Chapter II

Experimental

1. **Materials**

   1.1 **The Following Substances Obtaining from Commercial Sources**
   - hydrochlorothiazide (China National Chemicals Imp.& Exp.Co., China)
   - α-lactose monohydrate (Tablettose[R], Meggle Milch Industrial GMBH & Co., Germany)
   - dibasic calcium phosphate dihydrate (Emcompress[R], Edward Mendell Co., USA)
   - corn starch (Pharmaceutical Science, Thailand)
   - Explotab[R] (Edward Mendell Co., USA)
   - Ac-Di-Sol[R] (FMC, USA)
   - Kollidon CL[R] (BASF, Germany)
   - ECG 505[R] (Daicel Ltd., Japan)
   - L-HPC type LH-11 (Shin-Etsu Chemical, Japan)
   - magnesium stearate (Pharmaceutical Science, Thailand)
   - ethanol, absolute (E.Merck, Germany)
   - hydrochloric acid, analytical grade (E.Merck, Germany)

1.2 Preparation of Scaphium Powders

Treatment 1 (S_1)

The dried fruits (2 kg) of *Scaphium macropodum* Beumée was separated from the seeds. The pulps was then washed with alcohol to remove dust and other materials. The treated pulp was dried in an oven at $50^\circ C$ and ground in an electric blender to give a light-brown coarse powder. The powder was further ground in a ball mill (2.5 litres, Dascal Engineering Co.,Ltd., England) for 18 hours to give a very fine powder (total yield of 600 g).

Treatment 2 (S_2)

The dried fruits (2 kg) of *Scaphium macropodum* Beumée was allowed to absorb and fully swell in water. The thin layer of pericarps as well as the stony seeds were separated from the swollen mucilagenous pulp of the fruits.

The wet pulp was drained through a piece of cloth and washed several times with alcohol to remove water and some colour materials. The treated pulp was then dried at $50^\circ C$ and ground in an electric blender to give a light-brown coarse powder. The powder was further ground in a ball mill for 18 hours to give a very fine powder (total yield of 400 g).

2. Methods

The following testing methods were employed to assess the physical properties of the materials in relation to disintegrating efficiency.

2.1 Evaluation of Physical Properties of Scaphium Powders

2.1.1 Morphology Examination

Size and shape of Scaphium powders were examined using scanning electron microscope (JSM-35 CF, JEOL, Japan) and electron photomicrographs were taken.

2.1.2 Size Distribution and Specific Surface Area

Size distribution, specific surface area and average diameter of particle of Scaphium powders were examined using laser diffraction particle size analyzer (Malvern particle sizers series 2600C, UK).

2.1.3 Bulk and Tapped Density

Samples were passed through 20-mesh screen before density determination. Bulk density was performed by pouring 25 g material into a-100 ml graduated cylinder and measuring volume to nearest ml. Tapped density was performed by dropping graduated cylinder containing the sample powder onto a hard wood surface form a height of 5 cm until the powder attained a constant tapped volume.

The bulk and tapped density were then calculated by dividing the weight of the sample (grams) by bulk volume (millilitres) and final tapped volume

(millilitres) of the sample contained in the cylinder, respectively.

Percent compressibility of the powder was calculated from tapped density and bulk density as follows :

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

The results of bulk density, tapped density and % compressibility presented as an average of two determinations.

2.1.4 True Density

True density was determined using a 50-ml pycnometric bottle (Wertheim, Germany). Five grams of samples were accurately weighed and transfered into the pycnometer. Amount of ether was added to fill up the pycnometer, and the sample-solvent mixture was shaken. The whole bottle was accurately weighed and the true density was calculated as follows :

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

when ρ = true density

W_2 = weight of solvent in a pycnometer

W_3 = weight of sample in a pycnometer

W_4 = weight of sample-solvent mixture in a pycnometer

True density was an average of two determinations.

2.1.5 Moisture Determination

Five grams of sample was accurately weighed and evenly distributed over the surface of an 89-mm tared petri-dish. The sample was placed in a hot air oven (Memmert, Germany) and the temperature was maintained between 105°-110°C until the weight of sample was constant.

