CHAPTER III

EXPERIMENTAL

Materials

The following materials obtained from the commercial sources were used as received.

1. Model Drug

- Acetaminophen (Lot & control number PA42/ 909, Mallinckrodt Chemical Inc., USA)

2. Starches

- Corn starch (High Grade, Ocean Foods (Thailand) Co., Ltd. Bangkok, Thailand).
- Fully pregelatinized corn starch (National 1551®, National Starch and Chemical Corporation., USA)
- Fully pregelatinized rice starch (Era-Gel®, Erawan Pharmaceutical Research and Laboratory co.,Ltd,Thailand)
- Glutinous rice starch (Pharmaceutical Grade, Erawan Pharmaceutical Research and Laboratory Co., Ltd., Thailand)
- Partially pregelatinized corn starch (Starch 1500®, Colorcon, Ltd., England) Chemical Corporation., USA)
- Tapioca starch (Wide Trade Co., Ltd.)

3. Others

- Hydrochloric acid (HCl Approx. 37%, Mallinckrodt, AR Grade, USA)
- Magnesium stearate (Lot No. MAF 05/360, Italy).
- Monobasic potassium phospahte (Lot No. F8K437, Ajax Chemicals, A division of Clyde Industries Ltd., Australia).
- Sodium hydroxide pellet, AR grade (Mallinckrodt Baker, S.A. de C.V., Mexico)
- Sodium starch glycolate (Explotab®, Lot No. E4222, Mendell, USA)

- Talcum (Osmanthus brand, China)

All materials were used without further purification.

Apparatus

1. Analytical balance (Model A200S, Sartorious GmbH, Germany; Model PB3002 and PB303, Mettler Toledo, Switzerland)
2. Brabender amylograph (Model PT100, Duisburg, Germany)
3. Ultracentrifugation (Beckman, L-80 Ultracentrifuge, USA)
4. Differential scanning calorimeter (Model DSC7, Perkin Elmer, USA)
5. Dissolution test apparatus (Model DT6R, Erweka, Germany)
6. Disintegration test apparatus (Model DT6R, Erweka, Germany)
7. Drum dryer (F.A.E. Trading Ltd. Part., Thailand) with adjustable frequency A.C. motor drive (T-Verter-N2 series, Taian Electric Co., Ltd., Republic of Chaina)
8. Drum hoop mixer (Model AR 400, Erweka, Germany)
9. Fourier transform infrared spectrometer (Model 1760X, Perkin Elmer, USA
10. Friabilator (Model TAR20, Erweka, Germany)
11. Haake viscometer (Model Rotovisco® RV 20, Germany)
12. Hardness tester (Model TBH 30, Erweka, Germany)
13. Muffle Furnace (Gallenkamp, Size 2, USA)
14. Laser particle size distribution analyzer (Model Mastersizer-S,Malvern Instruments Ltd., UK)
15. Mechanical sieve shaker (Josef Deckelmann, Aschaffenburg, Germany)
16. Moisture determination balance (Model MA 30, Sartorius, Germany)
17. pH meter (Model 292, Pye Unicam Ltd., England)
18. Pin mill (Nara Jiyu Mill, MPV-2 Type, Nara Machinery Co., Ltd., Japan)
19. Polarizing microscope (MTV-3) (Model PM 10-AD)
20. Camera back 35 mm (C-35AD4)(Olympus Optical Co., Ltd., Japan)
21. Powder characteristic tester (Model PT-N, Hosokawa Micron Corporation, Japan)
22. Scanning electron microscope (Model JSM-6400 LV, Jeol Ltd., Japan)
23. Single punch tabletting machine (Yiuhang Engineering, Thailand) with strain meter (model DA-12A, TML Instruments, Tokyo Sokki Kenkyuju, Japan) and X-Y Recorder (Sekonic Japan)

24. UV spectrophotometer (UV-160 Shimadzu, Japan)
25. X-ray powder difractometer (Model JDX-8030, Jeol Ltd., Japan)
26. Stirrer (Erweka-Apparatebau-G.m.b.H., Germany)

Methods

The methods studied were divided in two parts, the starch powder study and the preparation of acetaminophen tablet formulations and granules, respectively.

Part I

Starch Powder Study

1. Properties of Starting Native Starch

There are three types of native starch used in this study, corn, glutinous rice and tapioca starch. The physical and chemical properties of these starches were investigated according to the USP 23, specifications. All test methods were performed by Food Division, Department of Medical Sciences, Ministry of Public Health, Thailand.

