CHAPTER III

MATERIALS AND METHODS

Materials

All materials were used as following;

1. Alpha starch® (Thai Wah Public Company Limited, Thailand)
2. Dicloxacillin sodium (Aurobindo Pharma Co., Ltd, India, batch number EDX 0330067)
3. Gelatin (Type B, 250 bloom. Gelita Deutschland GmbH, Germany, lot number 618063 and 616211)
4. Glutinous rice starch (Erawan™ brand, Thailand)
5. Glycerin USP (S Tong Chemicals CO.,LTD, Thailand, lot number 12821203)
6. Elastigel 1000J® (National Starch and Chemical Company, lot number JAB 5905)
7. Elastigel 2000C® (National Starch and Chemical Company, USA, lot number FKX-00-70)
8. Elastigel 3000M® (National Starch and Chemical Company, Thailand, lot number ECB 2032)
9. Eragel® (Erawan Pharmaceutical Research and Laboratory Co., Ltd, Thailand, lot number G4505080)
10. Rice starch (Erawan™ brand, Thailand)
11. Sorbitol 70% USP (S Tong Chemicals CO.,LTD, Thailand, lot number 70-211145)
12. Sodium lauryl sulfate (S Tong Chemicals CO.,LTD, Thailand, lot number 10420)
13. Tapioca starch (Thai Wah Public Company Limited, Thailand)
14. Ultra-pure water (The Scientific and Technological Research Equipment, Chulalongkorn University)

Instruments

Rotation viscometer (International Rheology Viscometer, Ireland, model RI:2:H2)

Analytical balance (Sartorius, Germany, model 1518)

Analytical balance (Mettler Toledo, Switzerland, model AG 285)

Analytical balance (Mettler Toledo, Switzerland, model PL6001-S)

Dial gauge micrometer (Mitutoyo, Japan, No 2046F)

Digital micrometer (Starrett, Germany, No 734M)

Disintegration apparatus (Erwaka, Germany, model ZT31)

Dissolution apparatus (Sortax, Switzerland, model S17)

Hot air oven (Memmert, Germany, type U10)

Hot air oven (Hot pack, USA, model 435314, serial 75310)

Hard capsule dipping I™ (STREC, Chulalongkorn University Thailand)

Hard capsule dipping II™ (STREC, Chulalongkorn University Thailand)

Lloyd (model LR10K)

ProGloss 3 (Germany)

Sonacater (Transsonic Digitals, Germany, model TP 680DH)

Water bath (Heto DT Hetotherm, Denmark, Type 23DT-2)

TLC holder (Biddy Sterilin Ltd.)

TLC spreader (Biddy Sterilin Ltd.)

Methods

1. Preparation of gelatin/starch-gelatin solutions, films and hard capsules

1.1 Gelatin solution

Gelatin powder was dispersed in ultra-pure water with resistivity of 18.2 MW cm at 33% w/w concentration to make total of 500 g. in a beaker and was heated to 60-65°C in water bath until gelatin completely dissolved. Gelatin solution was sonicated in a sonicator (Transsonic Digitals, model TP 680DH) to remove air bubbles at 60-70°C and let it cool down to 50 ± 2°C before the viscosity measurement and film casting respectively.

1.2 Starch-gelatin solution

Eight starches were studied and could be classified into three types: 1) native starches i.e., rice flour, glutinous flour and tapioca starch 2) pregelatinized starches i.e., pregelatinized tapioca starch (Alpha starch®) and pregelatinized rice starch (Eragel®) and 3) modified starches i.e., Elastigel 1000J®, Elastigel 2000C® and Elastigel 3000M®. Gelatin was substituted by 5% increment by each type of starches until starch-gelatin mixture shows the visible phase separation or viscosity over 3000 mPas. Starch-gelatin solutions were prepared at fixed concentration at 33% w/w in total amount of 500 g.

Each starch was suspended in ultra-pure water with resistivity of 18.2 MW cm and was heated to 90°C for 30 minutes with vigorously stirring using magnetic stirrer. The starch solution was cooled down to 70°C and gelatin was added. The mixture was kept in the water bath at 60-70°C and was continuously stirred with stirring rod until gelatin completely dissolved. Each starch-gelatin solution was sonicated in a sonicator (Transsonic Digitals, model TP 680DH) to remove air bubbles at 60-70°C and let it cool down to 50 ± 2°C before the viscosity measurement and film casting respectively.

1.3 Gelatin film

Preparation of gelatin film was carried out by casting method. Ten by twenty square centimeter-sized glass plates (10x20 cm) were fixed in TLC holder. Warm gelatin solutions (50 ± 2°C) were poured down onto glass plates and 0.75 mm.-thickness films were cast by TLC spreader. Plates were dried in an oven (Hot pack, model 435314, serial 75310) at the controlled temperature of 30°C and relative humidity 50% for 2 hours.

1.4 Starch-gelatin films

Preparation of starch-gelatin films was carried out by casting method same as described in gelatin film preparation.