2.1.6 Hydration Capacity

A 200 mg sample was placed into a 40-ml centrifuge tube tarred with stopper. Forty millilitres of distilled water (pH 6-7) was added, and the tube was stoppered and shaken vigorously to suspend the sample thoroughly. The suspension was allowed to stand for 10 minutes. During the time, it was mixed by inverting three times at the end of 5 and 10 minutes. The stopper was then removed and the tube was centrifuged for 15 minutes at 10,000 r.p.m. 20°C using refrigerated centrifuge apparatus (Hitachi, SCR 20B, Japan) The centrifuge was allowed to stop without breaking. The supernatant was carefully decanted and the tube was inverted to allow draining. The tube was then restopped and the contents were weighed. The hydration capacity was calculated in the following manner :

$$\text{hydration capacity} = \frac{(\text{weight of tube+sediment})-(\text{weight of tube})}{\text{weight of sample (dry basis)}}$$

Hydration capacity presented as an average of two determinations.

2.1.7 Moisture Sorption of Disintegrants

Five grams of sample was accurately weighed and evenly distributed over the surface of an 89-mm tared petri-dish. The sample was then placed in a desiccator containing saturated salt of potassium sulphate in the reservior. The desiccator was then stored in a laboratory oven (Memmert, Germany) and the temperature was maintained constant at $35^{\circ}\pm0.5^{\circ}C$ in which the relative humidity of 96.7% was indicated (71). At various time intervals, the weight gained by the exposed samples was recorded and the amount of water absorbed was calculated form the weight difference. The rate of moisture sorption was an average of two determinations.

2.1.8 Bulk Swelling of Disintegrants

The apparatus (as shown in Figure 1) employed to test bulk swelling was modified from the method reported by Rudnic et al.(66). A sample of each 500 mg was packed in the graduated glass tube which was placed upon the moist glass filter plate and the volume of the swelling powder due to the intake of water was observed (both measured in ml) at pre-determined time intervals. Distilled water was used as a test media.

The bulk swelling of the sample, V_b, was estimated as a percent of original volume using equation as follows :

$$V_b = [(h_t-h_o)/h_o]\ 100$$

When h_o = the original height of powder bed

h_t = the height of powder bed at time t

2.1.9 Ash Content

Each of sample about 2-3 g was weighed in a tared crucible dish and ignited in the furnace (Maffle Furnace, Gallenkamp size 3, England) at 600°C until the color of the ash was white. The ash was weighed and % ash was calculated based on the initial dry weight. The ash content was an average of two determinations.

2.2 Preparation of Tablets for Disintegration Time Study

2.2.1 The Instrumentation of a Tablet Machine

A Stokes Model A -3 single punch tablet machine (Viuhang Engineering, Thailand), driven at a constant speed through 1.5 horse powers electric motor was instrumented so that the compression force could be determined as shown in Figure 2.   The strain guages (Kyowa, type KFC-5-Cl-11 L30, foil, Japan) were used for dynamic force measurements.   Two strain gauges (one an active guage and the other a compensating resistance gauge) for measuring the applied force of upper punch   were assembled to the modified upper punch holder. They were mounted with a cyanoacrylate adhesive (Shinkoh, Type Y-10, Japan), then coated with moisture proofing wax (Kyowa, Type C-IA, Japan).

The active gauge and compensating resistance gauge were formed two arms of a Wheatstone Bridge (Bridge Box : Tokyo Sokki Kenkyujo Co.,Ltd., Japan) which connected to one chanel strain indicator amplifier (Tokyo Sokki Kenkyujo Co.,Ltd., Japan). When stress was applied, the resulting
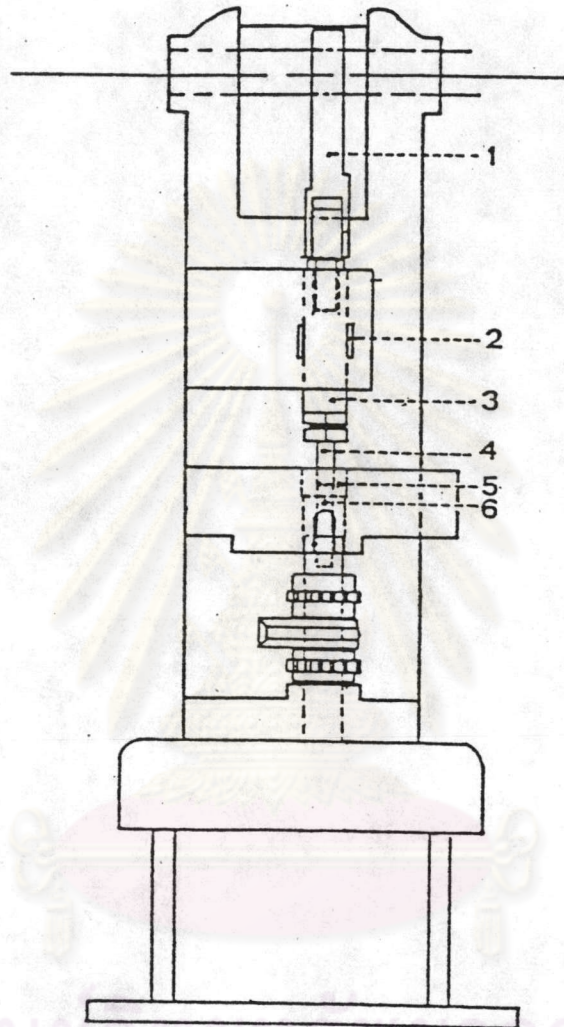
Figure 2   Schematic Drawing of the Instrumented Tablet Machine
           (Reference 72,73)
               1 Eccentric Sheave   2 Strain Gauge
               3 Upper Plunger   4 Upper Punch
               5 Die   6 Lower Punch

