CHAPTER II

EXPERIMENTAL

Materials

The following materials were obtained from commercial sources except for Ispaghula husk procured from India.

Active Ingredients

- Paracetamol (BP 1988 , Lot 3202129102 , China)
- Nicotinamide (USP XX , Lot 306297 , Roche , Switzerland)

Diluents

- Lactose (Wyndale , New Zealand)

Binders

- Ispaghula Husk (India)
- PVP K30 (BASF , Germany)
- Hydroxypropyl cellulose type L (NISSO , Japan)
- Corn starch (Pharmaceutical Science Co., Ltd., Bangkok ,Thailand)
- Starch 1500 ® (Colorcon , England)
- Gelatin (Pharmaceutical Science Co., Ltd., Bangkok , Thailand)

Lubricant

- Magnesium stearate (Pharmaceutical Science Co., Ltd., Bangkok , Thailand)

Miscellaneous

- Hydrochloric acid (E. Merck , Darmstadt , Germany)
- Potassium dihydrogen phosphate (E. Merck , Darmstadt , Germany)
- Sodium hydroxide (Nobel Industries , Sweden)

Equipments

- Analytical balance (Sartorius , Model A200S , Germany)
- Bridge box (Tokyo Sokki Kenkyujo Co ., Ltd., Japan)
- Cube mixer (Erweka , Germany)
- Disintegration apparatus (Hanson Research , Model QC-21, USA .)
- Dissolution apparatus (Hanson Research , Model SR 2, USA.)
- Erweka friabilitor (Erweka , Type TAP , Germany)
- Hardness tester (Schleuniger , Model 2E / 205 , Germany)
- High speed chopper ((Janke & Kunkel , Kika Werk, Germany)
- Hot air oven (Memmert , Type UL80 , Germany)
- Micrometer (Telclock Corp ., Japan)
- Moisture determination balance (Mettler , LP16 , USA .)
- Nest of sieve (Endecotts Ltd . , England)
- Oscillating granulator (Kan Seng Lee Ltd ., Part, Bangkok, Thailand)
- Planetary mixer (Kenwood , USA .)
- pH meter (Hanna Instruments , Model HI8417 , USA .)
- Scanning electron microscope (Jeol , T220A , Japan)
- Sieve shaker (Josef Deckelman , Aschaffenburg , Germany)
- Single punch tabletting machine (Viuhang Engineering , Model A3 , Bangkok , Thailand)
- Spectrophotometer (The Bausch & Lomb , Spectronic 2000, New York ,USA .)
- Strain gauge (Kyowa , Type KFC-5-C1 11 L30 , Japan)
- Strain meter (Tokyo Sokki Kenkyujo Co ., Ltd., Japan)
- Strip chart recorder (S. C. Siam Engineering , Bangkok , Thailand)

Methods

1. Fraction of Ispaghula Husk for Employing as Binder

Ispahula seed husk was dried at 50 °C for 24 hours,pulverized in a high speed chopper (Janke & Kunkel, Kika Werk,Germany) and the fraction passing through sieve cut 100/200 and fracture retained on sieve No.200 was used .

2. Preparation of Granules

2.1 Solution Incorporation Method

All binder solutions employed in the study were freshly prepared with sufficient purified water at the concentration of 0.5 % , 1 % and 2 % weight by weight of the formula . Ispaghula husk was dissolved in purified water and stirred until exactly hydrated before used. Other binder solutions were prepared by the method previously mentioned.

Each 700 g of granules were prepared following to the formulation in Table 3 . First , drug and lactose were mixed in a cube mixer for 5 minutes at a rotation speed of 30 rpm . Second , the powder mixed were gradually and uniformly moistened with binder solution in the planetary mixer at a fixed speed of NO. 1. Mixing continued for 5 minutes. Third, the damp mass was granulated by oscillating granulator through a # 16 mesh sieve and dried in a hot air oven for 5 hours at 50°C. Finally, the granules were resieved through a # 20 mesh sieve and kept in the desiccator until used.

