

## CHAPTER V

### SYNTHESIS AND STUDY OF SERICIN-G-PLA: COMPARISON OF MASS RATIOS BETWEEN SERICIN AND LA MONOMER

#### 5.1 ABSTRACT

In this study we present an experiment for bulk synthesis of the sericin-g-PLA by using Sn(Oct)<sub>2</sub> as catalyst and study the effect of the mass ratios between Nang Lai (NL) sericin and LA monomer on properties of the sericin-g-PLA. We investigated the chemical structure of the grafted copolymers by using FTIR and GPC. Moreover, the grafting percentage was determined by Soxhlet extraction. The IR spectra of extracted sample showed almost similar absorption bands. The broad absorption band around 3600 cm<sup>-1</sup> that attributed to the -OH group of sericin was increased with the increasing of sericin content. Also, the molecular weight of the grafted copolymers was in range from 2.0 to 5.2 kg/mole. And sericin-g-PLA with 8 wt% of NL sericin and 92 wt% of PLA showed the highest grafting percentage of the grafted copolymers.

**Keywords:** Nang Lai sericin; Poly(lactic acid); Grafting percentage

#### 5.2 INTRODUCTION

Silk sericin (SS) enveloped the fibroin is a second type of silk protein, which contains 18 amino acids including essential amino acids and is characterized by the presence of 32% of serine. The total amount of hydroxy amino acids in sericin is 45.8%. There are 42.3% of polar amino acid and 12.2% of non-polar amino acid residues (Hu, 2011). SS can be cross-linked, copolymerized, and blended with other macromolecular materials, especially synthetic polymers, to produce materials with improved property (Sarovart et al., 2003).

Aliphatic polyesters represent an important family of biodegradable polymers. Poly(lactic acid) (PLA) is the best known polymer of this family (Garlotta, 2001). Its low toxicity, along with its environmentally benign

characteristics, has made PLA an ideal material for food packaging and film wrap and for other consumer products (Auras *et al.*, 2004, Weber *et al.*, 2002). PLA is characterized by excellent optical properties and high tensile strength but unfortunately, it is rigid and brittle at room temperature due to its glass transition temperature ( $T_g$ ) close to 55 °C (Lim *et al.*, 2008).

This work studies the different action on properties of sericin-g-PLA with various mass ratios between NL sericin and PLA. Sericin-g-PLA was synthesized by bulk polymerization with stannous octoate as the catalyst. The functional groups of the graft copolymers were determined by Fourier Transform Infrared Spectroscopy (FTIR), the grafting percentage by Soxhlet extractor, the molecular weight (MW) and molecular weight distribution index (MWD) by Gel Permeation Chromatography (GPC), and also the solubility in water and chloroform.

### 5.3 EXPERIMENTAL

#### 5.3.1 Materials

Thai silk cocoon (*Bombyx Mori*); Namg Lai was purchased from local silk sericulture in Thailand. L-lactide monomer (99.5% purity) was purchased from Shenzhen Brightchina Industrial Co., Ltd. Stannous (II) octoate used as catalyst was purchased from Sigma Aldrich Corp., Japan.

#### 5.3.2 Extraction of Silk Sericin

Silk sericin was extracted by using hot water degumming process. Silk cocoons were rinsed with water to eliminate contaminated matter. 20 g of silk cocoons were cut into small pieces (about 5×5 mm<sup>2</sup>) and mixed with 300 ml of purified water. Silk cocoons were autoclaved under pressure of 0.8-0.9 atm at 120 °C for 60 min. The silk fiber (fibroin) was filtered out to obtain the sericin aqueous solution. Then, silk sericin solution was frozen in glass shells at -40 °C for 12 hr and this glass shell was attached in a freeze-dryer maintained at -110 °C for 48 hr under vacuum to obtain silk sericin powder.

#### 5.3.3 Preparation of Sericin-g-PLA

Stannous (II) octoate ( $\text{Sn}(\text{Oct})_2$ ) (0.2 wt%) was added into sericin (NL) powder (2, 4, 6 and 8 wt%). Then, they were mixed with lactide monomers (98, 96,

94 and 92 wt%, respectively) and stirred at 400 rpm, 140 °C for 10 hr. After 10 hr of mixing, the mixture was cooled down to the room temperature to get the crude synthesized product. Then, the crude synthesized product was grinded into powder.