.strain in the upper punch was shown by strain gauges consequently causing a change of resistance of the gauges and an unbalanced in the bridge. The unbalanced potential which was directly proportional to the force applied on upper punch was then amplified by strain indicator amplifier and were recorded on an oscilloscope (HAMEG, Model HM 203-6, 20 MHZ, Geramany). Responses were read directly as unit of signal deflection by XY/XY-T recorder (Watanabe Instrument Corp.Model WX 440 Series, Japan). The diagram of tablet instrument was shown in Figure 3.

The strain gauges mounted on the upper punch holder were calibrated under static condition by using hydraulic press over a range of force 250 pounds up to 3750 pounds. The compressed force during tabletting process could be determined from this strain-force calibration curve.

2.2.2 Preparation of Tablets without Drug Substance

Tablets were manufactured by direct compression method using two types of directly compressible vehicles,α-lactose monohydrate and dibasic calcium phosphate dihydrate which were water soluble and water insoluble excipients, respectively. The study of disintegrating efficacy of the fruitpulp of *Scaphium macropodum* Beumee were made in comparison with the following disintegrants : Ac-Di-Sol[R], corn starch, ECG 505[R], Explotab[R], Kollidon CL[R] and L-HPC. The concentrations of disintegrants were varied in the range from 0 to 5%.

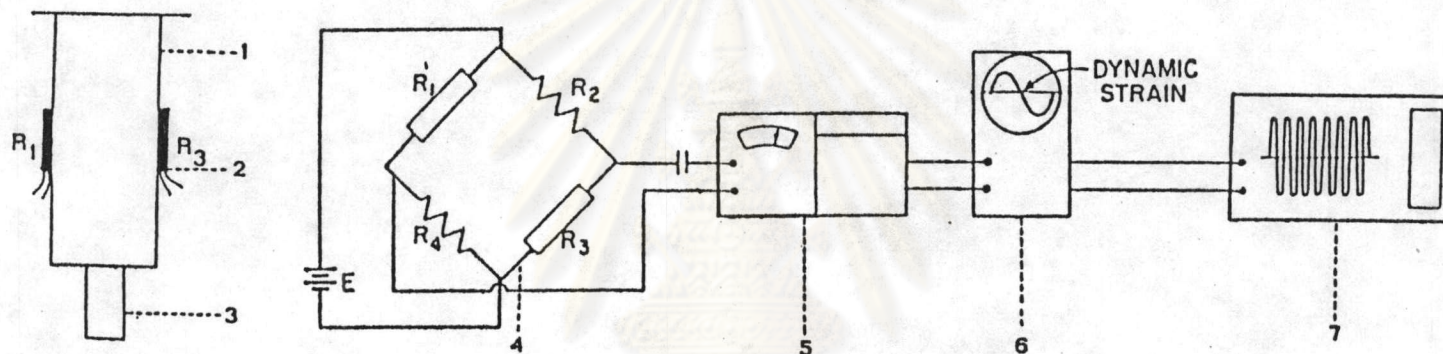Figure 3   Function Block Diagram of Press and Associated Measuring
            System (Reference 73)
            1 Upper Plunger   2 Strain Gauge
            3 Upper Punch   4 Wheatstone Bridge
            5 Dynamic Strain Indicator   6 Oscilloscope
            7 Recorder

