# CHAPTER III

# EXPERIMENTAL

## 1. Equipments

1.1 Autoclave, Gettinge, Model GEV, Sweden
1.2 Analytical balance, Sartorius
1.3 Hot air oven, Memmert, Germany
1.4 Centrifugal separator, Thailand
1.5 Muffle furnace, Gallenkamp, Tactical 308, Cat. No. FSE621 110H, England
1.6 Ball mill, Petkasem Ceramic Machine Co., Ltd., Thailand
1.7 Hammer mill, U.S.A. Economic Development Co. Ltd., Thailand
1.8 Nest of sieves, Endecotts, England
1.10 IR spectrophotometer, Perkin Elmer, FTIR spectrophotometer spectrum 2000
1.11 Hydraulic press, Thailand
1.14 Scanning electron microscope, JEOL, JSM 5410 LV scanning microscope, Japan
1.15 Mastersizer Scirocco 2000, Model ADA 2000, Malvern, England
1.16 X-ray diffraction spectrometer, JEOL, XRD JDX-8030, Japan
1.17 pH meter, MP 230 pH meter, Mettler Toledo, Switzerland
1.18 Differential scanning calorimeter, Mettler Toledo,
1.19 Thermogravimetric analyzer, Mettler Toledo,
1.20 Micrometer, TECLOCK, Japan
1.20 Hardness tester, Schleuniger 2E, Switzerland
1.21 Friabilator, ERWEKA TAP Nr.27635, Germany
1.22 Tablet disintegration tester, Manesty TD 63 T170, England

## 2. Materials

### 2.1 Chemical substances

Analytical grade sodium hydroxide (NaOH) and hydrochloric acid (HCl) 37% were obtained from E.Merck Dramstadt, Germany.

Reagent grade sodium hydroxide (NaOH) 50% solution and hydrochloric acid (HCl) 35% solution were obtained from Movic Inter Connection, Thailand.

Reagent grade sodium hydrochlorite (NaOCl) 10% solution was obtained from EMC-IMEX Co., Ltd., Thailand.

Analytical grade sodium chloride (NaCl) was obtained from E.Merck Dramstadt, Germany.

Microcrystalline cellulose (Avicel PH101®) was obtained from Asahi Kasei Corporation, Japan.

95% ethanol was obtained from the Excise Department of Thailand.

### 2.2 Fiber residue of durian fruit-hulls

Fruit-hulls of durian was cleaned in water, ground and dried in hot air oven at 50-60°C. Dried ground fruit-hulls was used to extract Polysaccharide Gel (PG) using acidified water, the mixture was filtered and fiber residues were collected, and dried fiber residues were used for isolation of microcrystalline cellulose.

## 3. Methodology

### 3.1 Isolation of microcrystalline cellulose (MCC) from durian fruit-hulls

#### 3.1.1 Laboratory-scale experiments

The schematic isolation procedure is shown in Figure 3. Polysaccharide gel (PG) extraction was processed according to a modified procedure of Pongsamart et al (1998). Fiber residue after PG extraction was cleaned by soaking in 75% ethanol for 10 minutes, filtered through cotton cloth and then dried in hot air oven at 60°C. The dried fiber residue was pulverized by hammer mill and retained as starting material in this experiment. Thirty grams of fiber residue was hydrolyzed in 1.5% (w/v) of sodium hydroxide solution 300 mL or 10 volume of raw fiber weight, autoclaved at

