CHAPTER II

EXPERIMENTAL

1. MATERIALS

All of the materials employed in the study are commercial sources except for durian rind extracts

active ingredients

- paracetamol powder (USPXX, Lot 6088988 M 362, China)
- pyridoxine HCl (USP, Lot 94-1378, BASF, Germany)

diluent

- lactose hydrous (Wyndale, New Zealand)

binders

- durian rind extracts (D_1 : alcohol extraction, D_2 : acid alcohol extraction) (Biochemistry laboratory, Faculty of Pharmaceutical Sciences, Chulalongkorn University)
- PVP K30 (BASF, Germany)
- corn starch (Pharmaceutical Sciences, Bangkok, Thailand)
- Starch 1500$^{(R)}$ (Colorcon Inc , USA)
- gelatin (Pharmaceutical Science , Bangkok, Thailand)
- Methocel E15LV$^{(R)}$ (Premium grade, Colorcon Inc, USA)

lubricant

- magnesium stearate (Pharmaceutical Sciences, Bangkok, Thailand)

miscellaneous

- potassium phosphate, monobasic (E.Merck , Dermstadt, Germany

- sodium hydroxide (E, Merck, Dermstadt, Germany)

- hydrochloric acid (Riedel-de Hain , Germany)

2. **EQUIPMENTS**

- Carver laboratory Press (Perkin Elmer model C, Fred & Caver Inc., USA)

- Scanning electron microscope (JEOL, JSM-35CF, Japan)

- Moisture determination balance (OHAUS, Scale Corp., USA)

- Analytical balance (Sartorius, Germany)

- Hardness tester (Schleuniger 2E, Germany)

- Roche Friabilator (Erweka, Germany)

- Micrometer (Teclock Corp., Germany)

- Disintegration apparatus (Hanson Research model QC-21, USA)

- Dissolution apparatus (Hanson Research , USA)

- Spectrophotometer (The Bausch & Lomb, New York, USA)

- Cube mixer (Erweka, Germany)

- Planetary mixer (Kenwood , USA)

- Oscillating granulator (Kan Seng Lee Ltd., Part ,Bangkok, Thailand)

- Hot air oven (Mammertt, Germany)

- Sieve shaker (Josef Deckelman , Germany)

- Nest of sieve (Endecotts Ltd., London, England)

- Strain meter (Tokyo Sokki Kenkyujo Co,Ltd, Japan)

- Bridge box (Tokyo Sokki Kenkyujo Co,Ltd, Japan)

- Oscilloscope (HAMEG Model HM 203-6,20MHZ,Germany)

- Strain guage (Kyowa, Japan)

3. METHODS

3.1 Preparation of Granulles

3.1.1 Solutiuon Incorporation Method

All binders solutions employed were freshly prepared with sufficient purified water at the concentration of 1,2 and 4 % dry weight of the formula. Durian rind extracts were dissolved in the purified water and stirred until extremely hydrated before used. The other binders were prepared by the method previously mentioned.

A batch of 500 g were prepared according to the formulation present in Tables 2 and 3. Active ingredient and lactose were dried mixed for 5 minutes in a cube mixer at a rotation speed of 30 rpm. Then the mixtures were gradually and uniformly moistened with binder solution in the planetary mixer at a fixed speed of NO. 1. Mixing continued for 5 min and the wet 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 $^\circ$C and then resieved through a 20 mesh sieve.

3.1.2 Dry Incorporation Method

Only PVPK30 , Starch 1500$^{(R)}$, D_1 and D_2 were employed as 2 % dry weight of the formula. 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 distilled water in planetary mixer at a fixed speed of NO. 1. Mixing continued for 5 minutes and the wet 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 then resieved through a 20 mesh sieve.

Table 2

Formulation of Paracetamol Tablet Used in This Study

Ingredients	% dry weight per tablet		
	A	B	C
Paracetamol	60	60	60
Lactose	37	36	34
Binder*	1	2	4
Magnesium Stearate	2	2	2
Distilled Water, ml	qs.	qs.	qs.

