb, U.S.A.)

2.12 Automatic Voltage Stabilizer (Quasar Model AVS-4002B, U.S.A.) Equip with Spectronic-2000

2.13 X-Ray Diffractometer (Phillips Eindhoven, Netherlands)

2.14 Fisher Sub-Sieve Sizer (Model 95)

3. Methods

3.1 Preparation of Drug-Diluent Mixtures

3.1.1 Simple Blend Method

A. Unmilled Drug

The unmilled diazepam was manually bottle-tumbling with 5-fold, 10-fold and 20-fold of diluent for 15 minutes. The diluents used were mannitol, sucrose, dibasic calcium phosphate and microcrystalline cellulose. All diluents used in the experiment were passed individually through a 40-mesh sieve before preparing the drug-diluent mixtures. The drug-diluent mixtures were kept in the dessicator using silica gel as adsorbent.

B. Milled Drug

The milled diazepam was prepared by comminuting the drug in a ball mill which was half filled with twenty-five 19 mm. diameter porcelain balls, sixty 12mm. diameter porcelain balls and one hundred 9 mm. diameter porcelain balls for twenty hours. The milled diazepam was then manually bottle-tumbling with 5-fold, 10-fold and 20-fold of diluent for 15 minutes. The diluents used were mannitol, sucrose, dibasic calcium phosphate and microcrystalline cellulose The drug-diluent mixtures were kept in the dessicator using silica gel as adsorbent.

3.1.2 Ball Milling Method

Drug-diluent mixtures were prepared as follows; diazepam and a 5-fold, 10-fold and 20-fold excess of diluents were blended

and transferred to a ball mill. The ball mill was half-filled with twenty-five 19 mm. diameter parcelain balls, sixty 12 mm. diameter porcelain balls and one hundred 9 mm. diameter procelain balls. The diluents used were mannitol, sucrose, dibasic calcium phosphate and microcrystalline cellulose. Drug-diluent mixtures were comminuting continuously. Drug-diluent mixtures were sampling at the interval of time until the X-Ray diffraction patterns of drug-diluent mixtures appeared to indicate the amorphous nature of diazepam in the ground mixture. Crystalline powder of diazepam was also ground in a similar manner, but in the absence of diluents. The change of crystallinity of crystalline powder of diazepam at the interval of time was also determined by X-Ray diffraction pattern. All of the ball milled samples were kept in the dessicator, using silica gel as adsorbent.

3.1.3 Solvent Deposition Method

Drug-diluent mixtures prepared by dissolving diazepam in sufficient chloroform and uniformly wetting a 5-fold, 10-fold and 20-fold excess of diluents in a motar. The diluents used were mannitol, sucrose, dibasic calcium phosphate and microcrystalline cellulose. The drug-diluent mixtures were dried at 37°C for 6 hours, then passed through a 40-mesh sieve to break up any agglomerate and bottle blended to ensure homogemeity. The drug-diluent mixtures were kept in the dessicator, using silica gel as adsorbent.

3.2 Determination of The Crystallinity by X-Ray Diffractometer.

Unmilled diazepam, milled diazepam, diazepam-diluent mixture, mannitol, sucrose, dibasic calcium phosphate and microcrystalline cellulose were determined. Samples for X-Ray diffraction studies were firmly packed into the cavity of a thin rectangular metal plate using two glass slides which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, before taken the prepared sample to expose the X-Ray in the X-Ray diffraction chamber. The X-Ray diffraction patterns were recorded at the rate of 2° per minutes from 4° to 36° in the term of 2θ angle.

3.3 Determination of The Specific Surface Area of The Diluents by Fisher Sub-Sieve Sizer.

Before particle measurements could be made, the Fisher Sub-Sieve Sizer should be allowed to warm up for 20 minutes to give it a chance to stabilize and the following procedure was to be checked. First the manometer level and pressure regulator standardpipe were adjusted to the standard level. Drying agent was also to be checked. A blue color of drying agent indicated that the agent was satisfactory. Then sample was weighed out equal in grams to the true density of the sample and packed into the sample tube with the aid of two porous plugs. At the selected sample height and porosity, the sample tube was removed to the rubber-cushion support and locked into place, making an air-tight seal at both ends of the tube. After that the water level in the manometer tube would rise slowly and reach a maximum height with 30 seconds to several minutes. After the water

in the manometer reached its maximum level, the particle size was read off the chart and the specific surface area was calculated from the relationships expressed in the equation

$$Sw = \frac{6 \times 10^4}{dp.}$$

Sw = specific surface area in sq cm per gm of material

p = true density of material

d = average particle size in micron taken from calculator chart.