2.2 Dry Incorporation Method

Just PVP K30 , Starch 1500 ® and Ispaghula husk were employed in the study according to formulae in the Table 4 . Active ingredient, lactose and dry binder were mixed for 5 minutes in a cube mixer at a rotation speed of 30 rpm. Then the mixtures were gradually moistened with sufficient purified water in planetary mixer at a fixed speed of NO.1. Mixing continued for 5 minutes and the damp mass was granulated by oscillating granulator through a # 16 mesh sieve. The wet granules were dried in a hot air oven for 5 hours at 50°C and resieved through a # 20 mesh sieve. After finishing these processes, the granules were stored in the desiccator until used.

A batch of blank was prepared in the same manner but without binder.

Table 3 Formulation of Paracetamol and Nicotinamide Tablets Prepared by Solution Incorporation Method

Ingredients (gram)	Amount per 700 g Batch		
	% Dry Weight of the Binder in the Formula		
	0.5	1.0	2.0
Model Drug *	455.0	455.0	455.0
Lactose	227.5	224	217.0
Binder $^{+}$	3.5	7.0	14.0
Magnesium stearate	14.0	14.0	14.0
Purified Water	qs.	qs.	qs.

Table 4 Formulation of Paracetamol and Nicotinamide Tablets Prepared by Dry Incorporation Method

Ingredients (gram)	Amount per 700 g Batch		
	% Dry Weight of the Binder in the Formula		
	1.0	2.0	4.0
Model Drug *	455.0	455.0	455.0
Lactose	224	217.0	203.0
Binder $^{+}$	7.0	14.0	28.0
Magnesium stearate	14.0	14.0	14.0
Purified Water	qs.	qs.	qs.

Model Drug * are Paracetamol and Nicotinamide
Binder $^{+}$ are corn starch , gelatin , hydroxypropyl cellulose (HPC type L) , Ispaghula Husk , polyvinylpyrrolidone (PVP K 30) and pregelatinized starch (Starch 1500 $^{®}$)

3. Evaluation of Granules Prepared from Binders at Various Concentrations of Studies

3.1 Morphology Examination

All granule samples were coated with gold prior to the microscopic examination using ion sputtering. Photomicrographs of sample were taken with scanning electron microscope (SEM) at appropriate magnification .

3.2 Particle Size Distribution

Particle size distribution was determined by sieve analysis. The 100 g of granules were put on the top sieve of a sieve series ranging from 850, 450, 250, 180 to 150 μm, respectively. The nest of sieve was placed on the sieve shaker for 10 minutes . The average result from three determinations were reported in percentage of weight retained on each sieve size . The average granule size given corresponding to 50 % size on the cumulative percentage undersize axis (El-Gindy, Samaha and El-Maradny, 1988).

3.3 Bulk Density , Tapped Density and Compressibility Determination

Bulk density was determined by careful pouring the weight of each sample (about 40 g) into a 100 ml graduated cylinder and the bulk volume was recorded. Tapped density was determined by dropping the graduated cylinder containing the sample onto a hard wood surface from a height of 5 cm until a constant tapped volume was attained. Both densities were calculated by dividing the weight of sample with its bulk volume and tapped volume, respectively. The percentage of compressibility was calculated from the Equation 1(Hiestand and Peot, 1974). The results were average from three trials .

$$\text{Compressibility (\%)} = (1 - D_B/D_T)\,100 \qquad \text{Eq . (1)}$$

When D_T and D_B were tapped and bulk density , respectively.

3.4 Flow Rate and Angle of Repose Determination

Angle of repose was determined by fixed base cone method. An amount of 40 g of each sample was filled in a glass funnel with 6 mm internal stem diameter fixed on a clamp. The time was recorded when the granules start to flow from the height of 8 cm until finish . The round heap was produced and angle of repose was calculated from the Equation 2.

$$\alpha = \tan^{-1}(H / R) \qquad \text{Eq .(2)}$$

α = angle of repose (degree)
H = height of heap (cm)
R = radius of heap (cm)

3.5 Comparison of Percent Fine

The 40 g of each granules was placed on the sieve NO. 100 and the fine was removed by shaking on the sieve shaker for 30 seconds. The content of remaining was reweighed. The difference between before and after shaking was calculated as the percentage of fine which obtained from three determinations .