* Binders are D_1, D_2, PVP K30, corn starch, Starch 1500[R], gelatin and Methocel E15LV[R]

Batch size : 500 g

Table 3

Formulation of Pyridoxine Hydrochloride Tablet Used in This Study

Ingredients	% dry weight per tablet		
	A	B	C
Pyridoxine Hydrochloride	60	60	60
Lactose	37	36	34
Binder*	1	2	4
Magnesium Stearate	2	2	2
Distilled Water, ml	qs.	qs.	qs.

* Binders are D_1, D_2, PVP K30, corn starch, Starch 1500[R], gelatin and Methocel E15LV[R]

Batch size : 500 g

A batch of blank was prepared in the same manner as previously described but without binder. All the granules produced were kept in the desiccator until used.

3.2 Calibration of Instrumented Single Punch Tablet Machine

The upper punch bounded with strain guages to either side were statically calibrated on a hydraulic press over a range of force between 300 to 3600 pounds. The excellent linear relationship as shown in Figure 3 was obtained over the range force test (r = 0.9994).

3.3 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 paracetamol granules were compressed into 500 mg tablets using 11 mm diameter round plane faced punch at the compression pressure of 3,000 pounds. Pyridoxine hydrochloride granules were compressed into 300 mg tablets using 9 mm diameter round plane faced punch at compression pressure of 2,400 pounds on instrumented single punch machine. The prepared tablets were kept in the desiccator until used.

3.4 Granules Evaluation

3.4.1 Determination of Granule Appearance

Photomicrographs of granule samples were taken with scanning electron microscope . The samples were coated with gold prior to the microscopic examination using ion sputtering.

3.4.2 Particle Size Distribution

Particle size distribution was determined by sieve analysis (48) . The 100 g of granules was put on the top sieve of a sieve series ranging from 850 ,425, 250, 180 to 150 um, respectively. The nest of sieve was placed on the sieve shaker for
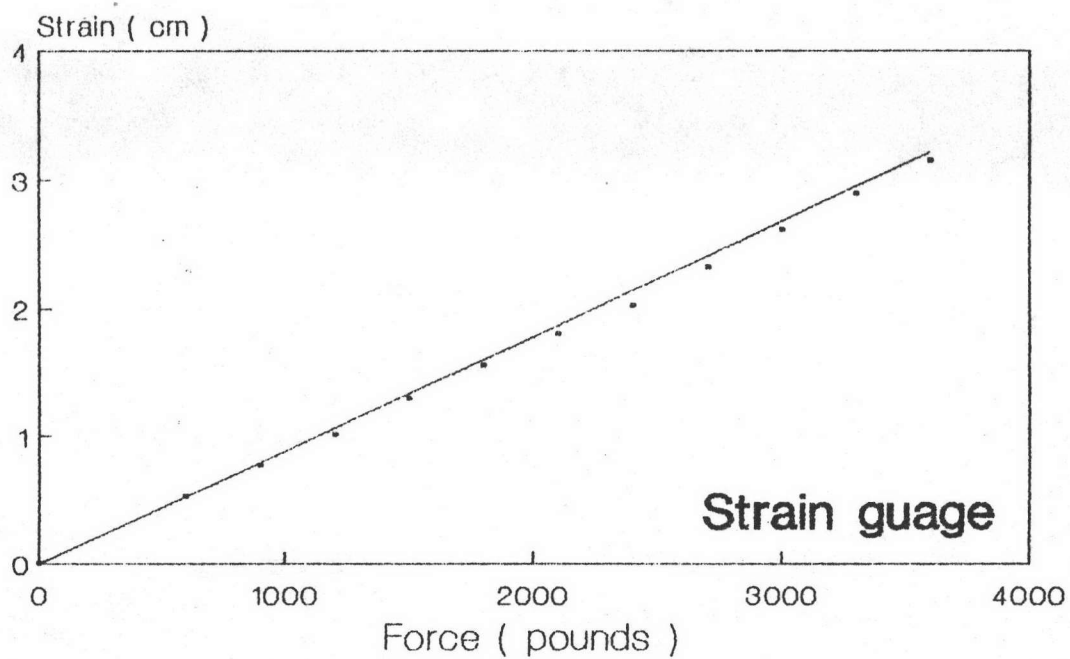
Figure 3   Calibration   Curve   of   Strain   Guage   Bounded   on   the Instrumented Upper Punch ($Y = 0.0009X - 0.0202$, $r^2 = 0.9994$).