#### 5.3.4 Characterizations

##### 5.3.4.1 *Fourier Transform Infrared Spectroscopy (FTIR)*

The functional groups of extracted sericin-g-PLA were analyzed by Thermo Nicolet Nexus 670 FTIR spectrometer. About 1-2 mg of sample powder was ground with KBr and was pelletized into the pellet with the thickness less than 0.5 mm. The spectra were recorded over the wavenumber range from 400-4,000  $\text{cm}^{-1}$  with the resolution of 4  $\text{cm}^{-1}$  and the number of scan at 64.

##### 5.3.4.2 *Soxhlet Extractor*

The grafting percentage was determined by soxhlet extractor (VELP SCIENTIFICA, SER 148) with paper thimble. Chloroform and water were used to extract the crude copolymer at 180 °C for 3 hr. After that the remaining yield was dried in vacuum oven at 60°C and weighed. This method was repeated at less 3 times for each sample. The grafting percentage, homo PLA percentage, and free sericin percentage were calculated as shown in Eqs. 5.1-5.3, respectively.

$$\text{grafting percentage} = \frac{M_3}{M_1} \times 100 \quad (\text{Eq. 5.1})$$

$$\text{homo PLA percentage} = \frac{M_1 - M_2}{M_1} \times 100 \quad (\text{Eq. 5.2})$$

$$\text{free sericin percentage} = \frac{M_2 - M_3}{M_1} \times 100 \quad (\text{Eq. 5.3})$$

where  $M_1$  was the sample weight before Soxhlet extraction,  $M_2$  was the sample weight after Soxhlet extraction with chloroform and  $M_3$  was the sample weight after Soxhlet extraction with chloroform and water.

#### 5.3.4.3 Gel Permeation Chromatography (GPC)

The molecular weight (MW) and molecular weight distribution index (MWD) were determined by gel permeation chromatography (GPC). GPC was carried out in Tetrahydrofuran (THF) solvent as the mobile phase using a Water Styragel HR 6E 0F 7.8mmID × 300 MM column packed. From the synthesized product, the chloroform and water extracted sample of 0.01 g was dissolved in 5 ml solvent (0.2 %w/v). The prepared solution was filtered through syringe filter and injected to the injection loop of 60  $\mu$ L. The conditions of this machine were temperature of 40  $^{\circ}$ C, flow rate of 1ml/min, and runtime of 15 min. The molecular weight was calibrated according to polystyrene standards calibration curve:  $\log M = -17938t + (2 \times 10^6)$ .

#### 5.3.4.4 Solubility

The solubility of the extracted sericin-g-PLA in water and chloroform solvent were obtained. A dried sample powder ( $\sim 0.1$  g) was immersed in 40 mL of solvent. After 24 hr of immersion at 25  $^{\circ}$ C, the remnant was dried at 60  $^{\circ}$ C for another 24 hr to determine the weight of insoluble dry matter ( $W_2$ ). The weight of the dry matter dissolved in solvent was calculated by subtracting the remaining dry matter from the initial dry matter ( $W_1$ ). The amount of the solubility percentage was calculated as shown in Eq. 5.4.

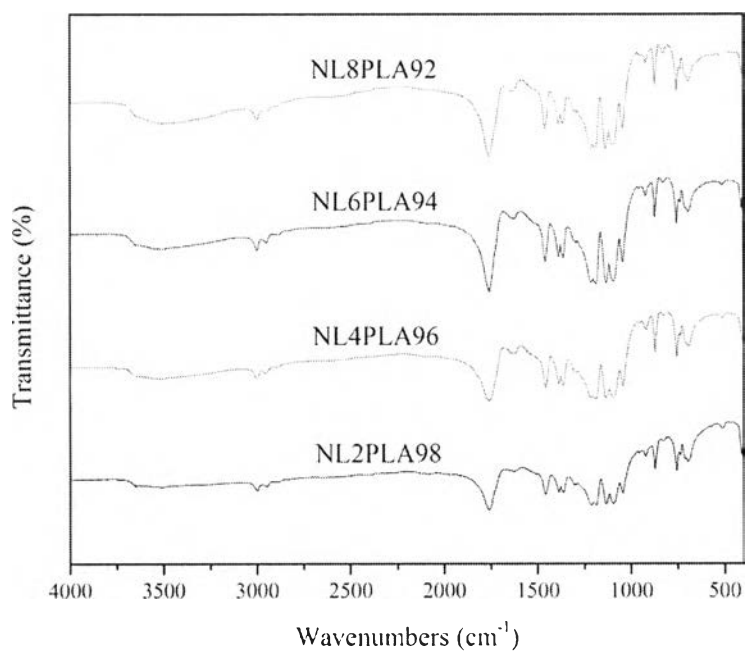
$$\text{solubility percentage} = \frac{W_1 - W_2}{W_1} \times 100 \quad (\text{Eq. 5.4})$$