121°C, 15 lb/in$^2$ for 30 minutes. Solid fiber residue was collected by filtering through cotton sheet and washed with deionized water until pH of filtrate equals to 7 and appeared colorless. The solid fiber was then bleached in sodium hypochlorite solution (25 g chlorine/L) 20 volume of dried solid weight at room temperature for 4 hours, filtered and washed with deionized water until no residual chloride ion detected in the filtrate. The white solid fiber was then hydrolyzed in 1.0 Molar hydrochloric acid 20 volume of dried solid weight, autoclaved at 121°C, 15 lb/in$^2$ for 30 minutes. After acid hydrolysis, the solid fiber was filtered and washed with deionized water until the filtrate became neutral. The solid fiber was bleached repeatedly in sodium hypochlorite solution (18 g chlorine/L) 20 volume of dried solid weight at room temperature for 4 hours, and washed with deionized water until no residual chloride ion detected in the filtrate. After second bleaching, the solid fiber was treated in 0.5 Molar sodium chloride solution 20 volume of dried solid weight and stirred occasionally for 3 hours, filtered and washed until filtrate was free from chloride. The white solid fiber was then soaked in 0.1 Molar hydrochloric acid 20 volume of dried solid weight and stirred occasionally for 2 hours. The white solid fiber was filtered and washed with excess deionized water until filtrate became neutral. Dried at 60°C and weighed the white polysaccharide fiber (PF) product and then milled using ball mill apparatus. The fine powder then passed through 60 mesh sieve. The white powder of cellulose product was obtained.

For pH and residual chloride ion detection, filtrate was obtained at the end of each isolation step. The pH of water was measured using pH paper, and residual chloride ion was measured by dropping 1-2 drops of 1% silver nitrate ($AgNO_3$) solution into washing filtrate and recorded the score of silver chloride (AgCl) precipitate from 0 to +5. The end point needed for each washing step were pH 7 and no residual chloride ion detected (score of AgCl precipitate = 0).

### 3.1.2 Pilot-scale experiments

The MCC isolations scale-up were carried out with 300-g and 3000-g batch size with the principles of geometric similarity using time and temperature for the digestion of raw fibers, mixer velocity, and after-treatment end point detection concerning pH and residual chloride ion as similarity factors and scale-up criterion (Table 5). The isolation procedure was the same as the laboratory-scale experiments and shown in Figure 3. Scale-up was considered successful when the physicochemical

Durian rinds (Durio zibethinus Merr.) after PG extraction

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Soak in 75% ethanol for 10 min, filter and dry at 50-60°C

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Pulverize with hammer mill

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Alkali hydrolysis in autoclave at 121°C, 15 lb/in$^2$ for 30 min
in 1.5% sodium hydroxide solution (10 volume of raw fiber weight), wash in DI water

↓

Bleach in sodium hypochlorite solution (25 g chlorine/L)
(20 volume of solid weight) for 4 hr, wash in DI water

↓

Acid hydrolysis in autoclave at 121°C, 15 lb/in$^2$ for 30 min
in 1.0 M hydrochloric acid (20 volume of solid weight), wash in DI water

↓

Bleach in sodium hypochlorite solution (18 g chlorine/L)
(20 volume of solid weight) for 4 hr, wash in DI water

↓

Wash in 0.5 M sodium chloride solution
(20 volume of solid weight) for 3 hr

↓

Wash in 0.1 M hydrochloric acid
(20 volume of solid weight) for 2 hr

↓

Dry at 60°C

↓

Mill in ball mill

↓

Pass through 60 mesh sieve

**Figure 3** Schematic presentation of the established isolation of microcrystalline cellulose (MCC) from durian fruit-hulls.

properties of prepared MCC (infrared spectra, powder X-ray diffraction pattern, differential scanning calorimetric thermogram and thermogravimetric profile) were similar to the commercial MCC (Avicel PH101®).

**Table 5** Scale-up criterion for the isolation of MCC from durian fruit-hulls.

| Scale-up criterion used | Values |
|---|---|
| Mixer velocity | 1250 rpm |
| Digestion temperature | 121 °C (15 lb/in$^2$ pressure) |
| Digestion time | 30 min |
| After-treatment end point: | |
| - pH | 7 |
| - Chloride ion | No silver chloride precipitate appeared (Score 0) |

## 3.2 Physicochemical characterization of prepared microcrystalline cellulose (MCC)

### 3.2.1 Particle morphology

Particle morphology of polysaccharide fiber, shape and size, was determined by scanning electron microscope (JEOL, JSM 5410LV scanning microscope, Japan). Powder of sample was a sprinkled onto a stub and coated with gold. Photomicrographs of three-dimensional structure of particle morphology were evaluated under scanning electron microscope.

