Chapter II

Experimental

1. Materials

The following substances were obtained from commercial sources.

- Chitin (Kyowa Technos Co.,Ltd., Japan supplied by G.T. Chemical, Thailand)
- Sodium hydroxide, commercial grade (Thai Asahi Chemical, Ltd. Thailand)
- Sodium hydroxide, analytical grade (Riedel-de Haen AG,Germany)
- Hydrochloric acid (E. Merck, Germany)
- Methanol, analytical grade (LabGuard, USA)
- Glacial acetic acid (E. Merck, Germany)
- Paracetamol BP. (China, supplied by Srichand-United Dispensary Ltd. Partnership , Thailand)
- Lactose (The Lactose Company of New Zealand, Hawera, New Zealand)
- Magnesium stearate (supplied by Pharmaceutical Sciences, Bangkok,Thailand)

2. Equipment

- Fizt mill (Kan Seng Lee factory Ltd., Thailand)
- Mechanical sieve shaker (Josef Deckelmann, Aschaffenburg, Western Germany)
- Magnetic stirrer (model SP-18420, Nuova 7 stir-plate, Sybron Thermolyne, USA)
- Hot air oven (Memmert, Germany)
- Analytical balance (Satorious, Germany)
- Moisture determination balance (Mettler LP16, Switzerland)
- Carver press (model C, Perkin Elmer, USA)
- Hardness tester (Schleuniger, Switzerland)
- Micrometer (Teclock Corp., Japan)
- Disintegration tester (Hanson-Research, USA)
- Refrigerated centrifuge (Himac, SCR 20B Hitachi, Japan)
- Brookfield viscometer (model LVT DV-II , Brookfield Engineering Laboratories,Inc., USA)
- Fourier transform infrared spectrometer (model 1760X Perkin Elmer, USA)
- Mass spectrometer (model JMS-DX 300, Jeol, Japan)
- Thermal analyzer (model DT-30, Shimadzu, Japan)
- Scanning electron microscope (model JSM-T 220A, Jeol, Japan)
- X-ray diffractometer (model JDX 8030, Jeol, Japan)

3. Methods

The experiment could be divided into three sections: deacetylation of chitin, evaluation of physicochemical characteristics of chitin and chitosan having different degree of deacetylation, and evaluation of their tablet-disintegrating properties.

3.1 Deacetylation of Chitin

Chitin from crab hulls was used as the dry starting material. It was pulverized with Fitz mill (Kan Seng Lee factory Ltd., Thailand) and sized through 60/200 mesh.

3.1.1 Method A. (deacetylation at 110 °C, under air atmosphere)

Chitin was deacetylated by following procedure.

1. Sodium hydroxide solution 50%(w/v) was prepared. First, 650 grams of Sodium hydroxide (commercial grade) were placed in a 2000-ml Pyrex beaker that placed on a magnetic stirrer. (Nuova 7 stir-plate, model SP-18420) After that, deionized water was added to dissolve and to make solution volume 1,300 ml.

2. Stirring-speed was set at No.7 and the heater-controller was adjusted to make reaction temperature 110 °C.

3. Chitin of 100 grams were added into the solution and then the beaker was covered with aluminium foil to prevent the evaporation of water. The reaction temperature was checked periodically.

4. The reaction time was varied to produce chitosan in different percentage of degree of deacetylation.

5. After reaching the reaction time as required, the suspension of chitosan was transferred from the beaker into a sintered-glass filter No.1 that

connected with Erlenmeyer flask and vacuum pump. The process of suction and washing chitosan with deionized water was repeated until the filtrate was neutral to pH-paper.

6. Chitosan was removed from sintered-glass filter and dried in a hot air-oven (Memmert, Germany) at 70 °C for 6 hours.

7. Dried chitosan was sieved through No.60 mesh screen, and stored in tight glass-bottle in desiccator.

3.1.2 Method B.(deacetylation at 110 °C, under nitrogen atmosphere)

Method B was the same as method A but the deacetylation reaction occurred under nitrogen atmosphere instead of air. After finishing step 2 as described in method A, the solution was purged with nitrogen for 10 minutes before chitosan was added. Purging the reaction with nitrogen resulted in chitosan products having higher molecular weight distribution than those prepared in an air atmosphere.