1.5 Hard gelatin or starch-gelatin capsules

Gelatin or starch-gelatin solution was warmed in a water bath at 50 ± 2°C while lubricating capsule moulds were lubricated with greaser. Hard capsule dipping I™ machine was used to prepare capsules. Capsule #1 pin bars (cap or body) were gently lowered into the solution until the distance from pin bar base to the solution surface was about 1 cm and then pin bar were lifted up slowly (35-38 seconds for caps and 45-48 seconds for bodies). Gelatin or starch-gelatin was picked up on the mould pins. After withdrawal immediately, there was an accumulation of gelatin or starch-gelatin on the tops of the pins formed when the pin breaks free from the surface of the solution. To spread this gelatin or starch-gelatin evenly over the surface of the mould pins, the pin bars were rotated about a horizontal axis as forward and backward for 3 cycles. The coated moulds were dried in an oven at 30°C and relative humidity 50% for 30 minutes. The capsule shells were removed from the pins by metal jaws. The jaws were closed and pulled back along the pin. Lengths of body and cap were specified at 16.5±0.5 mm. and 9.9±0.5 mm for body length and cap length respectively. Capsules were cut to meet the specified lengths.

2. Effects of plasticizers and sodium lauryl sulfate (SLS) on films and hard capsules

2.1 Effects of plasticizers on films and hard capsules

2.1.1 Effects of plasticizer on gelatin films

Plasticizers (sorbitol and glycerin at 0.1, 0.5 and 1% by weight of solution) were used to study for gelatin film. Plasticizer was mixed in ultra-pure water with resistivity of 18.2 MW cm until homogeneous before gelatin was added as described in preparation section. The physical properties were evaluated

2.1.2 Effects of plasticizer on starch-gelatin films

Selected substituted starches from section 1 were used for this study. Plasticizers (i.e., sorbitol at 1, 2, 3, 4, 5, 6, 7, and 10% w/w and glycerin at 1, 2, 3, 4, and 5% w/w) were also used. Plasticizer was mixed in ultra-pure water with resistivity of 18.2 MW cm until homogeneous before adding gelatin or starch in order to prepare starch-gelatin solutions as described in preparation section. The physical properties were evaluated.

2.1.3 Effects of plasticizer on the stability of gelatin and starch-gelatin films

Plasticizers (i.e., sorbitol at 2 and 4% w/w and glycerin at 2, and 4% w/w) were studied. Plasticizer was mixed in ultra-pure water with resistivity of 18.2 MW cm until homogeneous before adding gelatin or starch was added to prepare either gelatin solution or starch-gelatin solutions as described in preparation section. Plasticizer effects on film properties were studied in both gelatin and starch-gelatin formulations. The physical properties were evaluated.

2.1.4 Effects of plasticizer on hard capsules

Selected substituted starches from section 1 were used for this study. Plasticizers (i.e., sorbitol at 1, 2 and 3% w/w and glycerin at 1, 2 and 3% w/w) were also used. Plasticizer was mixed in ultra-pure water with resistivity of 18.2 MW cm until

homogeneous before adding gelatin or starch in order to prepare starch-gelatin solutions and make capsule as described in preparation section. The weight and thickness of capsule were determined.

2.2 Effects of sodium lauryl sulfate (SLS) on films and hard capsules

Two SLS concentrations were studied (i.e., 0.1 and 1 % w/w of solid content in the formulation) as summarized in table 3.1 SLS and plasticizer (glycerin) were mixed in ultra-pure water with resistivity of 18.2 MW cm until homogeneous before gelatin or starch was added to prepare starch-gelatin solutions as described in preparation section. Films and hard capsules properties in starch-gelatin formulations were evaluated effects of SLS.

Table 3.1 Summarized formulation which added SLS 0.1 and 1% w/w of solid content

SLS part of solid content (% w/w)	solid content		glycerin (% w/w)	Ultra-pure water (% w/w)
	Gelatin (% w/w)	Starch (% w/w)		
Elastigel 2000C®-gelatin				
0.000	21.45	11.55	0	67.000
0.033	21.45	11.55	0	66.967
0.330	21.45	11.55	0	66.670
Eragel®-gelatin				
0.000	24.75	8.25	1	66.000
0.033	24.75	8.25	1	65.967
0.330	24.75	8.25	1	65.670
Elastigel 3000M®-gelatin				
0.000	26.40	6.60	2	65.000
0.033	26.40	6.60	2	64.967
0.330	26.40	6.60	2	64.670

3. Evaluations of films and hard capsules properties

3.1 Physical properties

3.1.1 Measurement of viscosity

The procedure for viscosity measurement was cool down before viscosity measurement. Then the gelatin or gelatin/starch solutions were performed as following: The gelatin solution was loaded onto rotational viscometer (International Rheology Viscometer, model RI:2:H2) equipped with a spindle No.2 used in the interactive mode and programmed via a computer controlled Rheocontroller RI:2:H2 module. Samples were taken about 250 ml. into the beaker and allowed to rest for 5 min. at the desired temperature (50±2°C) and were subjected to a programed shear rate of 90 rpm. Apparent viscosity data were recorded.