3.6 Comparison of Percent Friability

The percent friability determination method was modified from previous work (Puttipipatknachorn ,1987). Ten grams of granules retained on 20/40 mesh out and five stainless spheres (each sphere weigh 2.05 g and diameter 6.94 mm) were filled into the polyvinylchloride container 9.5 cm in length and 6 cm in diameter. The container was tightly closed with the cap and firmly sealed with tape. Then the container was put on the cube mixer and rotated for 5 minutes . The granules finer than 80 mesh was sieved off by shaking on the sieve shaker for 5 minutes . The percent friability values calculated as percentage of weight loss was obtained from three determinations.

3.7 Moisture Determination

Two grams of granules was accurately weighed on a pan of Mettler LP 16 moisture determination balance and dried until constant weight was obtained . The result was shown as percent moisture content . The percent moisture content was averaged from two determinations .

4. Calibration of the Instrumentation for the Tabletting Machine

Two strain gauges , one was an active gauge and the other was a compensating resistance , were mounted on the upper plunger holder for measuring the applied force of an upper punch .

The active gauge and compensating resistance gauge formed two arms of a wheatstone bridge which connected to one channel strain indicator amplifier. When stress was applied to the upper punch , the resulting strain caused a change in resistance of the gauges and unbalanced the bridge. The potential difference which was directly proportional to the force was then amplified by strain indicator amplifier and the signal was recorded on graphic paper of the strip chart recorder. The diagram of press and associated measuring instrumentation for tablating machine is given in Figure 2 .

The strain gauges mounted on the upper punch holder were calibrated under static condition using hydraulic press over a range of force between 200 upto 4,000 pounds. A good linear relationship between applied force (pounds) and strain (microstrains) is shown in Figure 3.

5. Preparation of Tablets

Each batch of granules was mixed with 2 % w/w of magnesium stearate in cube mixer for 5 minutes at a rotation speed of 30 rpm . The granules were compressed into 500 mg tablets using single punch tabletting machine equipped with 12.7 mm diameter round plane faced punch at the compression pressure 2,800 pounds. The prepared tablets were kept in dessicator until used .

6. Evaluation of Tablets Prepared from Binders at Various Concentrations of Studies

6.1 Weight Variation

For the test, twenty tablets from each batch were individually weighed, using an analytical balance (Sartorius, Model A200S, Germany) and determined for average weight and standard deviation .

