# CHAPTER III

# EXPERIMENTAL

## 3.1 Materials

Commercial grade L-lactic acid (88 % w/w) obtained from fermentation was used in this research. L-lactide was recrystallized in ethyl acetate and dried in vacuum oven for 5 hours prior to use. All of the initiators, and other chemicals from the suppliers were used without further purification. AR grade ethyl acetate and methanol, and commercial grade solvents were distilled before use.

1. L-lactic acid : Commercial grade, Archer Daniels Midland Company
2. L-lactide : AR grade, Aldrich
3. Toluene-4-sulfonic acid monohydrate : AR grade, Fluka
4. Stannous(II) 2-ethylhexanoate : AR grade, Sigma
5. Creatine hydrate : AR grade, Sigma
6. Ethyl acetate : AR grade, Fischer
7. Methanol : AR grade, Merck
8. Dichloromethane : Commercial grade, Merck
9. Chloroform-d : AR grade, Aldrich
10. Tetrahydrofuran : AR grade, Merck

11. Nitrogen gas : High purity 99.99%, Thai Industrial Gas

12. 1,6-Hexamethylene diisocyanate : AR grade, Aldrich

13. Tolylene 2,4-diisocyanate terminated poly 1,4-butanediol prepolymer : AR grade, Aldrich

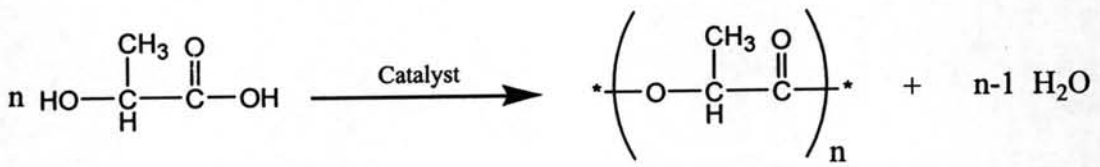
**3.2 Instruments**

| Instrument | Model | Company/Country |
|---|---|---|
| Nuclear Magnetic Resonance Spectrometer (NMR) | : Varian mercury-400 spcetrometer | Varian, USA |
| Gel Permeation Chromatograph (GPC) | : Waters 150-CV<br>Degas: ERC-3415α<br>Column: Waters Styragel HR columns (HR 1, 3, and 4), PL-gel 10 μm<br>Pump: Waters 600 Controller<br>Refractive Index Detector: Waters 2414 | Waters, USA |
| Differential Scanning Calorimeter (DSC) | : NETZSCH DSC204F1 Phoenix | Phoenix, USA |
| Centrifuge | : KR-20000T | Shimadzu, Japan |
| Vacuum Drying Oven | : DP 41 | Yamato Scientific, Japan |

### 3.3 Methodology

L-lactide synthesis was divided into two steps. The first step was to produce low molecular weight PLLA and the second step was L-lactide ring formation by decomposition of low molecular weight PLLA.

#### 3.3.1 Synthesis of low molecular weight polylactide using toluene-4-sulfonic acid monohydrate (PTSA) as a catalyst

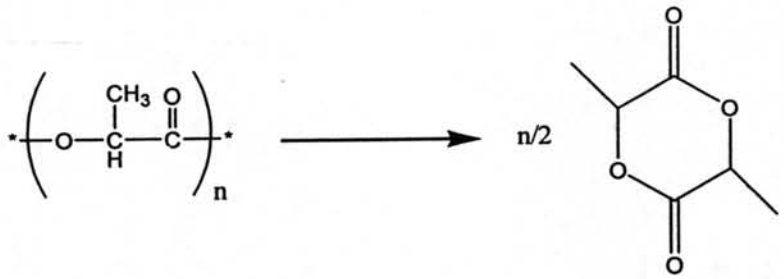



L(+)-lactic acid Low mol. wt. PLLA

**Scheme 3.1** Synthesis of low molecular weight poly(L-lactide).

First L-lactic acid solution (400 g) was heated with toluene-4-sulfonic acid monohydrate (4.0 g) at 140°C for two hours. The weight ratio of catalyst to L-lactic acid is 1:100. High vacuum was applied in this step for 30 minutes to remove water which initially presented in L-lactic acid solution and obtained from polymerization. Later, the reaction mixture was heated to 160°C until no water was condensed to obtain low molecular weight poly(L-lactide).