3.1.2 Measurement of film properties

a. Appearance of the film

By visual observation such as toughness, brittle, hardness etc.

b. Film thicknesses

The thickness of each sample was measured at ten different points with a digital micrometer (Starrett, No 734M) and the averaged value was taken.

c. Mechanical properties

Measurement of films were performed using a Lloyd (model LR10K) with loading force of 100 N. and a constant crosshead speed of 10 mm./min. Five specimen of 50 × 5 mm^2 rectangular film were cut from each sample and used to study the elongation at break and maximum stress which were computed from loading force versus distance curve.

d. Gloss

ProGloss 3 (Germany) was used to measure ten points of gloss film at geometries 60°. Ten average values were evaluated.

e. Moisture content

The moisture content was determined according to Thailand industrial standard for hard gelatin capsule by following 1) accurately weighing the 1-gram film sample into the crucible 2) drying loaded crucible in the oven (Memmert, type U10) at 105±2°C over 17 hrs and 3) allowing it to cool down to the room temperature in a desiccator before final weighing.

$$\% \text{ Moisture content} = \frac{m_0 - m_1}{m_0} \times 100$$

Where m_0 = weight of sample (g.)

m_1 = weight of sample after drying (g.)

3.1.3 Measurement of hard capsule properties

a. Appearance of the hard capsule

By visual observation such as toughness, brittle, hardness etc.

b. Hard capsule thicknesses

Each cap was measured at 2 sides of walls with a digital micrometer (Starrett, No. 734M) to total 10 caps and measurement the same method with body. The averaged value was taken.

Final stability of capsule thickness was measured at 3 points of walls with a Dial gauge micrometer (Mitutoyo, Japan, No 2046F) to total 10 cap and measurement the same method with body. The top wall was also measured for each cap at one point to total 10 caps and measurement the same method with body. The averaged value was taken.

c. Weight of hard capsule

Weight of cap and body using analytical balance (Mettler Toledo, model AG 285) weight cap 10 pieces and body 10 pieces and the averaged value was taken.

d. Moisture content

The same method for moisture content determination was used as described earlier.

3.2 Dissolution testing of hard capsules

Dicloxacillin was used as a model drug filled in capsules. The dissolution properties of dipping gelatin and starch-gelatin capsule were compared with the commercial hard gelatin capsule in the same conditions.

The dissolution test was conducted using the USP dissolution apparatus I for dicloxacillin capsules (USP 25, 2002). One capsule of dicloxacillin was placed in each vessel of the dissolution tester (Sortax, S17) containing 900 ml of water at 37 ± 0.5°C as dissolution medium. The apparatus was operated at the rate of 100 rpm. Five milliliter dissolution samples were withdrawn after the apparatus was operated at time 4, 7, 10, 15, 20, 30, 45, 60, and 90 minutes, respectively. The equivalent amount of water equilibrated at 37±0.5°C was added immediately after each sampling to maintain a constant volume of dissolution medium. Six capsules of each formulation were tested. The amount of dicloxacillin dissolved in each sample was quantitated by using a spectrophotometer at the wavelength of 274 nm. The amount of dicloxacillin dissolved was calculated by using the calibration curve. The dissolution profiles were then constructed by plotting percent dicloxacillin dissolved of each formulation versus time.

Calibration curve

Standard stock solution of dicloxacillin (1mg/ml) was prepared in water. Serial dilutions were made at final concentrations of dicloxacillin (0.08, 0.1, 0.12, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/ml). The absorbance of the solution was monitored at 274 nm by a spectrophotometer. The straight line calibration curve was plotted between the absorbance of dicloxacillin at 274 nm versus the dicloxacillin concentrations using linear regression.

3.3 Disintegration testing of hard capsules

The disintegration testing was conducted using the USP disintegration apparatus (USP 25, 2002). An empty hard capsule was placed in each open-ended transparent tubes

of basket-rack assembly and a disk was added to each tube which contained water at 37 ± 2°C as fluid medium. Six capsules of each formulation were tested. The basket-rack assembly moves vertically along its axis in the immersion fluid at a constant frequency rate between 29-32 cycles per min. Observe all the capsules had disintegrated no fragment from the capsule shell within 15 minutes means tested sample disintegrated completely and recorded the exact disintegration time.

4. Stability of hard capsules

Hard capsules was kept in two conditions: 1) inside LDPE bag and 2) without LDPE bag.

Hard capsule dipping II™ machine was used to prepare dipping gelatin capsules and starch-gelatin capsules. Dipping gelatin capsules, starch-gelatin capsules, commercial hard gelatin capsules were stored under 2 conditions that were at 30°C: 75%RH and 40°C: 75%RH for 3 months. Capsule properties, such as weight, thickness, and disintegration time, were evaluated after 1 and 3 months storage.