10 minutes. The results, which averaged from two determinations were reported as percentage of weight retained on each sieve size. The average granule size given corresponding to 50 % size on the cumulative percentage undersize axis (10).

3.4.3 Bulk Density , Tapped Density and Compressibility Determination

The bulk density and tapped density were determined from the weight of 40 g sample , carefully charged into a 100 ml graduated cylinder and the volume was recorded. The powder was tapped from the height of 5 cm until a constant volume was obtained. Both densities were averaged from 3 determinations. The compressibility index was calculated from the following equation (51)

$$\text{Compressibility \%} = \frac{(P_T - P_B)}{P_T} \, 100 \qquad (1)$$

where P_T and P_B are tapped and bulk density, respectively.

3.4.4 Flow Rate and Angle of Repose Determination

An amount of 40 g of granules 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 until finish. Flow rate was calculated in g/min and angle of repose was calculated from the following equation (14)

$$\propto \; = \; \tan^{-1} \frac{H}{R} \qquad (2)$$

where $\propto$ is the angle of repose ; H and R are the height and radius of the granule pile, respectively.

3.4.5 Comparison of Percent Friability

Granule friability determination method was modified from previous work (50). Granules retained on 20/40 mesh cut 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 firmly closed with the cap, put on the cube mixer and rotated for 5 minutes. The granules finer than 80 mesh was sieved off. The percent friability was calculated as percentage of weight loss.

3.4.6 Comparison of Percent Fine

A 40 g of granules was placed on the sieve NO.100 and the fine was removed by shaking on the sieve shaker for 30 seconds. The content remaining on sieve was reweighed. The difference in the two weight was calculated as the percentage of fine which obtained from two determinations.

3.4.7 Moisture Determination

The moisture content of granules was determined by using OHAUS moisture determination balance . The 10 g of sample was exposed to an IR lamp set at 1.5 inch mark with an intensity of approximately 150° C until constant weight was reached. The percent moisture content was calculated from the following equation :

$$\text{percent moisture content} = \frac{(\text{wet mass} - \text{dry mass})}{\text{wet mass}}\ 100 \qquad (3)$$

3.5 Tablet Evaluation

3.5.1 Weight Variation

For the test, twenty tablets were individually weighed and the average weight was calculated.

3.5.2 Tablet Hardness

The hardness was measured using the Schleuniger-2E hardness tester and the mean of ten determinations was calculated.

3.5.3 Tablet Thickness

The thickness was measured by using micrometer. The mean was averaged from ten determinations.
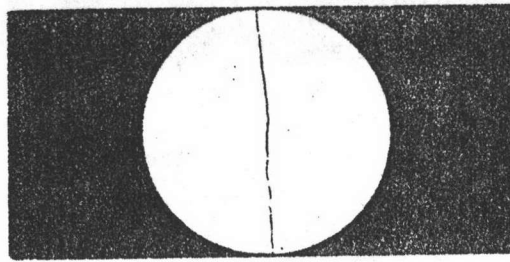
3.5.4 Tablet Tensile Strength

The tensile strength was determined by the diameteral compression test. The tablets were compressed diametrically on a modified Heberlein hardnessd tester. In order to minimize the shear and compressive stress below the loading area, the platen width is limitted to 1/10 of the diameter of the tablet (51-53). 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 were converted to tensile strength in the manner of Fell and Newton (54). The tensile strength (σ_o) is given by :

$$\sigma_o \quad = \quad \frac{2F}{\pi Dt} \tag{4}$$

where σ_o is tablet tensile strength (MN/m^2),
F is the force applied diametrically at fracture (Newton), D and t are diameter and thickness of the tablet (m), respectively.
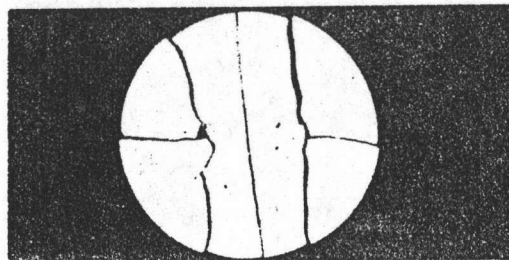
The mode of failure was determined visually by checking the shape of the fragments after fracture. If the compact splits into two equal halved, tensile strength has recorded (Figure 4). All tensile strength reported are base on 10 determinations.