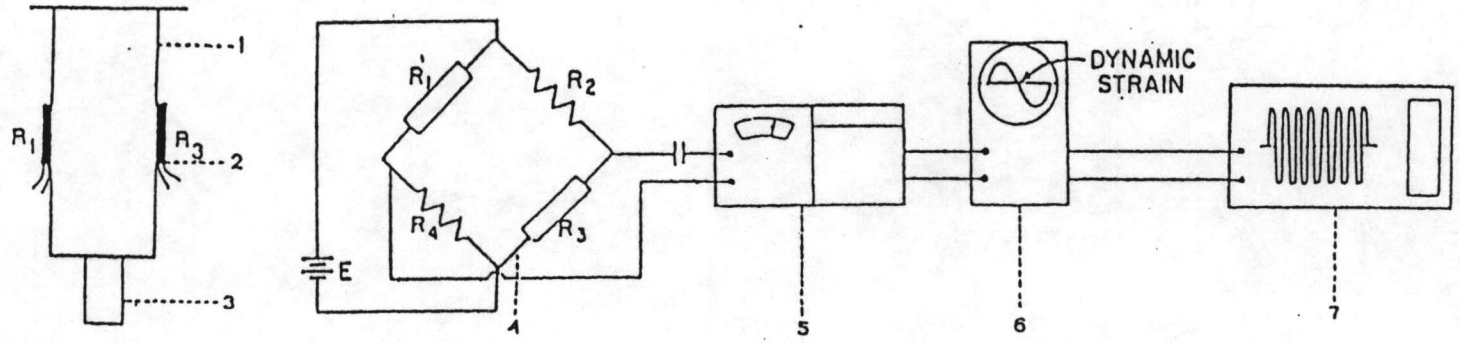

Figure 2 Function Block Diagram of Press and Associated Measuring System

1. Upper plunger
2. Strain gauge
3. Upper punch
4. Wheastone bridge
5. Dynamic strainmeter
6. Oscilloscope
7. Recorder

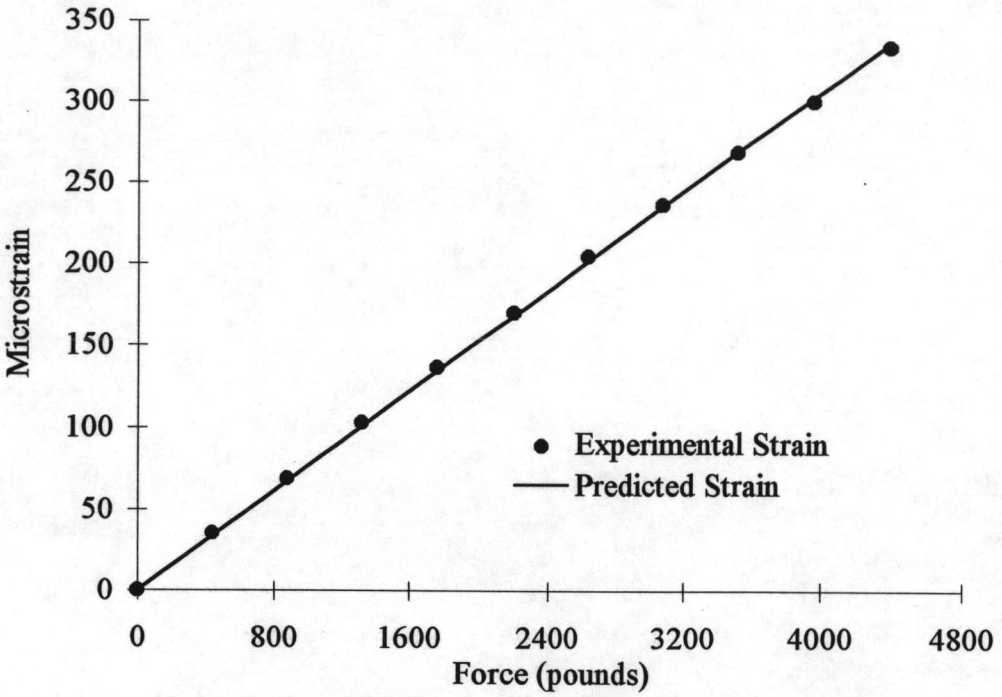

Figure 3 Calibration Curve between Strain and Applied Forces of Guages Bound on Instrumented Upper Punch (Y = 0.0764 X + 0.002 ; R square = 0.9997)

6.2 Tablet Hardness

The hardness was measured by using the hardness tester (Schleuniger - 2E, Germany) and expressed in kilopounds (kps) units. The average of ten determinations was calculated.

6.3 Tablet Thickness

The thickness was measured by using a micrometer (Telclock, Japan) and expressed in mm. The average thickness of ten observations was determined.

6.4 Tablet Tensile Strength

The tablet tensile strength was determined by the diametrical compression test. The tablets were compressed diametrically on a modified Herberlein hardness tester. In order to minimize the shear and compressive stress below the loading area, the platen width is limited to 1/10 of the tablet diameter (Alderborn and Nystrom,1984 ; Fell and Newton,1970 ; Hiestand and Poet ,1974). The motor was operated to apply an increasing force to the tablet at constant rate. When the tablet failed, the tester stopped automatically. The force reading was converted to tensile strength in the manner of Fell and Newton (1970). The tensile strength (σ_0) is calculated by the Equation 3 .

$$\sigma_0 = 2F / \pi Dt \qquad \text{Eq. (3)}$$

σ_0 = tablet tensile strength (kp/cm^2)
F = the force applied diametrically at fracture (kilopounds)
D = diameter of the tablet (cm)
t = thickness of the tablet (cm)

The mode of failure was determined visually by checking the shape of the fragments after fracture. If the compact splits into two equal halved , tensile strength has been recorded (are shown in Figure 4) . All tensile strengths reported are based on ten determinations.

6.5 Tablet Friability

Twenty preweighed tablets or at least 6 grams were placed in Erweka friabilitor rotated at 25 rpm for 4 minutes. Loss of their weight with respect to the initial value was calculated as percent weight friability.