### 3.2.2 Particle size distribution

Determination of particle size distribution was performed on the principle of laser diffraction by the Malvern Mastersizer instrument. The light from a 1 mW He-Ne laser was passed through a dispersion of MCC particles in water as medium. The diffracted light was focused onto a multiple element annular ring detector. The intensity maxima in the diffraction pattern was determined for particle size distribution.

### 3.2.3 Infrared spectrophotometry

IR spectra were monitored by Perkin Elmer FTIR spectrophotometer, model spectrum2000, in transmission mode over the range 400-4000 $cm^{-1}$, using the KBr disc technique (1 mg sample/300 mg KBr).

### 3.2.4 Powder X-ray diffractometry

The X-ray diffraction pattern of samples was determined using JEOL XRD JDX-8030 diffractrometer (Japan), MCC sample was pressed into a holder, and powder X-ray diffraction profile was measured under monochromatic $CuK_{\alpha}$ radiation at a step size of 1.6° $2\theta$/min over the range 5-55° $2\theta$. The spectra were obtained at 35 mA with an accelerating voltage of 45 kV.

### 3.2.5 Thermal analysis

Differential scanning calorimetry (DSC) and thermogravimetry (TG) was analyzed using NETZSCH DSC 200 and NETZSCH STA 409 instruments, respectively. The heat evolved during the heating process, at a heating rate of $10^{o}$C/min from 20 to 400°C, was recorded as a function of temperature. Nitrogen atmosphere was used at a rate of 60 mL/min.

## 3.3 Pharmaceutical properties of prepared microcrystalline cellulose (MCC) according to USP26/NFXXI

### 3.3.1 Identification

Mixed 30 g of sample with 270 mL of water. Performed the mixing for 5 minutes in a single-speed, high-speed power blender that has a clover-shaped jar design. Transfered 100 mL of the dispersion to a 100-mL graduate cylinder, and allowed to stand for 3 hours: a white, opaque, bubble-free dispersion, without supernatant water on top, was obtained.

### 3.3.2 pH

Shaked approximately 5 g of sample with 40 mL of water for 20 minutes, and centrifuged: the pH of supernatant water was determined by pH meter to obtain pH between 5.0 and 7.0.

### 3.3.3 Loss on drying

Performed loss on drying of about 3.0 g sample by moisture balance: loss on drying should not more than 7.0% of its total weight.

### 3.3.4 Residue on ignition

Weighed accurately 2 g of the sample in a suitable tared crucible. Ignited gently at first until the sample was thoroughly charred, cooled and then moistened the residue with 1 mL of sulfuric acid, heat gently until white fumes was no longer evolved and ignited at 800±25°C until all the carbon was consumed. Cooled in a desiccator, weighed and calculated the percentage of residue: the results should not be more than 0.05% of its total weight.

### 3.3.5 Water-soluble substances

Shaked 5 g of sample with 80 mL of water for 10 minutes, filtered through filter paper (Whatman No.42 or equivalent) into a tared beaker, evaporate on a steam bath to dryness, and dried in hot air oven at 105°C for 1 hour: not more than 12.0 mg (0.24%) of residue was obtained.

## 3.4 Bulk pharmaceutical properties of prepared microcrystalline cellulose (MCC)

### 3.4.1 Bulk density and tapped density

Bulk density and tapped density were determined by pouring accurately 5 g of sample into a graduate cylinder which has an inside diameter of 30.0±2.0 mm via a large funnel at a rate suitable to prevent clogging and the bulk volume was recorded. The bulk density is a division of weight (g) by bulk volume (mL). Dropping the cylinder on a hard surface from a height of 5 cm until a constant volume was obtained. Tapped density is a division of weight by tapped volume. The compressibility could be calculated using the following equation;

$$\% \text{ Compressibility} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

### 3.4.2 Flowability

The flowability of powder sample was assessed by pouring 5 g of sample into a glass funnel (0.9 cm orifice diameter). The powder was released from the funnel by quickly removing the stopper from the orifice stem. Time and mass was recorded and calculated for the flow rate in g/sec.

