CHAPTER II

MATERIALS AND METHODS

MATERIALS

1. Model Drug

-Prednisolone USP, anhydrous micronized (Upjohn, U.S.A.) Batch no.221UK

2. Drug Carriers

-Starch 1500 (Colorcon, West Point, PA, U.S.A.)

-Elcema G.250 (DEGUSSA, West Germany)

-Tablettose (Meggle, West Germany)

3. Lubricating Agents

-Magnesium stearate (Pharmaceutical Sciences, Bangkok, Thailand)

-Aerosil L.200 (MEGGLE, West Germany)

4. Others

-Absolute methyl alcohol, AR (J.T.Baker Chemical Co., Philburg)

-Distilled Water

Apparatus

1. Analytical Balance (Satorius, Model 2442, Germany)

2. Mortar and Pestle

3. Cube Mixer (Datamach Pharma Supplies L.T.D. Part, Thailand)

4. Sampling Thief (Chareon Karnchang, Thailand)

5. Single Punch Tablet Machine (Viuhang Enginerring, Thailand)

6. Hardness Tester (Schleuniger, Model 2E/205, Dr.K.Schleuniger Co., Switzerland)

7. Erweka Friabilator (Type T.A.3 Nr.26603, West Germany)

8. Disintegration apparatus (Manesty T.D. 65T 170, Manesty Machines Ltd., England)

9. Dissolution apparatus U.S.P. Type I (Hanson Research Corporation, Model 500-230, Northridge, California, U.S.A.)

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

11. U.S. Standard sieve series no.20, 40, 60, 80, and 100 mesh (Endecotts Ltds., England)

12. Mechanical sieve shaker (Josef Deckelmann, West Germany)

13. Hot air oven (Memmert TV400 UL 998001)

14. Scanning Electron Microscope (Model JSM-T20, JOEL Co.,Ltd., Japan)

METHODS

1. Particle Size Determination

1.1 Particle Size Distribution

The direct compression vehicles of 100 grams weight were each subjected to particle size analysis, using a mechanical sieve shaker consisting of sieve no. 20, 40, 60, 80 and 100 mesh with an opening size of 840, 420, 250, 177 and 149 µm. respectively, operated for 10 minutes. The powder retained on each sieve was weighed and the size distribution data was calculated.

1.2 Scanning Electron Microscope Studies

Prednisolone and all of the direct compression vehicles were individually studied for the particle size and surface characteristics, by using a Scanning Electron Microscope. After preparing prednisolone ordered mixes, a sample from each mixing batches was subjected to Scanning Electron Microscope to study the adherence characteristic of the particles.

2. Preparation of Ordered Mixture

All of the direct compression vehicles were each passed through sieve no.18 in order to break lumps. Magnesium stearate and aerosil L.200 were passed through sieve no.80.

Mixing of each formulation was performed in a cube mixer (15×15 cm.) revolving at a speed of 50 r.p.m., and was loaded with about 500 g. powder containing 2.5% micronized prednisolone resembled in the formulation shown in Table 1.

2.1 Mixing Homogeneity Studies

Prednisolone and drug carrier(s) were incorporated into the mixer and mixing was performed. Then 10 samples from different positions in the mixer (each approximately 100 mg.) were withdrawn at the interval of 2, 5, 10, 20, 30, and 50 min.mixing time by using a sampling thief. After mixing to 50 minutes, magnesium stearate and aerosil L.200, which were premixed were incorporated into the mixture, and the mixing was continued for 10 minutes. Then 10 samples were withdrawn.

The content of prednisolone in each sample was determined using UV Spectrophotometer by weighing each sample accurately, transfer to a suitable volumetric flask and dissolved with absolute methanol, adjusting to 25 ml. then filtered to remove the solids. The absorbance was measured at 242 nm. using absolute methanol as a blank. The prednisolone contents were calculated from the standard curve shown.

The standard curve of prednisolone in absolute methanol was performed, having a concentration ranging 3-20 mcg./ml. Absorbance obtained at 242 nm. versus known concentration were plotted and shown in Figure 4.

Table 1 Experimental Formulations of Three Differences Mixing Batches.