3.1.3 Method C. (deacetylation at room temperature, under nitrogen atmosphere)

Method C was the same as method B but the reaction occurred without heating. By using 50%(w/v) Sodium hydroxide as the reactor, the temperature of the suspension was approximately 30°C. This condition produced the highest molecular weight of chitosan among all methods.

3.2 Evaluation of Physicochemical Characteristics

3.2.1 Determination of degree of deacetylation of chitosans

3.2.1.1 Colloidal titration

Degree of deacetylation of chitosans were determined according to the method described by Hayes. (1978)

Preparation of chitosan hydrochloride

Chitosan hydrochloride was obtained by following procedure.

1. About 2.5 grams of chitosan were weighed and dissolved in 200-400 ml of 10% Acetic acid (depend on viscosity) by stirring at high speed for about 15 minutes.

2. Undissolved particles were removed by suction filtration using polyester cloth as a filter.

3. Concentrated hydrochloric acid (~18 ml) was added slowly to the chitosan solution with rapidly stirring until no further precipitation of chitosan hydrochloride was obtained. The temperature rose 6°C during the addition of acid.

4. The precipitate was filtered through polyester cloth. The solid was made into a slurry with 100 ml of methanol and refiltered. This operation was repeated until the washing was free of chloride ions. (test with 0.1% Silver nitrate solution). The total of 5 or 6 washings were required to remove the excess hydrochloric acid.

5. The chitosan hydrochloride precipitate was dried in a hot air oven at 50°C over night. The dry chitosan hydrochloride was light brown in color.

Titration of chitosan hydrochloride

1. Accurate weight (about 1 gram) of dried chitosan hydrochloride was dissolved in water and the solution was diluted to 250 ml in volumetric flask.

2. The solution of 50.0 ml was transfer to 125-ml Erlenmeyer flask. Next, the solution was titrated with standard Sodium hydroxide solution (0.0876 M) using phenolphthalein as an indicator.

3. The volume of Sodium hydroxide solution used was recorded as ml and percentage of degree of deacetylation of each chitosan sample could be calculated. The results were the means of three determinations.

3.2.1.2 Infrared spectrometry

Infrared spectra were examined by using a Fourier transform infrared spectrometer (Perkin Elmer, model 1760X, USA) and KBr disc.

3.2.2 Molecular weight comparison

3.2.2.1 Mass spectrometry

A mass spectrometer (model JMS-DX 300, Jeol, Japan) was used to obtain the mass spectra of chitin and chitosans. The spectra were performed on electron impact mode using an ionizing voltage of 70 V. and the temperature was set up from 150-200 °C.

3.2.2.2 Differential thermal analysis

DTA curves of chitin and chitosans were obtained by using a thermal analyzer (model DT-30, Shimadzu, Japan) with heating rate 10° C/min. in static air atmosphere (sealed cell).

3.2.2.3 Viscosity measurement

A Brookfield viscometer (Brookfield engineering Laboratories, Inc. USA, model LVT DV-II) with small sample adapter (spindle and chamber SC 4-31/13R) was used for this purpose. In all measurements, 50 ml of chitosan solution was prepared in 2% acetic acid at 10 g/l concentration. Measurements were made in triplicate at speed No.6 on solution at 28 ± 1 °C.

3.2.3 Morphology examination

Particle size and shape of chitin and chitosans were observed by scanning electron microscope (model JSM-T220A, Jeol, Japan) at 100 times magnification and SEM photomicrographs were taken.

3.2.4 Particle size distribution

Particle size distribution of chitin and chitosans were examined by sieved analysis, using a nest of sieve and a mechanical sieve shaker (Josef Deckelmann, Aschaffenburg, Western Germany). US standard sieves No. 80, 100, 120, 140, and 170 mesh were used. Ten grams of sample were placed in the top sieve and shaken for 20 minutes. Weight size was the product of the arithmetic mean size of the openings and the percentage retained on the smaller sieve.

3.2.5 True density determination

True density was determined using a 10-ml pycnometer bottle. Sample of 0.5 gram was accurately weighed and transfered into the pycnometer. The amount of benzene was added to filled up the pycnometer, and the sample-solvent mixture was shaken. The whole bottle was accurately weighed and the true density was calculated.