The compositions of the tablet formulations used in this study were presented in Table 2. All disintegrants and magnesium stearate were passed through 80-mesh handle screen sieve. The diluents, dibasic calcium phosphate dihydrate and α-lactose monohydrate were passed through 60-mesh handle screen sieve, then all excipients were dried in hot air oven at $60^{\circ}C$ (Memmert, Germany) for 30 minutes. A batch of 350 g of each formulation was prepared by mixing the excipients and disintegrants in a laboratory scale cube mixer (Erweka AR 400, type UG, West Germany) for 10 minutes at rotation speed of 20 r.p.m., magnesium stearate employed as lubricant was then added to the mixer. The materials were mixed for additional 5 minutes. The tablets were then compressed using instrumented single punch tablet machine equipped with 3/8 inch round flat faced punch. The weight of tablets for each formulation was 350 mg. Each tablet formulation was compressed at four different compressional forces of 1200, 1800, 2400 and 3000 pounds.

2.2.3 Preparation of Tablets Containing Drug Substance

Hydrochlorothiazide tablets containing S_2 were prepared according to Table 3. The powder of S_2 and magnesium stearate were separately passed through a 80-mesh handle screen sieve. The diluents, dibasic calcium phosphate dihydrate and α-lactose monohydrate were passed through a 60-mesh handle screen sieve. All the excipients

Table 2   Tablet Compositions for Evaluation of Disintegrant
           Efficacy

	% w/w per Tablet				
	A	B	C	D	E
Series 1 (Water Insoluble Diluent)					
Encompress[R]	99.0	98.5	98.0	96.0	94.0
disintegrant*	0	0.5	1.0	3.0	5.0
magnesium stearate	1.0	1.0	1.0	1.0	1.0
Series 2 (Water Soluble Diluent)					
Tablettose[R]	99.0	98.5	98.0	96.0	94.0
disintegrant*	0	0.5	1.0	3.0	5.0
magnesium stearate	1.0	1.0	1.0	1.0	1.0

*   disintegrants : S_1, S_2, Ac-Di-Sol[R], Corn Starch,
                      ECG 505[R], Explotab[R], Kollidon CL[R],
                      L-HPC

Table 3   The Formulations of Hydrochlorothiazide Tablets
           Using S_2 as Tablet Disintegrant

	% w/w per Tablet	
	A	B
Series 1 (Water Insoluble Diluent)		
Emcompress$^{(R)}$	85	84
HCTZ	12	12
S_2	2	3
magnesium stearate	1	1
Series 2 (Water Soluble Diluent)		
Tablettose$^{(R)}$	87	86.5
HCTZ	12	12
S_2	0	0.5
magnesium stearate	1	1

were dried at 60°C for 30 minutes. A batch of 350 g of various formulations from Table 3 were prepared by mixing each of diluents, hydrochlorothiazide (HCTZ) and S_2 using laboratory scale cube mixer at rotation speed of 20 r.p.m.. After mixing for 10 minutes, magnesium stearate was added, mixing for an additional 5 minutes, then compressed into tablets using single punch tabletting machine (Modified Stroke Model A-3, Thailand) equipped with 3/8 inch round flat faced punch. The weight of tablets for each formulation was 350 mg. The target hardness was controlled within the range 6 ± 1 Kp.

2.2.4 Evaluation of Tablets

The following physical properties of tablets were examined.

2.2.4.1 Weight Variation

Twenty tablets of each batch were individually weighed, using an analytical balance (Sartorious, A-200S, Germany). The average, standard deviation and relative standard deviation were determined.

2.2.4.2 Tablet Thickness

The thickness of tablet was determined by using a micrometer (Teclock Corp, 0.01 mm, Japan) and expressed in millimetre. The thickness value was an average of ten determinations.

2.2.4.3 Tablet Hardness

The hardness of the compressed tablet was determined by using a hardness tester (Schleuniger-2 E, Switzerland) and the hardness recorded was an average of ten determinations.

2.2.4.4 Tablet Friability

Twenty previously weighed tablets were placed in the Erweka friability apparatus (Erweka, Germany) and rotated at 25 r.p.m. for 4 minutes. The tablets were then reweighed and the weight loss was calculated in term of percent friability.