3.4 Preparation of Diazepam Capsules

The following formulas were used in preparing diazepam capsules (quantities for one capsule)

3.4.1 Control (Formula 0)

a. Diazepam	2.0	mg.
b. Spray dried lactose	80.0	mg.
c. Talc	1.0	mg.
d. Magnesium stearate	2.0	mg.

3.4.2 1:20 Drug-diluent Capsules (Formula 1-16)

a. Drug-diluent mixture	42.0	mg.
b. Spray dried lactose	80.0	mg.
c. Talc	1.0	mg.
d. Magnesium stearate	2.0	mg.

3.4.3 1:10 Drug-diluent Capsules (Formula 17-32)

a. Drug-diluent mixture	22.0	mg.
b. Spray dried lactose	80.0	mg.
c. Talc	1.0	mg.
d. Magnesium stearate	2.0	mg.

3.4.4 1:5 Drug-diluents Capsules (Formula 33-48)

a. Drug-diluent mixture	12.0	mg.
b. Spray dried Lactose	80.0	mg.
c. Talc	1.0	mg.
d. Magnesium stearate	2.0	mg.

Detailed formulations of diazepam capsule were shown in table 2-13. The diazepam capsules were prepared according to the following procedures :-

Each ingredients was passed individually through a 40-mesh sieve to break up any agglomerate. Then diazepam-diluent mixture or pure diazepam and spray dried lactose were blended by geometric dilution technique. Talc and magnesium stearate were then added to the first portion ingredients. The powder mixture was transfered to a glass bottle and shaked for 15 minutes to ensure homogencity. The powder mixtures were filled into capsules no. 4 by using a capsule filling machine. The weight of diazepam capsule of the Formula no. 0, Formula no. 1-16, Formula no. 17-32 and Formula no. 33-48 was 85, 125, 105 and 95 mg. respectively.

Table: 2

1:20 Simple Blend of Diazepam (unmilled) Formula

Ingredients	Formula				
	#0	#1	#2	#3	#4
1:20 Simple Blend Diazepam-Mannitol mixture	-	42	-	-	-
1:20 Simple Blend Diazepam-Sucrose mixture	-	-	42	-	-
1:20 Simple Blend Diazepam-$CaHPO_4$ mixture	-	-	-	42	-
1.20 Simple Blende Diazepam-Avicel pH 101 mixture	-	-	-	-	42
pure diazepam (unmilled)	2	-	-	-	-
Spray dried lactose	80	80	80	80	80
talc	1	1	1	1	1
magnesium stearate	2	2	2	2	2
weight (mg)/capsule	85	125	125	125	125

Table: 3

1:20 Simple Blend of Milled Diazepam formula

Ingredients	Formula			
	#5	#6	#7	#8
Pure diazepam (Ball milled 20 hours)	2	2	2	2
Mannitol	40	-	-	-
Sucrose	-	40	-	-
$CaHPO_4$	-	-	40	-
Avicel-pH 101	-	-	-	40
Spray-dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight (mg)/capsule	125	125	125	125

Table: 4

1:20 Ball Milled Formula

Ingredients	Formula			
	#9	#10	#11	#12
1:20 Ball milled Diazepam-Mannitol mixture	42	-	-	-
1:20 Ball milled Diazepam-Soucrose mixture	-	42	-	-
1:20 Ball milled Diazepam-$CaHOP_4$ mixture	-	-	42	-
1:20 Ball milled Diazepam-Avicel pH 101 mixture	-	-	-	42
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight (mg)/capsule	125	125	125	125