3.2.6 Moisture determination

One gram of sample was evenly distributed on a pan of moisture determination balance (Mettler LP16, Switzerland). Weight of sample was automatically weighed. Then temperature was maintained at 100 °C. (Digital setting before placing sample) until moisture content become zero. The percentage of moisture content or percent loss on drying in sample was read directly from the balance.The results were the means of three determinations.

3.2.7 Hydration capacity (method of Kornblum and Stoopak, 1973)

Approximately 200 mg of sample was weighed in a 10 ml centrifuge tube that was accurately weighed. Then 10 ml distilled water was added. After that, the tube and stopper were balanced with the others . Next, the tube was closed with the stopper and shaken until all particles were well dispersed. Later, the tubes were allowed to stand for 10 minutes and mixed by inverting 3 times at the end of 5 and 10 minutes. After 10 minutes, the tubes were centrifuged in the refrigerated centrifuge (Himac, SCR 20B Hitachi,Japan) at 20°C, 15,000 rpm for 15 minutes. Subsequently, water was decanted and the tubes were inverted for 5 minutes allowing excessive water to drain. The tubes contained wet sediment were accurately weighed before drying at 100°C in a hot air oven until weight constant. Finally, weight of dry sediment in centrifuge tubes were measured. Hydration capacity could be calculated as follows.

$$\text{Hydration capacity} = \frac{\text{(wt. of tube + wet sediment) - (wt. of tube)}}{\text{(wt. of tube + dry sediment) - (wt. of tube)}}$$

3.2.8 Swelling power of particle (method of Visavarungroj, et al., 1990)

A sample of 0.5 gram was weighed in a 10 ml graduated cylinder. The initial volume (bulk volume) was noted before shaking with 8 ml of distilled water. After shaking until all particles were well dispersed, the dispersion was adjusted to volume and allowed to stand overnight. The sedimentation volume was read and compared. The swelling capacity was the ratio of the sedimentation volume to the bulk volume. The results were the mean of three determinations.

3.2.9 Rate of water uptake (The method modified from the method of Nagami, et al., 1969 and the method of Rhudic, et al.,1982)

The apparatus used to assess the water uptake characteristic of the powders was modified from the apparatus described by Nagami et al. (1969) The tube of sintered-glass filter was connected to a horizontal 5-ml graduated pipette by a rubber tube as shown in Figure 3. The whole assembly was immersed in a constant temperature water bath controlled at 37±1°C. A continuous water was maintained from the sintered glass filter to the end of the pipette.

A sample of 500 mg of each disintegrant was placed in the sample tube. (made of 5 ml plastic syringe, cut the ends, and close one side with Whatman filter paper No.1). After the sample was filled, the sample tube was tapped for 20 times to make equally packing of particles in all experiments.

A sample tube contained particles of disintegrant was placed on the top of the sintered-glass base. Then, the sintered-glass filter was covered with a petri-disk to prevent evaporating of penetration water. The volume of water uptake of disintegrant particles was read from the graduated pipette at

various time intervals. The rate of volume uptake was an average of five determinations.