2. Acid Treated Starches

2.1 Preliminary Study

2.1.1 Five hundred grams of each finely divided native starches were weighed accurately, dispersed in 1,250 ml of various hydrochloric acid concentration in deionized water. The reaction was held at the temperature to be studied for various

time intervals with maintenance of good agitation. To evaluate the effects of the factors influencing the acid modification of each starch, the conditions used were shown in **Table 3**.

Table 3 The acid treated conditions

Condition	Acid concentration (%v/v)	Temperature (°C)	Time (hr)
Corn starch			
1	0.5	35	0.5
2	0.5	35	1
3	0.5	35	2
4	1	35	0.5
5	1	50	0.5
6	2	35	0.5
7	2	50	0.5
Glutinous rice starch			
1	0.5	35	0.5
2	0.5	35	1
3	0.5	35	2
4	1	35	0.5
5	1	50	0.5
6	2	35	0.5
7	2	50	0.5
Tapioca starch			
1	0.5	35	0.5
2	0.5	35	1
3	0.5	35	2
4	1	35	0.5
5	1	50	0.5
6	2	35	0.5
7	2	50	0.5

After completion of these processes, the acid treated starches were recovered by filtration, washing with deionized water and repeated this step until the minimum amount of chloride remained in the filtrate which was tested by silver nitrate (1 N).

Dry the product in the hot air oven at 50°C for 10 hours. The dried product was screened through a 80 mesh-sieve and then kept in an air tight container.

The acid treated starches obtained were measured for their viscosity properties using Brabender viscoamylograph. High and low level viscosity of each starch were selected to prepared pregelatinized starches.

- The acid treated starch produced using 0.5% HCl acid, 35°C, 0.5 hr, possessed suitable high level viscosity properties of all types of starches.

- The acid treated starch produced using 2% HCl acid, 50°C, 0.5 hr, possessed suitable low level viscosity properties of all types of starches.

2.2 Scale-up

The preparations of acid treated starches selected from **2.1** were prepared in larger batch sized (1600 g) and rechecked for theirs viscosity by Brabender viscoamylograph.

3. Pregelatinized Starches

3.1 Double drum dryer setting

The double drum dryer (F.A.E. Trading Ltd., Part, Thailand) is the equipment used for production of pregelatinized starches in this study. The conditions used are described below.

- Distance between drums: 0.03 inch.

- Drum speed: 3 rpm.

- Steam pressure 60 psi.

3.2 Method of preparations

Native Starches and acid treated starches as described in section 2 were cooked to be 10% starch pastes before feeding to the double drum dryer. Since there were no feeding devices, the starch pastes were feed manually on the top of the drums and let it spread uniformly over the surface of each heat drums. The starches were dried as the drums rotated and then were removed by scrapers.

The pregelatinized starches obtained were then milled to fine particle by pin mill (Nara Jiyu Mill®, MPV-2 Type, Nara Machinery Co., Ltd., Japan). The screen used was No. M3 (0.3 N).

All starches obtained were labeled as presented in **Table 4**

Table 4 Starches used in this study

No.	Types of starches	CODE
	Corn Starch	
1	Native corn starch	CA
2	High level viscosity acid treated corn starch. (condition used: 0.5% HCl, 35°C,0.5 hr)	CB
3	Low level viscosity acid treated corn starch. (condition used: 2% HCl, 50°C,0.5 hr)	CC
4	Pregelatinized starch prepared from native corn starch.	CD
5	Pregelatinized starch prepared from high-level viscosity acid treated corn starch.	CE
6	Pregelatinized starch prepared from low-level viscosity acid treated corn starch.	CF
	Glutinous Rice Starch	
7	Native Glutinous Rice starch	GA
8	High level viscosity acid treated Glutinous Rice starch.(condition used: 0.5% HCl, 35°C,0.5 hr)	GB
9	Low level viscosity acid treated Glutinous Rice starch.(condition used: 2% HCl, 50°C,0.5 hr)	GC
10	Pregelatinized starch prepared from native Glutinous rice starch.	GD
11	Pregelatinized starch prepared from high-level viscosity acid treated Glutinous starch.	GE
12	Pregelatinized starch prepared from low-level viscosity acid treated Glutinous rice starch.	GF
	Tapioca Starch	
13	Native tapioca starch	TA
14	High level viscosity acid treated tapioca starch. (condition used: 0.5% HCL, 35°C,0.5 hr)	TB
15	Low level viscosity acid treated tapioca starch. (condition used: 2% HCL, 50°C,0.5 hr)	TC
16	Pregelatinized starch prepared from native tapioca starch.	TD
17	Pregelatinized starch prepared from high-level viscosity acid treated tapioca starch.	TE
18	Pregelatinized starch prepared from low-level viscosity acid treated tapioca starch.	TF
	Commercial Pregelatinized Starches	
19	Era-Gel	E
20	National 1551	N
21	Starch 1500	S

Evaluation of Physical Properties of Acid Treated Starches and Pregelatinized Starches in Comparison with Commercial Pregelatinized Starches.