Table: 5

1:20 Solvent Deposition Formula

Ingredients	Formula			
	#13	#14	#15	#16
1:20 Solvent deposition Diazepam-Mannitol mixture	42	-	-	-
1:20 Solvent deposition Diazepam-Sucrose mixture	-	42	-	-
1:20 Solvent deposition Diazepam-$CaHPO_4$ mixture	-	-	42	-
1:20 Solvent deposition Diazepam-Avicel pH 101 mixture	-	-	-	42
Spray dried lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight (mg)/ capsule	125	125	125	125

Table: 6

1:10 Simple Blend of Diazepam (unmilled) Formula

Ingredients	Formula			
	#17	#18	#19	#20
1:10 Simple Blend Diazepam-Mannitol mixture	22	-	-	-
1:10 Simple Blend Diazepam-Sucrose mixture	-	22	-	-
1:10 Simple Blend Diazepam-$CaHPO_4$ mixture	-	-	22	-
1:10 Simple Blend Diazepam-Avicel pH 101 mixture	-	-	-	22
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
magnesium stearate	2	2	2	2
weight(mg)/capsule	105	105	105	105

Table: 7

1:10 Simple Blend of Milled Diazepam Formula

Ingredients	Formula			
	#21	#22	#23	#24
Pure diazepam (Ball milled 20 hours)	2	2	2	2
Mannitol	20	-	-	-
Sucrose	-	20	-	-
$CaHPO_4$	-	-	20	-
Avicel pH 101 R	-	-	-	20
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	105	105	105	105

Table: 8

1:10 Ball Milled Formula

Ingredients	Formula			
	#25	#26	#27	#28
1:10 Ball milled Diazepam-Mannitol mixture	22	-	-	-
1:10 Ball milled Diazepam-Sucrose mixture	-	22	-	-
1:10 Ball milled Diazepam-$CaHPO_4$ mixture	-	-	22	-
1:10 Ball milled Diazepam-Avicel pH 101 mixture	-	-	-	22
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	105	105	105	105

Table: 9

1:10 Solvent Deposition Formula

Ingredients	Formula			
	#29	#30	#31	#32
1:10 Solvent deposition Diazepam-Mannitol mixture	22	-	-	-
1:10 Solvent deposition Diazepam-Sucrose mixture	-	22	-	-
1:10 Solvent deposition Diazepam-$CaHPO_4$ mixture	-	-	22	-
1:10 Solvent deposition Diazepam-Avicel pH 101 mixture	-	-	-	22
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/ capsule	105	105	105	105

Table: 10

1:5 Simple Blend of Diazepam (unmilled) Formula

Ingredients	Formula			
	#33	#34	#35	#36
1:5 Simple Blend Diazepam-Mannitol mixture	12	-	-	-
1:5 Simple Blend Diazepam-Sucrose mixture	-	12	-	-
1:5 Simple Blend Diazepam-$CaHPO_4$ mixture	-	-	12	-
1:5 Simple Blend Diazepam-Avicel pH 101 mixture	-	-	-	12
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	95	95	95	95

Table: 11

1:5 Simple Blend of Milled Diazepam Formula

Ingredients	Formula			
	#37	#38	#39	#40
Pure diazepam (Ball milled 20 hours)	2	2	2	2
Mannitol	10	-	-	-
Sucrose	-	10	-	-
$CaHPO_4$	-	-	10	-
Avicel pH 101	-	-	-	10
Spray dried Lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	95	95	95	95

Table: 12

1:5 Ball Milled Formula

Ingredients	Formula			
	#41	#42	#43	#44
1:5 Ball milled Diazepam-Mannitol mixture	12	-	-	-
1:5 Ball milled Diazepam-Sucrose mixture	-	12	-	-
1:5 Ball milled Diazepam-$CaHPO_4$ mixture	-	-	12	-
1:5 Ball milled Diazepam-Avicel pH 101 mixture	-	-	-	12
Spray dried lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	95	95	95	95

Table: 13

1:5 Solvent Deposition Formula

Ingredients	Formula			
	#45	#46	#47	#48
1:5 Solvent deposition Diazepam-Mannitol mixture	12	-	-	-
1:5 Solvent deposition Diazepam-Sucrose mixture	-	12	-	-
1:5 Solvent deposition Diazepam-$CaHPO_4$ mixture	-	-	12	-
1:5 Solvent deposition Diazepam-Avicel pH 101 mixture	-	-	-	12
Spray dried lactose	80	80	80	80
Talc	1	1	1	1
Magnesium stearate	2	2	2	2
weight(mg)/capsule	95	95	95	95