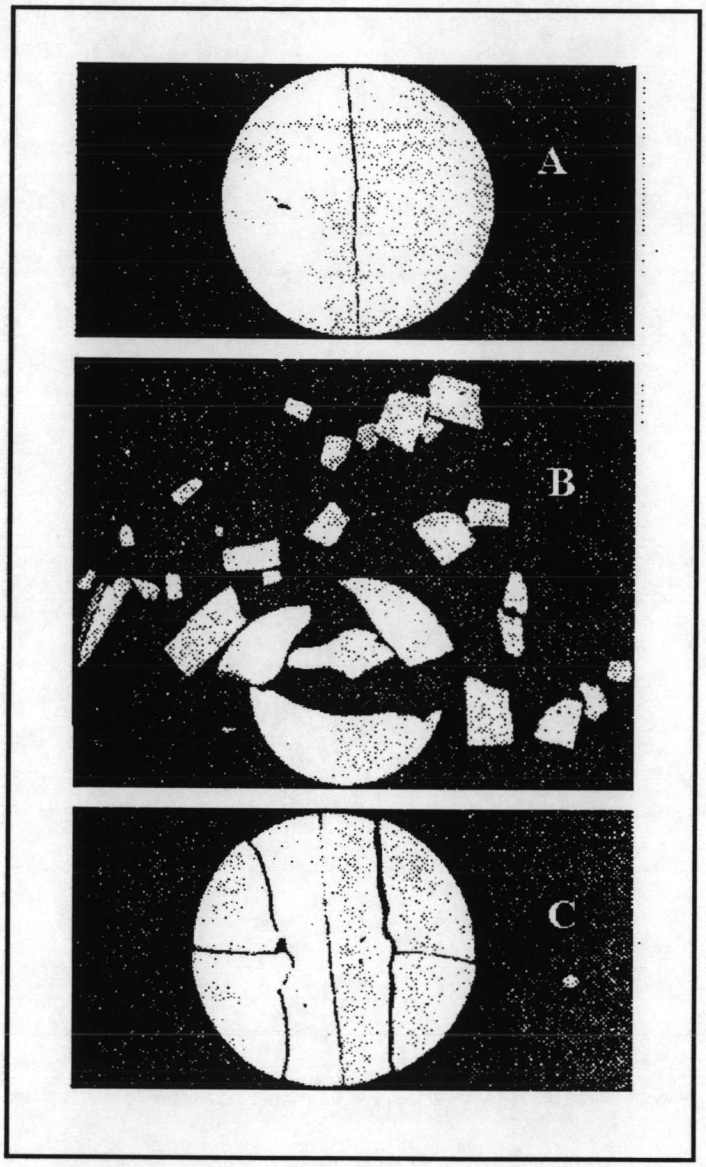

Figure 4 Fractured Tablet after Diametrical Compression
key : A) Accepted Normal Tensile Strength Failure
B) Shear and Compression Failure
C) Rejected Tensile Failure

6.6 Tablet Porosity

The porosity of the tablets were determined by using the method introduced by Seager et al and calculated from the Equation 4.

$$\text{Porosity} = 1 - \frac{\text{Apparent Density}}{\text{True Density}} \qquad \text{Eq. (4)}$$

The true density of tablets was determined by compressing the granules to their minimum volumes using 11mm punch at 4,000 pounds. This mass was approximately taken to have zero porosity and the true density was obtained by dividing the compact weight its volume.

The apparent density was determined similarly by dividing the tablet weight by the apparent volume calculated from the dimension of tablet.

6.7 Disintegration Time

Disintegration time was determined according to the USP XXIII by using disintegration apparatus (Hanson Research, USA) with purified water at 37± 2°c as disintegration fluid. The test was performed with disk and the disintegration time was averaged from six determinations.

6.8 Dissolution Time

Dissolution time was determined according to the USP XXIII by using dissolution apparatus (Hanson Research, USA.).

6.8.1 Paracetamol Tablets : The dissolution rate of each tablets was measured in 900 ml of phosphate buffer pH 5.8 at 37± 0.5 °c as the dissolution medium. Each tablet was placed in the vessel. The rotation speed of the paddle was 50 rpm.