2.2.4.5 Water Uptake of the Tablets

The apparatus used to assess the water uptake characteristic of tablets was modified from the apparatus described by Nogami et al. (30). The tube of sintered glass filter was connected to a horizontal graduated pipette by a rubber tube as shown in Figures 4,5. The whole assembly was immersed in a constant temperature water bath controlled at $37^{\circ}\pm1^{\circ}C$. A continuous water was maintained from the sintered glass filter to the end of the pipette. A tablet was placed on a piece of wetted filter paper which was on the top of the sintered glass base. The sintered glass filter was covered with a rubber bung to prevent evaporating of penetration liquid. The volume of water uptake of a tablet was read from the graduated pipette at various time intervals. The rate of volume uptake was an average of five determinations.
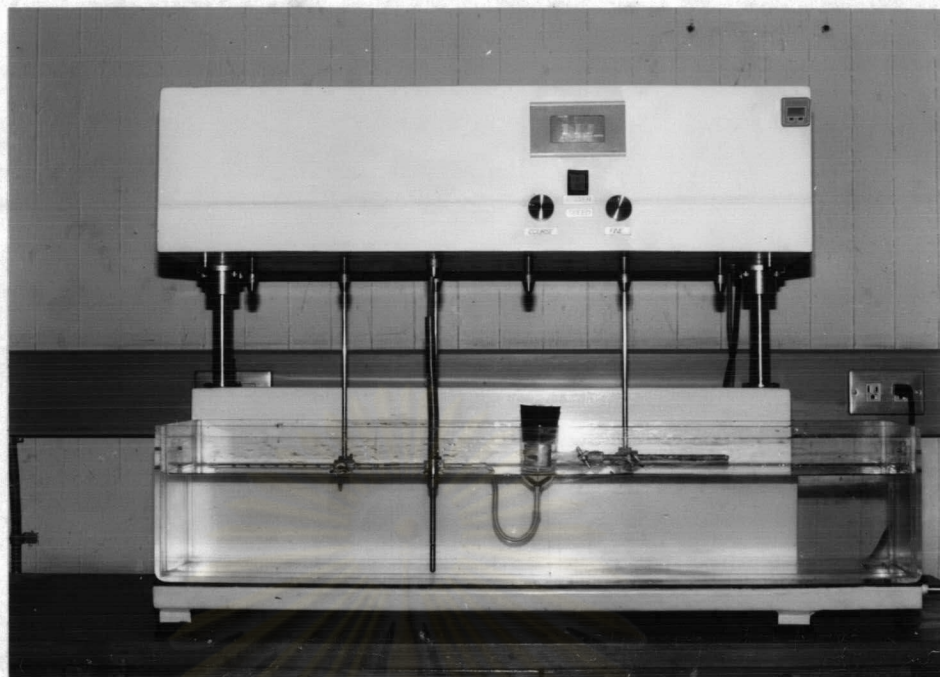
Figure 4 Photograph of Apparatus Set up for Determination of Water
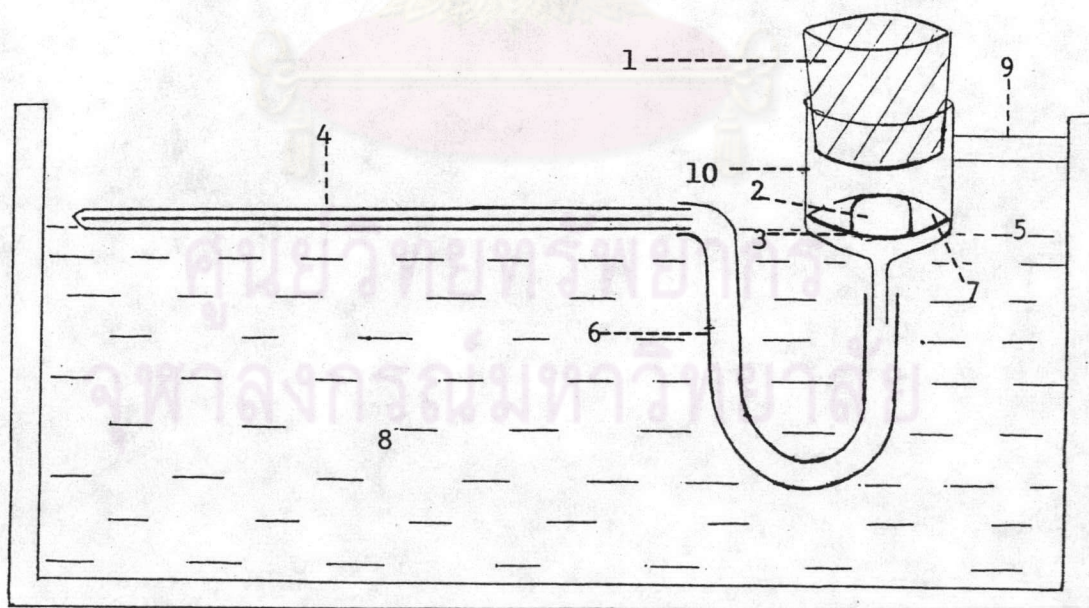Uptake of Tablets