3.5 Evaluation of Diazepam Capsules.

3.5.1 Weight variation of Diazepam Capsules.

Twenty diazepam capsules were weighed individually using an analytical balance. Care was taken to preserve the identity of each capsule and remove the contents of each capsule with the aid of a small brush or pledget of cotton. Then emptied shell, were weighed individually. The net weight of its contents were calculated by substracting the weight of the shell from the respective gross weight. The average weight and standard deviation of diazepam capsules were calculated.

3.5.2 Disintegration Time of Diazepam Capsules.

The Disintegration tester was used to determine the disintegration time of diazepam capsules. One capsule was placed in each of the six tubes of the basket. The apparatus was operated, using 0.1 N HCl maintained at 37±2°C as the immersion fluid. The capsules were observed within the time limit of 10 minutes. All capsules disintegrated except fragments from the capsule shell.

3.5.3 Percent Labeled Amount of Diazepam

The contents of not less than 20 diazepam capsules were weighed and determined the average weight per capsule . The combined contents were mixed, and transfered an accurtaly weighed portion of the powder, equivalent to about 10 mg of diazepam, to a 100-ml volumetric flask. A 5 ml of water was added, mixed and allowed to stand for 15 minutes.

A 90 ml. of 0.5% w/v solution of sulfuric acid in methanol was added and shaked for 15 minutes, then sufficient of the sulfuric acid solution was added to produce 100 ml. and filter. A 10 ml. of the filtrate was diluted to 100 ml. with the same solution. The absorbance of this solution and of a solution of diazepam reference standard in 0.5% w/v solution of sulfuric acid in methanol at concentration of about 10 ug/ml were concomitantly determined by a spectrophotometer in 1-cm cells at the maximum wavelength of 284 nm. The absorbance of the sample solution was recorded as Au and the reference standard solution as As. The quantity in mg of diazepam in diazepam capsule was calculated taken the formula : C (Au/As) in which C was the exact concentration in ug per ml. of the reference standard solution.

3.5.4 Dissolution Studies of Diazepam Capsules

The dissolution of diazepam capsules were determined by using a dissolution tester. A five hundreds ml. of 0.1 N. HCl was placed in the vessel and permitted to equilibrate to 37±1°C, Diazepam capsule was accurately weighed and placed in the basket and immersed into the vessel. At the moment of contact between diazepam capsule and dissolution medium, the motor and the timer were started simutaneously. The basket was rotated at the speed of 100 rpm. At various suitable time intervals, 8 ml. of the sample solution was withdrawn by a cornwall syringe and filtered through 0.45 um membrane filter before measuring the absorbance at the wavelength of 242 nm. with a spectrophotometer which equiped with automatic voltage stabilizer. The same quantity of

0.1 N HCl was added immediately after each sampling to keep the volume of dissolution medium constant during the course of the test. Since the diluent and capsule shell affected the diazepam absorbance value, placebo capsule containing an identical quantity of diluents was add to 0.1 N HCl. The filtrate was used as the blank. The dissolution experiment was conducted within one hours. The amount of diazepam dissolved was calculated from the calibrated concentration-absorbance curve. The dissolution profile was obtained by plotting the percent of diazepam dissolved against time. Time required for 85 percent of diazepam to dissolve was read from the dissolution profile. The average of at least two determinations was reported for each lot of diazepam capsules. The calibration concentration-absorbance curve of diazepam was constructed by preparing the standard solution at the concentration 1, 2, 3, 4, 5, 6, 7, 8 ug/ml. in 0.1 N HCl. The absorbance was measured at 242 nm. by spectrophotometer which equiped with automatic voltage stabilizer. The data were listed in table 14. The calibration concentration - absorbance curve of diazepam in 0.1 N HCl were shown in Figure 4.

Table: 14

The Absorbance of Standard Solution of Diazepam in 0.1 N HCl at Wavelength 242 nm.