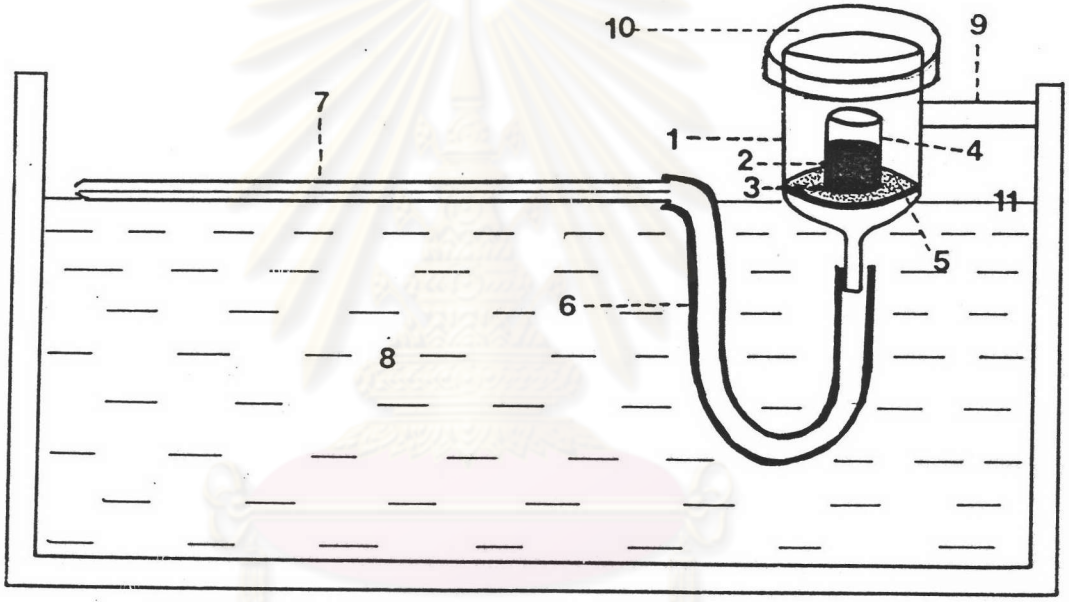

Figure 3 Diagramatic representation of apparatus for determination of water uptake of disintegrants.

1	sintered-glass filter	2	disintegrant powder	3	filter paper
4	sample tube	5	sintered-glass base	6	rubber tube
7	pipette	8	themostated at 37°C	9	clamp
10	petri-disk	11	water level		

3.2.10 X-ray diffraction

X-ray diffractograms were examined by the reflexion method with nickle-fittered CuKα radiation of Jeol diffractometer (model JDX 8030, Japan) operated in the ω - 2θ scanning mode between 5° and 20°.

3.3 Evaluation of Tablet-Disintegrating Properties

3.3.1 Preparation of paracetamol tablets

The compositions of paracetamol tablet formulations were presented in Table 4. All materials were first passed through No.40 mesh screen except Magnesium stearate which was passed through a No. 80 mesh screen. All disintegrants were dried at 60°C for an hour in a hot air oven before used. A batch size contained 50 grams of paracetamol was prepared. The required amounts of drug, tablet diluent, disintegrant and lubricant were accurately weighed. Paracetamol, lactose and intragranular disintegrant (50% of total amount of disintegrant in formulation) were mixed in a motar by means of geometric dilution. PVP K90 as a tablet binder was accurately weighed and dissolved in 17 ml of 70% ethanol. Binding solution was added gradually while mixing to produce a mass of proper consistency. The wet mass was passed through a No.12 mesh screen and dried in hot air oven at 60°C for an hour. The dried granules were sized through a No.20 mesh screen. The granules were mixed with extragranular disintegrant and Magnesium stearate in a bottle for 5 and 1 minutes respectively. The weights of tablets for each formulation were 540, 550, and 570 mg for tablets contained 3, 5, and 10% disintegrant respectively. The granules of drug were weighed by using analytical balance (Satorious, Germany) tablet by tablet. Then, the tablets were compressed using 1/2-inch round flat punch and hydraulic press (model C, Perkin

Elmer, USA). Each tablet formulation was compressed at four different compression forces of 2000, 3000, 4000, and 5000 pounds.

Table 4 The composition of paracetamol tablet formulation.

Ingredient	Amount (mg/tab.)
Paracetamol	500
Lactose	5
PVP K90	15
Disintegrant	3, 5, 10* % of drug
Magnesium stearate	5

* Only CTS3A, CTS7A, CTS2.5N, and CTS7N at 10% concentration

3.3.2 Evaluation of tablets

The following physical properties of tablets were examined.

3.3.2.1 Weight variation

Twenty tablets of each batch at every compression pressure were individually weighed, using an analytical balance (Satorious, Germany). The average and standard variation were examined.

3.3.2.2 Tablet thickness

The thickness of tablets were examined by using a micrometer (Teclock Corp., Japan) and expressed in millimeter. The thickness value was an average of six determinations.

3.3.2.3 Tablet hardness

The hardness of compressed tablets were determined by using a hardness tester (Schleuniger-2E, Switzerland) . The hardness recorded was the average of six determinations.

3.3.2.4 Disintegration time.

The disintegration time was measured by using the apparatus according to USP XXII (Hanson-Research, USA) with deionized water or 0.1 N. HCl at 37 ± 2 °C as disintegration fluids. The test were performed with disks. This value was measured in seconds. The mean of six determinations of each batch was presented.