The 5 ml of sample solution was withdrawn and filtered periodically at 10, 20, 30, 60, 90, 120, 150, 180, 240, 300 and 360 minutes interval. Only sample of Ispaghula husk was extended to 600 minutes. Then, the equal volume of buffer was substituted immediately. After suitable diluted with the same medium buffer, the sample was assayed by measuring the absorbance at 249 nm with spectrophotometer (Bausch & Lomb, USA.). The amoung of drug dissolved in each sample was calculated by comparing with standard curve present in Figure 5. The median dissolution time (T50%) was determined from dissolution profile.

6.8.2 Nicotinamide Tablets : The dissolution rate of each tablets was measured in 900 ml of purified water at 37± 0.5 °c as the dissolution medium . Each tablet was placed in the vessel . The rotation speed of the paddle was 50 rpm .

The 5 ml of sample solution was withdrawn and filtered periodically at 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes interval. Then, the equal volume of medium was substituted immediately. After suitable diluted with purified water, the sample was assayed by measuring the absorbance at 262 nm with the spectrophotometer. The amount of drug dissolved in each sample was calculated by using the standard curve present in Figure 6. The median dissolution time (T50%) was determined from dissolution profile.

6.9 Assay Procedure

The quantity or percent labeled amount of paracetamol tablets and nicotinamide tablets prepared in the study was determined by using spectrophotometer . The procedures for assay the quantity of paracetamol tablets and nicotinamide tablets were shown in Figure 7 and Figure 8 .

6.10 Binder Index Determination

The binder index for binding property evaluation, presented by El-Gindy et al. (1988), was calculated by Equation 5 .

$$\text{Ø}_b \text{ index} = \frac{\sigma_0 \cdot P}{T\,50\% \cdot F} \qquad \text{Eq . (5)}$$

Ø_b index = the binder index (kp /cm^2 .min)
σ_0 = tensile strength (kp /cm^2)
P = porosity in percentage
T50% = median dissolution time
F = friability in percentage

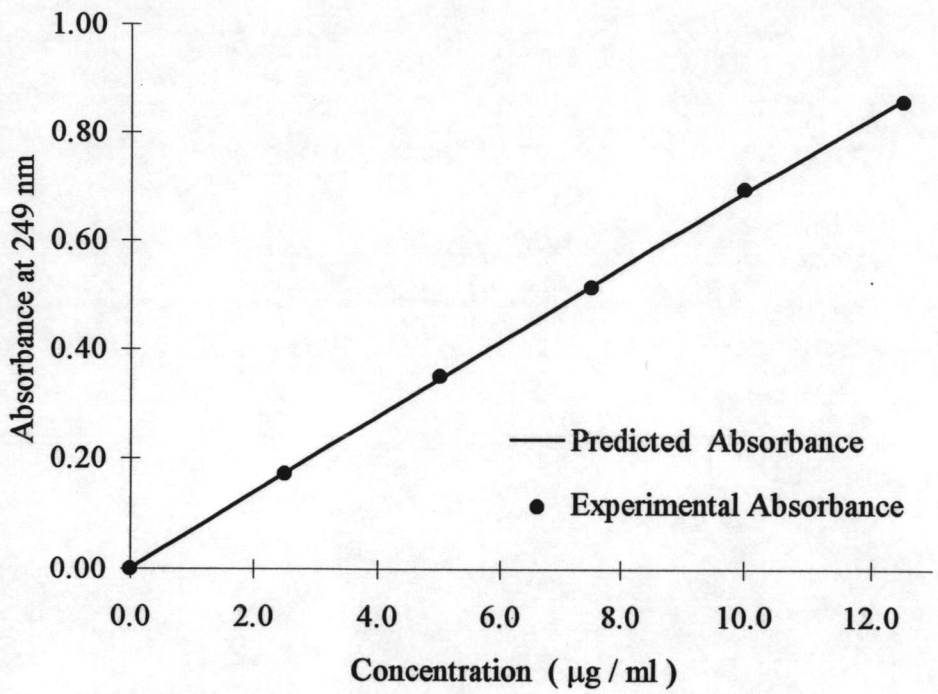

Figure 5 Standard Curve of Paracetamol in Phosphate Buffer pH5.8 at 249 nm ($Y = 0.067X + 0.00167, R^2 = .9998$)