Ingredients	Formulation 1	Formulation 2	Formulation 3
Prednisolone	12.5 g.	12.5 g.	12.5 g.
Starch 1500	485.0 g.	100.0 g.	100.0 g.
Elcema G.250	-	385.0 g.	-
Tablettose	-	-	385.0 g.
Magnesium stearate	1.25 g.	1.25 g.	1.25 g.
Aerosil L.200	1.25 g.	1.25 g.	1.25 g.

The average prednisolone content from each sampling interval were calculated. The degree of homogeneity of drug-drug carriers in the mixture were determined from the percent coefficient of variation (%C.V.).

3. Direct Compression of Prednisolone Tablets

Each of the prednisolone ordered mixture (500 g.) was transfered from the cube mixer into a hopper, subjected to a single punch tabletting machine, using punch size of 8/32". The tablets were compressed, weighing about 100 mg. each and hardness between 2-5 kp. Each tablet contained about 2.5 mg. of prednisolone.

3.1 Ordered Unit Segregation Studies

To investigate the ordered unit segregation of the mixture during compression which subjected to the vibration of the ordered mixture in the hopper, sampling of the first and the last interval of the compressed tablets were taken and 10 tablets of each sampling interval were subjected to content uniformity test.

3.1.1. Content Uniformity of Tablets

The drug content was calculated from individually 10 tablets. By transfered the tablet into a suitable volumetric flask, add absolute methanol to half volume. Gently shake until the tablet disintegrated, then adjust the volume, shake and filtered. Drug content was determined by UV Spectrophotometer. Calculated the average drug content and coefficient of variation.

3.2 Evaluation of Tablets

3.2.1 Weight Variation of Tablets

Weighed individually 20 tablets from each formulation using an analytical balance. The average weight and the weight variation were determined.

3.2.2. Hardness of Tablets

Six tablets from each formulation were randomly selected and individually subjected to a hardness tester. The average hardness was determined.

3.2.3. Friability of Tablets

Twenty tablets were randomly selected from each formulation, cleared the dust on the tablets'surface. The total weight of 20 tablets was detected using an analytical balance and then subjected to an Erweka friabilator rotating at a speed of 24 r.p.m. for 5 min. The tablets were cleared the dust again and detected the total weight. The percent of friability was determined.

3.2.4. Disintegration Time of Tablets

The disintegration time of tablets was determined using Manesty tablet disintegration tester. The temperature was maintained at 37±2°c. The average disintegration time was obtained from 6 tablets.

3.2.5. Dissolution Rate of Tablets

The dissolution rate of tablets was

performed according to USP dissolution type I. method. Using 900 ml. of deaerated distilled water as dissolution medium and was maintained at 37±1°C. The basket was adjusted to rotate at a speed of 100 r.p.m. Ten milliliters samples were withdrawn by a pipet at the interval of 5, 10, 15, 20 and 30 min. and then were filtered, using a Whatman Filter paper no.3, discarding the first 5 ml. of the filtrate and the subsequent filtrate were used as the sample for the further assay. After the samples were withdrawn at each intervals, the same volume of the dissolution medium was deposited into the medium. The filtrated samples were assayed by UV Spectrophotometer at 242 nm. by using deaerated water as a blank. The prednisolone contents were calculated from the standard curve.

The standard curve of prednisolone in dissolution medium was performed, having a concentration ranging 5-20 mcg./ml. Absorbances obtained at 242 nm. versus known concentration were plotted and shown in Figure 5.

4. Preparation of Prednisolone Tablets by Wet Granulation Methods

The certain amount of the drug and the direct compression vehicles according to Table 1 were passed through sieve no.18 to break lumps. The ingredients in each formulation were weighed and mixed thoroughly by mortar and pestle for 5 min. Then during mixing, purified water was incorporated little by little into the mixture, until a damp mass was obtained. The damp mass was passed

through sieve no.23 to form granules. The granules were tray dried in a hot air oven at 60°C. for one hour. The dried granules were again passed through sieve no.23. Then magnesium stearate and aerosil L.200 which were passed through sieve no.80 were incorporated to the granules and mixed.

4.1 Compression of Prednisolone Tablets

The granules were compressed into tablets, weighing about 100 mg. each and hardness between 2-5 kp. by a single punch tabletting machine, using punch size of 8/32". Each tablet contained about 2.5 mg. of prednisolone.

4.2 Dissolution Rate

The dissolution rate of tablet was performed, as described in 3.2.5.