A
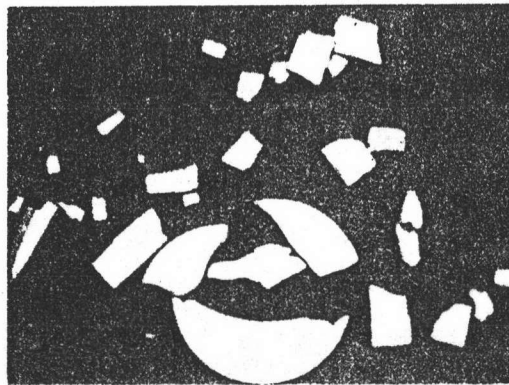
B

C

Figure 4   Fractured Tablet after Diametral Compression (A) Accepted
Normal Tensile Strength Failure (B) Shear and Compression
Failure (C) Rejected Tensile Failure.

3.5.5 Tablet Friability

Twenty tablets or not less than 6 g were weighed and subjected in Roche friabilator rotated at 25 rpm for 4 minutes. The tablets were reweighed and friability was calculated as percent weight loss.

3.5.6 Tablet Porosity

The total porosity of the tablets were determined using the method introduced by Seager et al (43) and calculated from the following equation :

$$\text{Porosity} = 1 - \frac{\text{Apparent Density}}{\text{True Density}} \qquad (5)$$

The true density of the tablets was detemined by compressing the granules to their minimum volumes using 11 mm punch at 7,000 pounds and 9 mm punch at 4,000 pounds for paracetamol and pyridoxine hydrochloride, respectively. This mass was approximately taken to have zero porosity and the true density was obtained by dividing the compact weight by its volume.

The apparent density was determined similarly by dividing the tablet weight by the volume calculated from the tablet dimension measured using a micrometer.

3.5.7 Disintegration Time

Disintegration time was determined according to USP XXII method (55). The average was calculated from six determinations.

3.5.8 Dissolution Time

Dissolution time was determined using apparatus

according to USP XXII. The average was calculated from three determinations.

3.5.8.1   Paracetamol Tablet : The dissolution rate of single tablets was measured in 900 ml of phosphate buffer pH 5.8 at 37 ± 0.5° C as the dissolution medium. A tablet was placed on the vessel and the paddle was rotated at 50 rpm. Sample of 5 ml were withdrawn periodically at 10, 20, 30, 60, 180, 240, 300 and 360 minutes interval.   The volume taken was substituted by an equal volume of prewarm buffer. After suitable diluted with phosphate buffer, the sample was then assayed spectrophotometrically by measuring the absorbance at 249 nm and the concentration was calculated from standard curve presented in Figure 5.   The medium dissolution time (T50%) was determined from dissolution profile.

3.5.8.2   Pyridoxine Hydrochloride Tablet : The dissolution rate of single tablets was determined in 900 ml of diluted hydrochloric acid (1 in 100) at 37 ± 0.5.° C as the dissolution medium. The basket containing a tablet was placed on the vessel and rotated at 100 rpm. Sample of 5 ml were withdrawn periodically at 2, 5, 10, 15, 20, 30, 40 and 60 minutes interval. The volume taken was substituted by an equal of prewarm diluted hydrochloric acid. After suitable diluted with diluted hydrochloric acid, the sample was then assay spectrophotometrically by measuring the absorbance at 290 nm and the concentration was calculated from standard curve presented in Figure 6. The medium dissolution time (T50%) was determined from dissolution profile.