Concentration (ug/ml)	Absorbance*
1.0	0.101
2.0	0.195
3.0	0.292
4.0	0.382
5.0	0.474
6.0	0.569
7.0	0.659
8.0	0.772

*Average of five determinations.

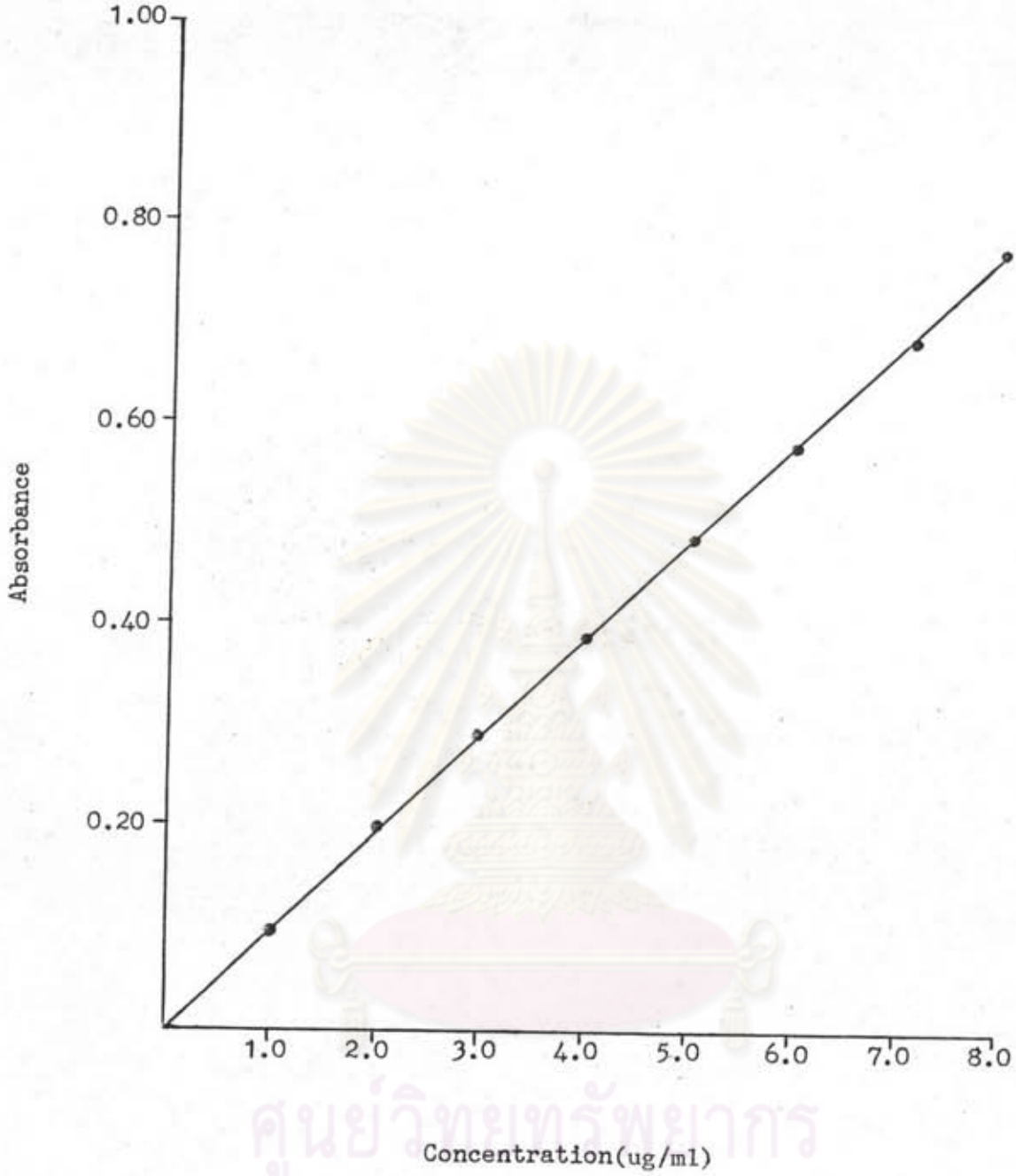

Figure 4 The calibration concentration-absorbance curve of the diazepam in 0.1 N HCl at wavelength 242 nm.