Figure 5 Diagramatic Representation of Apparatus for Determination
of Water Uptake of Tablets
1 Rubber Bung   2 Tablet
3 Filter Paper   4 Pipette
5 Water Level   6 Rubber Tube
7 Sintered Glass Base   8 Thermostated at 37°C
9 Clamp   10 Sinter Glass Filter

2.2.4.6 Disintegration Time

The disintegration time was measured using the USP XXII apparatus (Hanson, Research, USA) with purified water at $37^O \pm 2^OC$ as a disintegration fluid. The tests were performed with disks. This value was measured in seconds. The mean of six determinations of each batch was presented.

2.2.4.7 Dissolution Time (74)

The dissolution test of the tablets were carried out according to USP XXII (Hanson, Research, U.S.A.) A volume of 900 ml of 0.1 N hydrochloric acid was placed in the vessel that permitted holding the temperature at $37^O \pm 0.5^OC$ during the test. A tablet was placed in the basket and then immersed in the vessel. The rotation speed of basket was 100 r.p.m..

The 5 ml of sample solutions were withdrawn at various time intervals of 5,10,15,20,30,40,50, 60 minutes, then the equal volume of 0.1 N hydrochloric acid was added immidiately. The aliquot was diluted with 0.1 N hydrochloric acid to a suitable volume and the absorbance was measured in a 1-cm cell at the maximum absorbance wavelength of 272 nm with a spectrophotometer (Spectronic 2000, Bausch & Lomb, USA) using 0.1 N hydrochloric acid as a blank. The amount of drug dissolved in each sample was calculated by comparing with the standard calibration curve (Appendix 28). Dissolution profiles were obtained from the average of three determinations.

2.2.4.8 Content Uniformity

One tablet previously ground to fine powder was transferred to a 100-ml volumetric flask containing about 20 ml of ethanol, shake for 10 minutes. The final volume was made with ethanol, filtered and discarded the first 10 ml of the filtrate. A 2 ml of the filtrate was transferred to another 100-ml volumetric flask and make volume with ethanol. The absorbance of the sample solution was measured spectrophotometrically in a 1-cm cell using ethanol as a blank at the maximum absorbance wavelength of 269 nm (76). The amount of HCTZ was calculated from the standard calibration curve (Appendix 29). Ten tablets were determined individually. The average, standard deviation and relative standard deviation were determined.

2.2.4.9 Percent Labeled Amount

Weigh and thoroughly grind all of the content of 20 tablets. Weigh a portion of the powder equivalent to about 42 mg of HCTZ in a 100-ml volumetric flask containing about 20 ml of ethanol, shake for 10 minutes. The final volume was made with ethanol, filtered and discarded the first 10 ml of the filtrate. A 2 ml of the filtrate was transferred to another 100-ml volumetric flask and make volume with ethanol. The absorbance of the sample solution was measured spectrophotometrically in a 1-cm cell using ethanol as a blank at the maximum absorbance wavelength of 269 nm. The amount of HCTZ was calculated from

the standard calibration curve (Appendix 29). Percent labeled amount was an average of two determinations.

2.3 Disintegrating Characteristics of Tablets Containing Different Disintegrant Materials

Dibasic calcium phosphate dihydrate tablets containing various disintegrants made at the concentration of 3% was placed on 89-mm petri-dish. Water was slowly poured to petri-dish thus the water continuously penetrated into the tablet. The photographs were taken at various time intervals to show disintegrating characteristics after contacting with water.