3.5.9 Content Uniformity

3.5.9.1   Paracetamol tablets : Each tablet, which titurated individually to fine powder, was transferred to a 200 ml volumetric flask containing 100 ml of 0.1 M sodium hydroxide, diluted with 50 ml of water, shake for 15 minutes and add sufficient
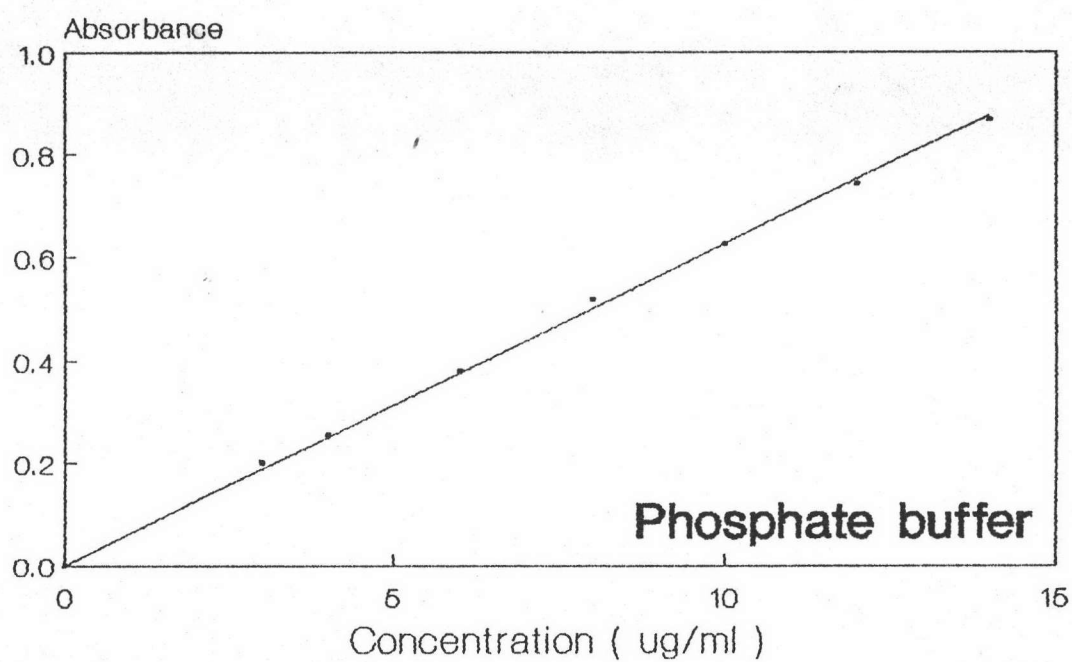
Figure 5   Standard Curve of Paracetamol in Phosphate Buffer pH 5.8
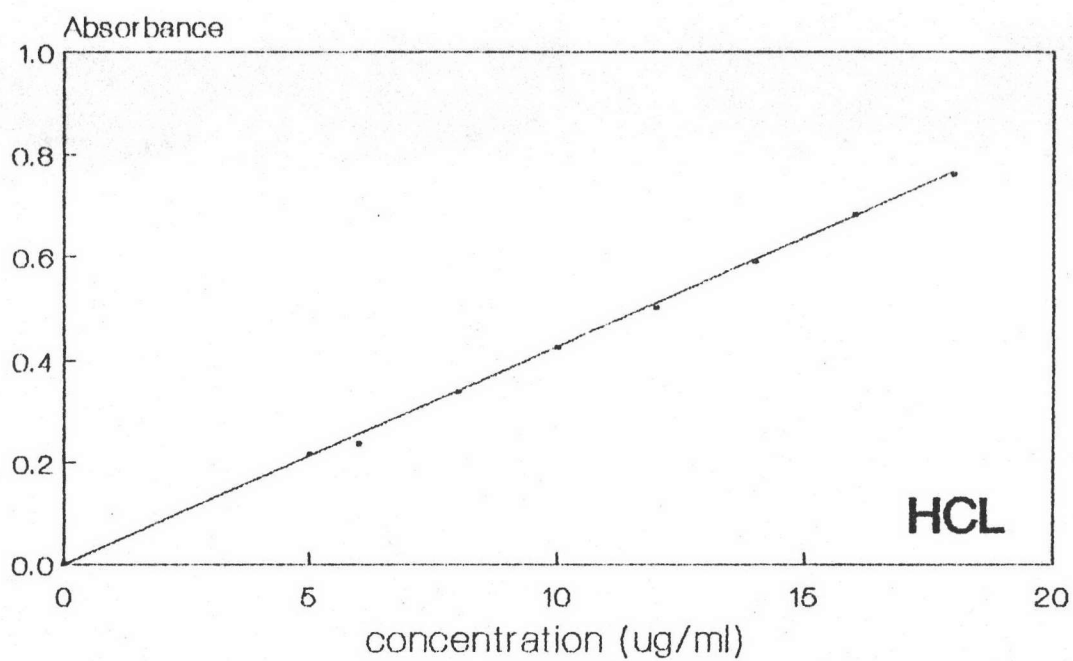at 249 nm ($Y = 0.0614X + 0.0102$, $r^2 = 0.9992$).