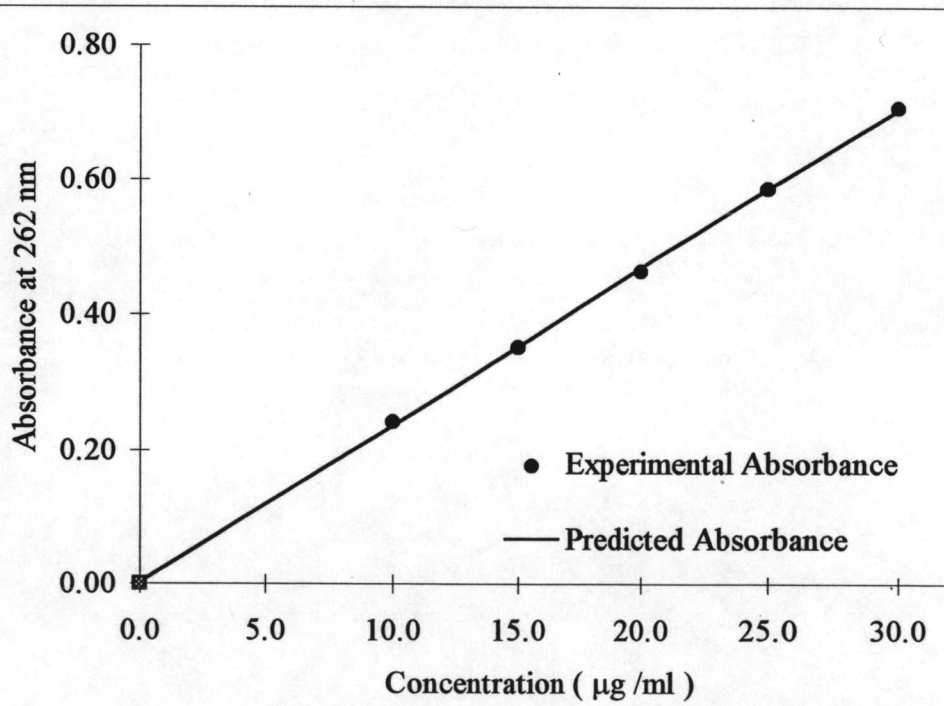

Figure 6 Standard Curve of Nicotinamide in Purified Water at 262 nm ($Y = 0.0234 X + 0.00164$, $R^2 = 0.9997$)