1. Viscosity Measurement

1.1 Branender® Viscoamygraph

The starch samples were prepared before measuring. Thirty grams of dried basis starch was suspended in deionized water and weight was adjusted to five hundred grams.

A mixture of starch and water was put into a beaker provide with a set of vertical pins. It was coupled to a synchronous drive, which turned around a vertical axis, and a stirrer was put in, also consisting of a set of vertical pins. The mixture was heating by a set of heating spirals cylindrically arrange around the beaker. The rate of heating and cooling was controlled by a contact thermometer with continuous changing adjustment. The temperature was raised at the pace of 1.5°C/min until a temperature of 95°C was reached. The starch paste was kept at a constant temperature for 15 minutes, after with a cooling spiral (with a tap also controlled by the thermometer) provides a constantly declining temperature until 50°C is reached. The torque of the stirring rod was registered by a dynamometer, so that the obtained curve was consistency plotted on viscograph against time (viz temperature).

1.2 Haake viscometer

The dried basis weight of starch was suspended in deionized water to make 6% w/w concentration. The starch samples were then loaded to the appropriated apparatus using M5 measuring system at 25°C.

The starch samples were divided in two groups, low viscosity and high viscosity, which had to use the different apparatus. The NV sensor was used for low viscosity starch sample while SV1 sensor was used for high viscous starch sample.

The low viscosity starch samples in this study are CA, CB, CC, CD, CE, CF, GA, GB, GC, GF, TA, TB, TC, TE and TF while the high viscosity starches samples are GD, GE and TD.

The starch suspension was loaded in the apparatus and the shear rate was then increased from 0 S^{-1} to 1000 S^{-1} in one minute for NV sensor and increased from 0 S^{-1} to 445 S^{-1} for SV1 sensor. The shear rate was held at 1000 S^{-1} (or 445 S^{-1}) for two minutes. Ten values of the constant viscosity of each sample were recorded. Three determinations were averaged of each starch sample.

The Microscopy of Starch

1.1 Starch Morphology by SEM

Morphology of powder samples were examined by scanning electron microscopy. The samples were coated with gold prior to the microscopic examination

using ion sputting. Size, shape and surface topography of powders were observed, and then photographed at appropriate magnification.

1.2 The Polarising Microscopy of Starch

The small amount of powder samples were placed on the microscope slide, mounted with a small drop of glycerin and drawing a drop of stain solution (0.1 N iodine solution) under the cover slip.

The microscope used was ordinary but having a polarising filter in its sub-stage carrier and could be rotated. The size and shape of starch granules were observed. The appearance of dark cross of the granules under polarised light is the determination of gelatinized and ungelatinized of starch powder. The photographs of the starch samples in both polarized and normal light were taken with a camera attached to the microscope.

2. X-ray Diffractometer

Before investigated by X-ray diffractometer (JDX-8030, Japan), the starch samples were packed on the cover slides until the smooth surface obtained, All diffraction spectra were scanned from 5-60° in term of the 2θ angle.

3. Differential Scanning Calorimeter

DSC thermograms were obtained from differential scanning calorimeter (Model DSC7, Perkin Elmer, USA). Since DSC was applied to measure heat of gelatinization of starches so samples used were prepared in 30% suspension of dried basis starches

The small amount of starch suspension contained 3 to 4 mg of total solid was transfered to the DSC pans which were hermetically sealed, water would not be lost from the system under normal operation temperature.

Calibration of the instrument was carried out with high-purity metal with accurately known enthalpy of fusion and melting point. The calibrant used is Indium (ΔH_{fusion} = 28.45 J/g, m.p.= 156.6°C)

Scanning was performed at the temperature range of 25-100°C with the scanning rate 10°C/min.