## 3.5 Evaluation of tablet characteristics derived from prepared microcrystalline cellulose (MCC)

### 3.5.1 Preparation of tablets

Two hundred and fifty milligrams of prepared MCC (from 3000-g batch isolation) and available commercial cellulose (Avicel PH101®) were accurately weighed without any actives or adjuncts and compressed with a hydraulic press for 3 seconds under force 500 lb/in$^2$ using a 10 mm diameter round flat-faced punch. The tablets were stored in desiccator 12 hours before the weight, thickness, hardness, friability and disintegration time were evaluated.

### 3.5.2 Tablet evaluation

#### 3.5.2.1 Weight variation

The weight of 20 tablets was determined individually and the mean weight (±SD) was calculated.

#### 3.5.2.2 Thickness

The determination of thickness was carried out with vernier caliper. An average of twenty determinations (±SD) was calculated.

#### 3.5.2.3 Hardness

Twenty tablets were measured for its hardness individually on a diametral compression test apparatus. An average value of hardness (±SD) was calculated.

#### 3.5.2.4 Friability

Weight loss through friability was determined for 20 tablets after 4 minutes in friabilator at 25 rpm. Percent friability was calculated from the lossed weight compared to initial weight of 20 tablets before determination.

#### 3.5.2.5 Disintegration time

The disintegration times of 6 tablets were measured in distilled water at 37°C in disintegration apparatus. The disintegration times were recorded as the time when the last of 6 tablets disintegrated and the average value (±SD) was calculated.

### 3.6 Unit cost analysis

The production details of MCC from 3000-g batch size were collected and used to calculate four main expenses which comprised of material cost, labor cost, electrical cost and depreciation cost.

Material cost is the cost of all input resources in production process in Appendix III.

Labor cost is the expenses paid to the employee in returns of services rendered including others monetary fringe benefit such as wage, salary and overtime. In this study, labor cost was calculated using rate 240 baht per 8 working hours per day and calculated from actual working time, not included waiting time during each isolation steps.

Production expense is all expenses excluding material and labor costs, for example, the water supply expenses, the electrical expenses, maintenance and depreciation. Generally, electrical expense was included. But in this study, it was calculated separately to present the main cost in MCC isolation using the equation:

$$\text{Electrical cost} = [(\text{watt} \times \text{working hour})/1000] \times \text{electrical cost per unit}$$

$$\text{When: electrical cost per unit} = 2.80 \text{ baht/unit}$$

Capital cost is the cost of resources which have a useful life of one year or more. These include the cost of buildings, equipments and vehicle. In this study, Capital cost was calculated and showed only the equipment depreciation cost, on the assumption that all equipments can be used for at least 10 years with 8 working hours per day. The equation for calculating depreciation cost of each equipment per minute is:

$$\frac{\text{Purchasing price of equipment}}{(10 \text{ year} \times 365 \text{ day} \times 8 \text{ working hour} \times 60 \text{ minute})}$$

The sum of four main expenses above will represent total cost. After the number of total cost was obtained, other expenses was calculated and added as a part of total cost to replace some expenses which could not exactly calculated such as factory renting, building and indirect cost. The equation for calculating other expenses is:

$$(\text{Material cost} + \text{Labor cost} + \text{Electrical cost} + \text{Depreciation cost}) \times 5\%$$

Thus, total cost was calculated from the equation:

$$(\text{Material cost} + \text{Labor cost} + \text{Electrical cost} + \text{Depreciation cost} + \text{Other expenses})$$

Unit cost was then calculated by divided the total cost with product output in unit of kilogram. The equation for calculating unit cost of prepared MCC is:

$$\text{Unit cost (baht per kg)} = \frac{\text{Total cost}}{\text{MCC product output (kilogram per batch isolation)}}$$