## 5.4 RESULTS AND DISCUSSION

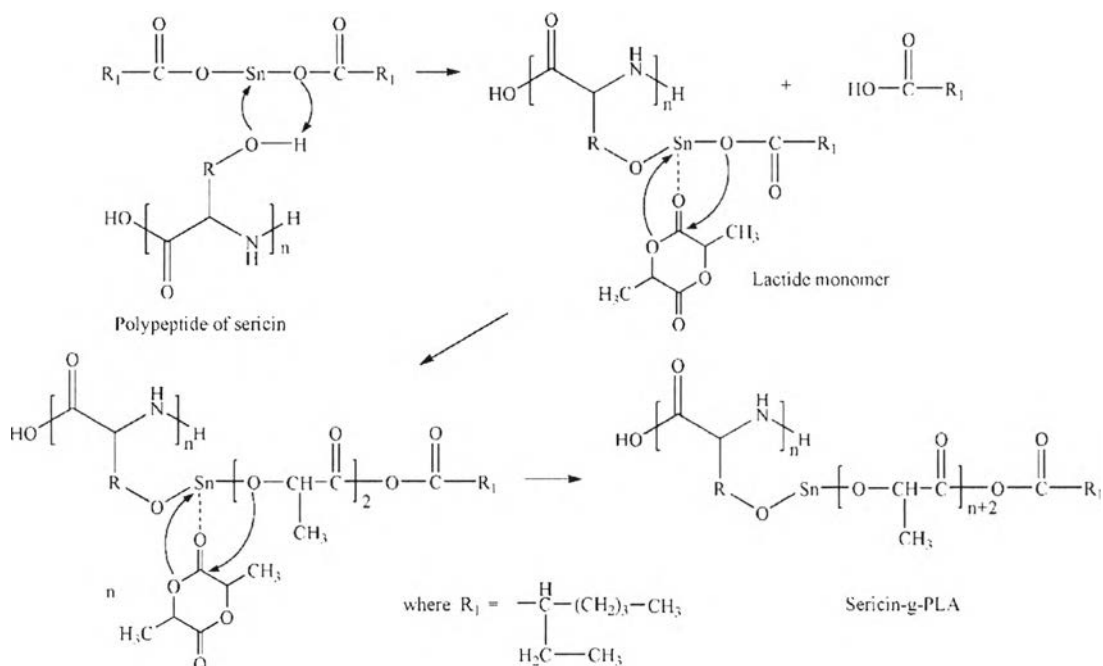
### 5.4.1 Chemical Analysis of Sericin-g-PLA

FTIR spectra of sericin-g-PLA with various mass ratios between NL sericin and PLA are shown in Figure 5.1. The IR spectra of extracted sample showed almost similar absorption bands. The broad absorption band around 3600  $\text{cm}^{-1}$  that attributed to the -OH group of sericin was increased with the increasing of sericin content. Comparing sericin-g-PLA copolymer with PLA polymer, sericin-g-PLA

copolymer shows the similar spectrum to PLA polymer. This result indicated the good compatibility of NL sericin and PLA molecules in the graft copolymerization (Deng *et al.*, 2013). In addition, the peak positioned  $1758\text{ cm}^{-1}$  and other two small peaks at  $1188$  and  $1215\text{ cm}^{-1}$  are assigned to the carbonyl group of the branched polylactide and symmetric C–O–C stretching modes of the ester group of polylactide, respectively. These peaks are obviously absent in the spectra of pure silk sericin. These evidences suggested that the lactide had been successfully grafted onto silk sericin (Song *et al.*, 2006). The mechanism for graft copolymerization is shown in Figure 5.2.