The degree of pregelatinization was calculated from gelatinization enthalpy(ΔH). Its native starch was used to represent 100% ungelatinized sample. The equation is

Degree of gelatinization = 100 - %UG

% UG = (ΔH of starch sample/ ΔH of native starch) × 100

4. Infrared Spectrophotometer

Infrared spectra of the starch samples were measured using infrared spectrophotometer. (Perkin Elmer 1760X). All samples were in the form of potassium bromide discs. The scanning time was 6 minutes.

5. Swelling Capacity

Ten gram of starch samples were poured into a 100 ml volumetric cylinder, measured the bulk volume (V_1), then added the deionized water 80 ml and the dispersions were well shaken for 5 min. Water was added up to 100 ml and allowed to stand for 24 hours before the sedimentation volume was read (V_2). The swelling volume was calculate as V_2/ V_1.

6. Amount of Solubles.

Ten percent starch slurry (w/v) of dried basis starch samples were prepared and then centrifuged by ultra centrifugation at 10,000 rpm, 25°C for 0.5 hour. 5 ml of the discarded supernatant was pipeted to the crucible and dried at 110°C in the muffle furnace until constant weight. The dry weight was defined as the amount of solubles and was expressed as the percentage of the initial weight of starch samples.

7. Moisture Determination

Three grams of starch samples were accurately weighed on a pan of the moisture determination balance (Model MA30, Sartorious, Germany), dried until constant weight was obtained. The result was shown as percent moisture content automatically.

8. Particle Size Analyzer

The instrument used was the laser particle size distribution analyzer (Model Mastersizer-S, Malvern Instruments, Ltd., UK). The analysis and calculation were performed by software package.

9. Bulk, Tapped density and Percent Compressibility

Both bulk and tapped densities were measured by the powder characteristic tester, which provided supporting measurements.

Aerated bulk density was obtained by dropping the starch samples through a vibrating chute to a fixed volume cup and then were weighed accurately automatically. (B)

Packed bulk density was obtained by tapping the sample 100 times from a constant height and further accurately weighed. (T)

Percent compressibility is determined by the measurement of aerated and packed bulk density. It was calculated from the following equation.

% Compressibility = (T-B)/ T x100

10. Angle of Repose and Flow Rate

The powder characteristic tester was also used to determine the angle of repose and flow rate of the starch samples.

The angle of repose was measured from a heap carefully built up by dropping the starch samples through a vibrating screen and glass funnel to the horizontal plate. When the angle of repose came to the desired condition, stopped the vibration and then moved the angle measuring arm moving lever by fingers, to a position at which the angle of repose could be measured in accordance with the display.

The flow rate measurement was applied from the determination of angle of repose above. But 30 g of the sample was accurately weighed before dropping to the glass funnel. The time was recorded when the powder started to flow until finished. The flow rate was expressed in gram/sec.

PART II

Preparation of Acetaminophen Tablet Formulations and Granules

Preparation of Acetaminophen Granules

1. **The compositions**

The composition of acetaminophen tablet formulations are given in **Table 5**

Table 5 The compositions of acetaminophen tablet formulation

Ingredients	Weight / Tablet	
	mg	%
Intragranula		
Actaminophen	500	83.33
Filler/Binder*	67	11.17
Explotab®	6	1.00
Purified Water	q.s.	.q.s.
Extragranula		
Magnesium staerate	3	0.50
Talcum	18	3.00
Explotab®	6	1.00

* The fillers/Binders used were all types of pregelatinized starches from PART I.

2. Method of preparation

2.1 Preparation of granules

For each batch of the formulation (300 g), the drug and the appropriate quantities of intragranula diluents were weighed and dry-mixed by the geometric dilution method using a porcelain mortar and pestle. After thoroughly mixed, purified water was gradually added to the mixture with constant mixing until homogeneous damp mass was obtained. The wet masses were then granulated by passing through a 16 mesh sieve. The granules were dried at 50°C for 4 h and the resulting dry granules were passed through a 18 mesh sieve. The dried granules obtained were divided in two parts, first for the study of physical properties and the other was thoroughly mixed with the extragranula excipients.

2.2 Compression of tablets

Approximately 570-600 mg of mixed granules from 2.1 was compressed in a 9.5 mm diameter die fitted with normal round, flat face punch using a single punch tablet machine (Yiuhang Engineering, Thailand), which was attached to the strain gauges and strain meter (Model DA-12A TML Instruments, Japan) in order to record the compression forces. The strain gauges was calibrated using testing machine (Shimudzu Universal Testing Machine DSS-10T, Japan.). The compression force was adjusted accordingly to have the tablet hardness of all formulations in the range of 6-8 Kp.