5. Physical Stability Studies

The physical stability of prednisolone direct compressed tablets was performed regarding to disintegration time, hardness and dissolution rate of the tablets prepared in each formulation. This is carried on 4th, 8th and 12th week by storing those tablets in polystyrene jar at room temperature.

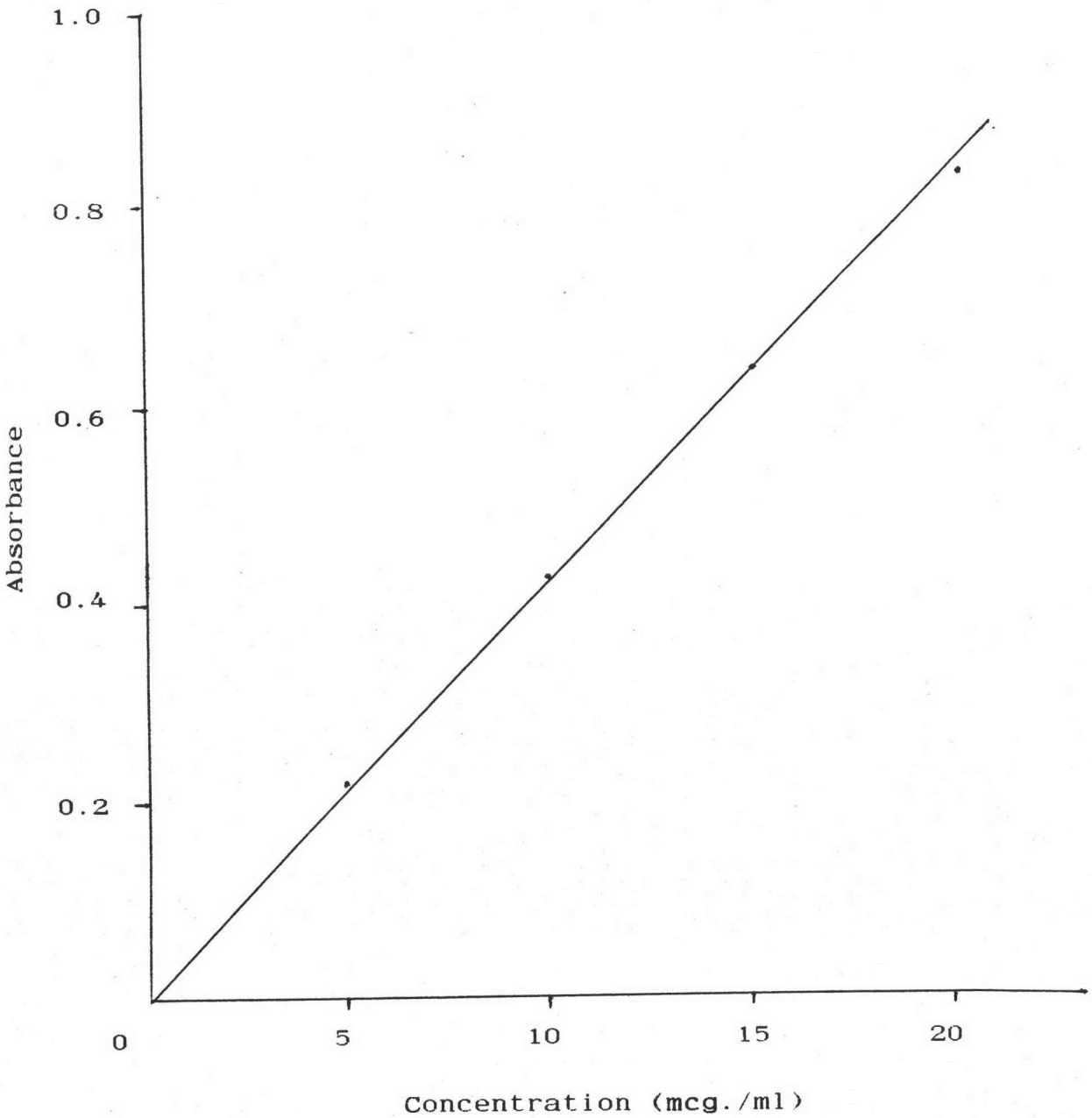

Figure 4 Standard curve plotting the concentration of prednisolone in absolute methanol versus absorbance at 242 nm.