#### 3.3.2 Ring formation of L-lactide using stannous 2-ethylhexanoate ($Sn(Oct)_2$) as a catalyst

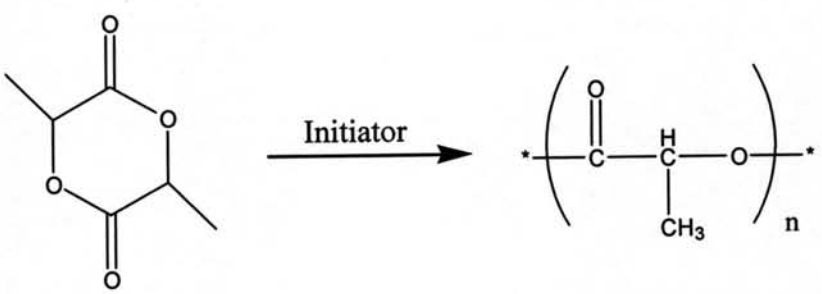



Low mol. wt. PLLA L-lactide

**Scheme 3.2** Ring formation of L-lactide.

In this step, low molecular weight of polylactide was heated with stannous (II) 2-ethylhexanoate (weight ratio of catalyst to low molecular weight polylactide is 1:100) at 160°C for 30 minutes. After that high vacuum was applied and the reaction mixture was heated to 220-230°C until L-lactide crystal was formed. The L-lactide crystal was removed and washed with cold de-ionized water to remove excess L-lactic acid and then vacuum filtered. L-lactide crude was dissolved in warm ethyl acetate to remove insoluble materials and filtered. The filtrate was then heated to evaporate the remaining ethyl acetate and precipitate white needle-like crystal of L-lactide. L-lactide crystal was dried in vacuum oven for 5 hours before use.

### 3.3.3 Ring opening polymerization of L-lactide




L-lactide Poly(L-lactic acid)

**Scheme 3.3** Polymerization of L-lactide.

L-lactide (2.0 g) and the initiator (0.006 g) at the weight ratio of 100:0.3 were stirred under nitrogen atmosphere at the certain time. The crude products were dissolved in dichloromethane and precipitated in methanol. Then, they were centrifuged to obtain white solid particles. White solid particles were dried in vacuum oven for 1 day.

Three parameters were studied in this work including type of initiator ($Sn(Oct)_2$, creatine hydrate), reaction time (12, 24, 48, and 96), and reaction temperature (100-160°C).

### 3.3.4 Increased PLLA molecular weight by chain extension

The low molecular weight PLLA (2.0 g) was placed in a round bottom flask 25 ml. Then, the flask was heated at 140°C in silicone oil bath. After 1 minute low molecular weight of PLLA was completely molten. Chain extender was added into the reaction mixture and stirred for 10 minutes. The ratios of PLLA to chain extender used in this study were 1:0.5, 1:1.1, and 1:2 respectively. The crude products were dissolved in dichloromethane and precipitated in methanol. Then, they were centrifuged to obtain white solid particles. White solid particles were dried in vacuum oven for 1 day.

## 3.4 Polymer characterization

### 3.4.1 Nuclear magnetic resonance spectrometer (NMR)

Proton ($^{1}H$) and carbon ($^{13}C$) nuclear magnetic resonance analysis were used to characterize L-lactide and PLLA products. The sample was dissolved in chloroform-d ($CDCl_3$) and vortexed until clear solution was obtained. The NMR experiment was carried out by using Varian mercury-400 spectrometer $^{1}H$ NMR operating at 400 MHz and $^{13}C$ NMR at 100 MHz. Chemical shifts (δ) were reported in parts per million (ppm) relative to the residual protonated solvent signal as a reference.

### 3.4.2 Gel permeation chromatograph (GPC)

Gel permeation chromatography (GPC) molecular weight of PLLA products average by weight ($\overline{M}_{w}$), by number ($\overline{M}_{n}$), and polydispersity index (PDI). The PLLA sample (15 mg) was dissolved in tetrahydrofuran (THF) (3 ml) and filtered by syringe filter (diameter 13 mm. 0.45 μm. nylon). GPC chromatogram of PLLA was obtained from Waters 150-CV chromatography equipped with PL-gel 10 μm mixed B 2 columns (MW resolving range = 500-10,000,000) at 35°C. Tetrahydrofuran was used as an eluent with the flow rate of 1.0 mL/min. Degassed THF mobile phase was passed through the column for 20 minutes before injected. The sample volume 100 μl

was injected and run for 40 minutes. Polystyrenes (MW = 5,460-1,290,000) were used as standards for calibration. The molecular weight was determined by a reflection index detector.

### 3.4.3 Differential scanning calorimeter (DSC)

Differential scanning calorimeter was determined by NETZSCH DSC204F1 Phoenix. Technique in which the difference in energy input into a substance and a reference material is measured as a function of temperature, while the substance and reference material are subjected to a controlled temperature program.

This technique presented physical property of semi-crystalline products. In this research, DSC technique was used to study about glass transition temperature ($T_g$) of PLLA products. PLLA was weighed in a sample pan about 10-13 mg. The temperature was started at 20°C, heated to 160°C (heating rate 20°C/min) and isothermal for 3 minutes. In this step PLLA would be completely molten. Liquid nitrogen was used to reduce temperature until -50°C (cooling rate 20°C/min) and isothermal for 3 minutes. Some semi-crystalline PLLA were precipitated quickly but the amorphous could not be precipitated in short time. The sample was heated to 200°C (heating rate 20°C/min) until completely molten again. The empty pan was used as standard calibration. The amorphous could be changed from glass-like to rubber-like at glass transition temperature.