3. Granules Evaluations

3.1 Scanning Electron Microscope (SEM)

Electron photomicrographs of acetaminophen granules containing various pregelatinized starches were taken with scanning electron microscope following procedure as described in **PART I**.

3.2 Sieve analysis

The granule size distribution and average granule size were determined by sieving through a set of four standard sieves (250, 500, 710 and 1000 μm), which were nested in descending order with respect to the screen opening.

Hundred grams of the granules were placed on the upper sieve, and the sieves were agitated for 5 min using sieve shaker. The granule size distribution could be established according to the amount (% w/w) of granules retained on each sieve. The average size (in μm) of granules retained on any particular sieve was determined by averaging the size of the sieves opening through which the granules were retained. The weight retained on each sieve was converted to percent retention and multiplied by the average size for that sieve. The sum of these products divided by 100 % yielded the average granule size. The geometric mean diameter was determined by the plot of cumulative percent frequency undersize (Z value) versus log particle size of acetaminophen granules.

3.3 Bulk, tapped density, percent compressibility, angle of repose and flow rate

All these evaluations were measured by the powder characteristic tester. The methods used were followed starch powder evaluations (**PART I**).

3.4 Granule friability

The friability of granules were determined by subjecting 10 g of the 250-500 μm granule samples to the friabilator which was applied from Erweka AR 400 filled with 6 stainless steel spheres (average weight 1.252 g and average diameter 6.275 mm) which were used to fall shocks the granules. After 10 min, the stainless steel spheres were removed and all remaining granules were placed on a 250 μm screen, which was put on the sieve shaker. The shaker was operated for 15 s. Material remaining on the screen was weighed and the percent friability calculated.

3.5 Moisture analyzer

The moisture content of the granules was followed procedure as described in **PART I.**

4. Tablet Evaluations

The following physical properties of tablets were examined.

4.1 Hardness

The hardness of the compressed tablets were determined by using the hardness tester (Erweka, Model TBH30, Germany). The hardness recorded was the average of 10 determinations.

4.2 Thickness

Ten of compressed tablets were measured for thickness by using a micrometer (Teclock, Corp., Japan). The thickness value was an averaged of all over those determinations.

4.3 Weight variation

Twenty tablets of each sample were individually weighed, using an analytical balance (Satorious , Germany). The average and standard deviation were examined.

4.4 Friability

Tablet friability was assessed using a friabilator (Erweka, Model TAR20, Geramany). The tablets were dusted, accurately weighed and transferred to the friabilator rotating at 25 rpm for 4 min, after that the tablets were dedusted and reweighed . The percentage loss in weight was recorded.

4.5 Disintegration time

Tablet disintegration time (data were mean of 6 tablets) was measured according to USP with the Erweka, Model ZT31 apparatus (Erweka, Germany) in deionized water at 37±2°C without disks.

4.6 Dissolution time

Dissolution of acetaminophen tablets was performed according to the USP 23. The paddle method was used and results obtained represent the mean of 3 tablets. A 900 ml of phosphate buffer, pH 5.8, was used as dissolution medium, which maintained at 37°C. The paddles were rotated at the speed of 50 rpm. Ten milliliters sample were withdrawn by syringe at the time intervals, 2, 5, 10, 15, 30, 45 and 60 min. The absorbances of sample were determined using ultraviolet spectrophotometer (UV-160A, Shimadzu Corp., Japan) at maximum wavelength, 243.0 nm and the contents were calculated from the absorbance-concentration relationship. To maintain constant volume of dissolution medium, a ten milliliters of fresh medium was replaced after removal of each sample.

Standard curve of acetaminophen

1. 100 mg of acetaminophen was accurately weighed and dissolved in phosphate buffer pH 5.8 . The solution was adjusted to 100 ml with the same buffer.

2. The solution in No.1 was individually pipetted 2, 4, 6, 8, 10 and 12 ml into 100 ml volumetric flask and diluted to volume with pH 5.8 buffer solution. The final concentration of each solution was 2, 4, 6, 8, 10 and 12 μg/ml, respectively.

3. The absorbance of known drug solution was determined using UV absorption spectrophotometer in a 1-cm cell at 243 nm. The pH 5.8 phosphate buffer was used as blank solution. Each concentration was determined in duplicated. The standard curve of acetaminophen was illustrated in **Appendix 8**.