$Y = 24.5658\ X - 0.3049 \quad (r^2 = 0.9998)$

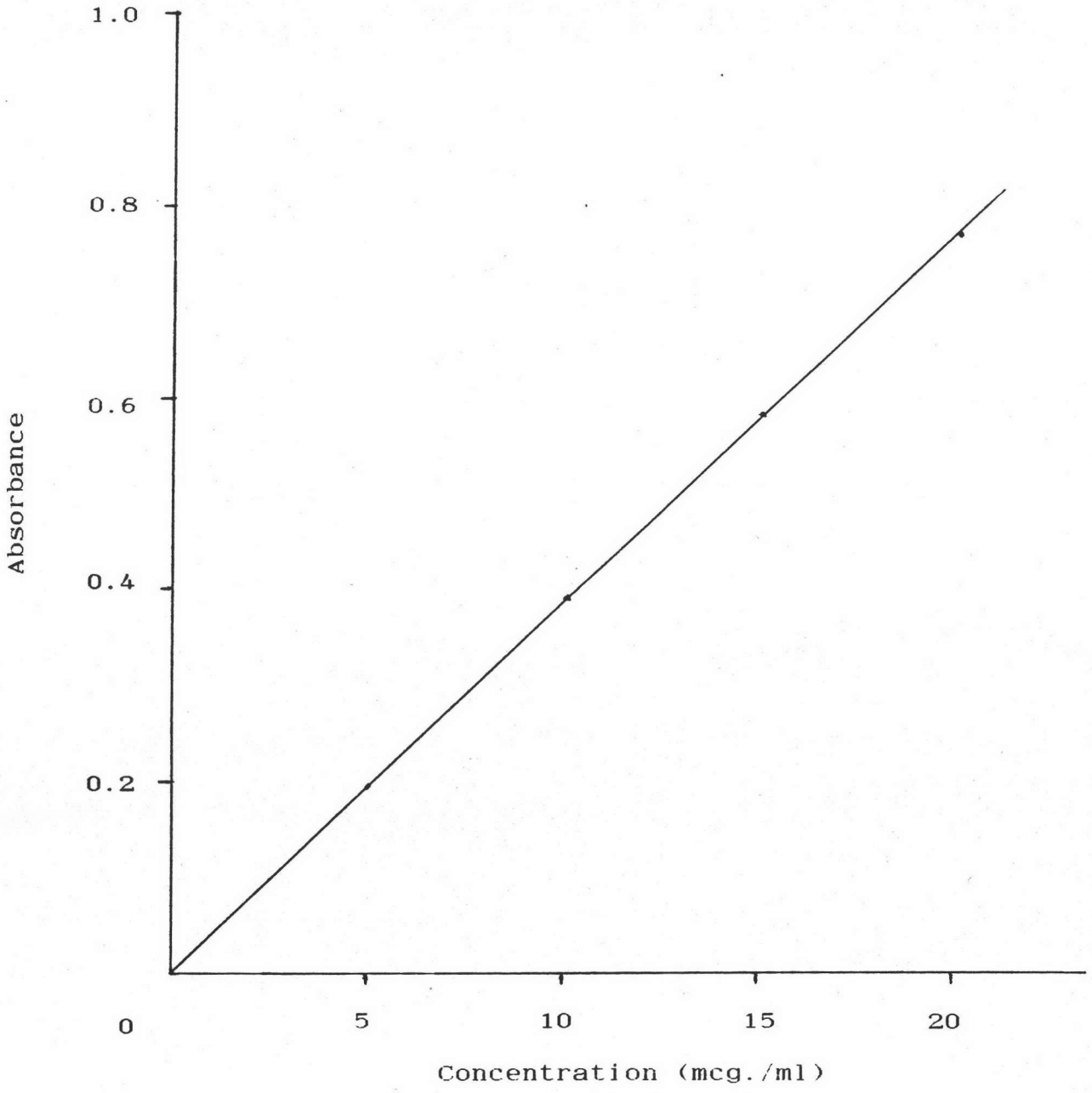

Figure 5 Standard curve plotting the concentration of prednisolone in deaerated distilled water versus absorbance at 242 nm.

$Y = 26.4082 X - 0.1759$ $(r^2 = 0.9996)$