Figure 6   Standard   Curve   of Pyridoxine Hydrochloride   in   Diluted
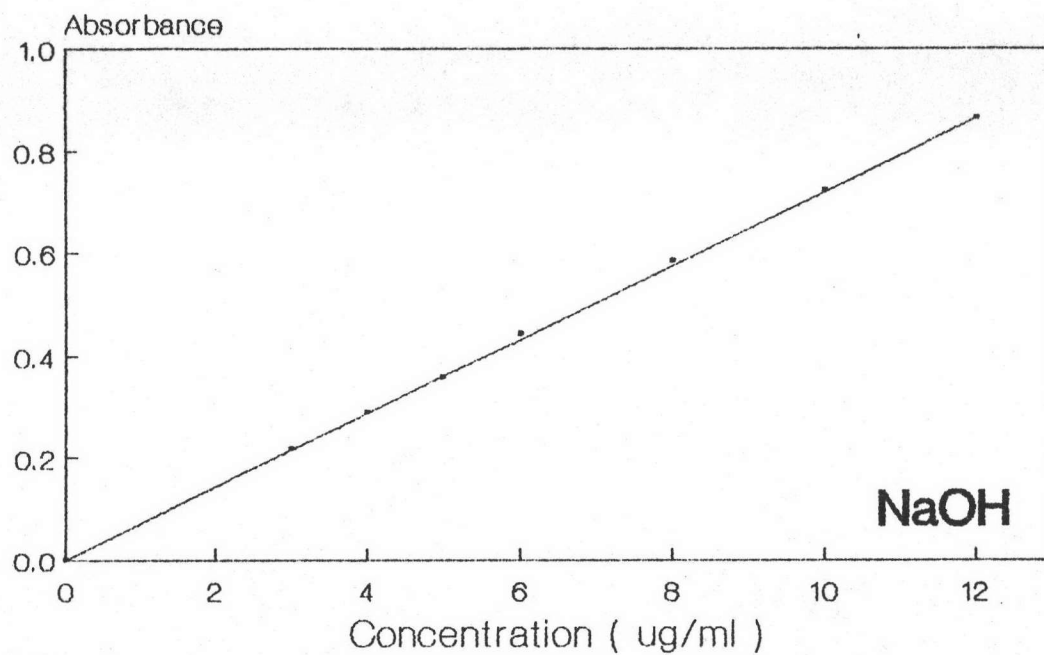Hydrochloric Acid (1 in 100) at 290 nm (Y = 0.0424X −
0.0033, r^2 = 0.9993).

Figure 7   Standard Curve of Paracetamol in 0.1 M Sodium Hydroxide
at 257 nm (Y = 0.0722X - 0.0014, r^2 = 0.9997).

water to produce 200 ml. Mix, filter and stepwise dilute with 0.1 M
sodium hydroxide and water until the concentration of paracetamol is
about 7.5 ug per ml. Measuring the absorbance of resulting solution
at 257 nm and the content was calculated from standard curve in
Figure 7.

3.5.9.2 Pyridoxine hydrochloride tablet :
Transfer tablet, previously finely powdered, to a 500 ml volumetric
flask containing about 300 ml of water, shake for 30 minutes, and
diluted with water to volume. Filter a portion of mixture
discarding the first 25 ml of the filtrate. Dilute a suitable
aliquot of the subsequent filtrate quantitatively and stepwise with
diluted hydrochloric acid (1 in 100) so that the conentration of
pyridoxine hydrochloride is about 10 ug per ml and calculated from
standard curve shown in Figure 6.

3.5.10 Binder Index Determination
The binder index for an overall binder activity
evaluation, presented by El-Gindy et al (10), is calculated
by following equation:

$$\theta_b \text{ index} = \frac{\sigma_o \cdot P}{T50\% \cdot F} \qquad (6)$$

where θ_b index is the binder index ($MN/m^2 \cdot min$),
σ_o is tensile strength (MN/m^2) P is porosity
in percentage, T50% is median dissolution time
(minutes), F is friability in percentage.