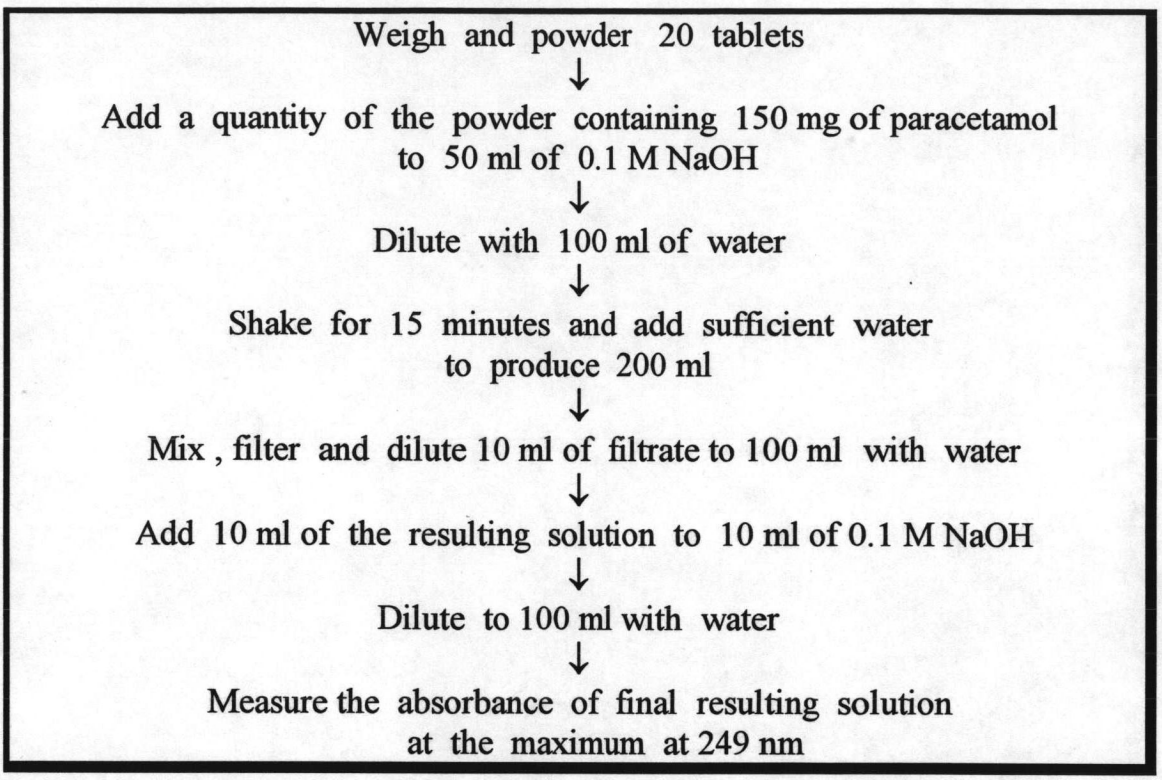

Figure 7 Diagram of the procedure for assay the quantity of paracetamol tablets

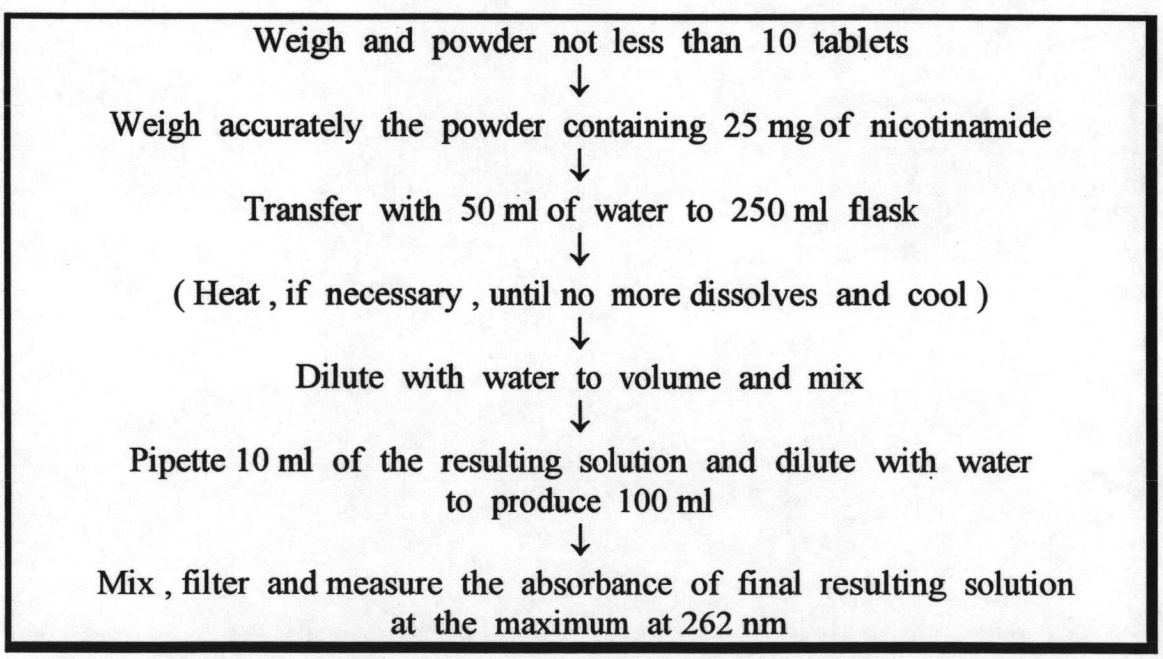

Figure 8 Diagram of the procedure for assay the quantity of nicotinamide tablets