**Figure 5.1** FTIR spectra of extracted sericin-g-PLA with different mass ratio (wt%).



**Figure 5.2** Purposed graft copolymerization mechanism between sericin and LA monomer.

#### 5.4.2 The Grafting Percentage of the Crude Polymerization Product

After reaction finished, the product was taken out from the reactor and was cooled down. The copolymer between sericin and PLA (sericin-g-PLA) was extracted by chloroform to get rid of pure homopolymer PLA and water to remove unreacted serine in Soxhlet extractor. The grafting percentage of the crude polymerization product with different mass ratios between sericin and PLA is presented in Table 5.1. Sericin protein was proposed to graft with the lactide monomers through the hydroxyl groups of protein. The increasing of NL sericin contents resulted in the increase of grafting percentage of the copolymer. This indicated that the raising of NL sericin content brought an increase of -OH active sites, which led to the increase of the number of grafting sites on silk sericin backbone (Song *et al.*, 2006).

**Table 5.1** The grafting percentage of sericin/PLA copolymer with different mass ratios between sericin and PLA

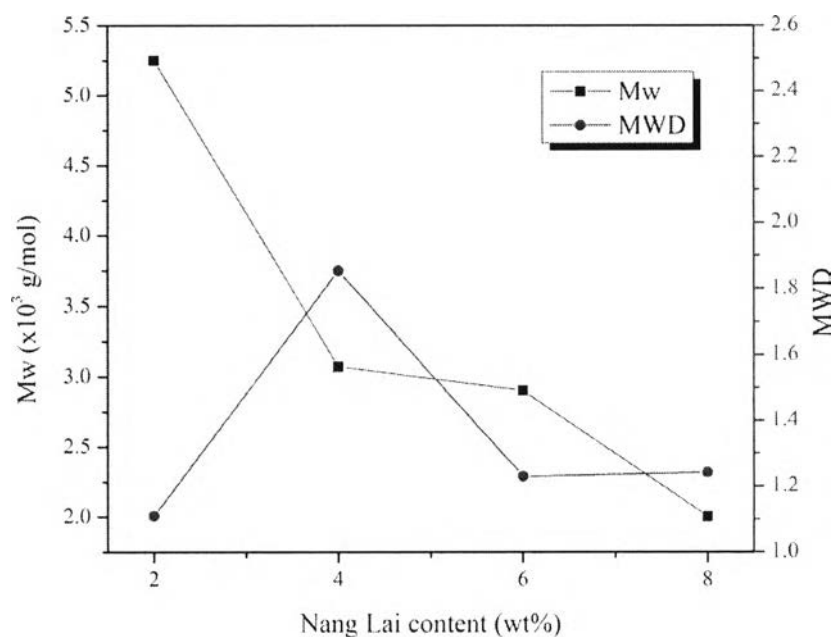
<b>Mass Ratio (sericin:PLA)</b>	<b>%Homo PLA</b>	<b>%Free sericin</b>	<b>%Grafting</b>
2:98	97.2	0.62	2.18
4:96	95.3	1.20	3.50
6:94	91.3	1.01	7.69
8:92	81.8	0.97	17.23

#### 5.4.3 Molecular Weight Measurement

The molecular weight (MW) and molecular weight distribution (MWD) of extracted sericin-g-PLA copolymers were analyzed by using the gel permeation chromatography (GPC). Molecular weight parameters of sericin-g-PLA copolymers synthesized with different mass ratios between sericin and PLA at 140 °C for 10 hr are shown in Table 5.2 and Figure 5.3. The increase of sericin content results in the decreasing of weight average molecular weight (Mw). This is due to increasing of sericin content brought an increase of active sites which can graft with PLA. Thus, grafting chain of PLA will have shorter which led to the decrease of Mw of molecules.

**Table 5.2** Mw and MWD of extracted sericin-g-PLA with various mass ratios between NL sericin and PLA

<b>Mass Ratio (sericin:PLA)</b>	<b>Mw (g/mol)</b>	<b>Mn (g/mol)</b>	<b>MWD</b>
2:98	5,249	4,941	1.11
4:96	3,072	1,657	1.85
6:94	2,903	2,360	1.23
8:92	2,002	1,612	1.24



**Figure 5.3** Molecular weight parameter of the extracted copolymers.

#### 5.4.4 Solubility of Sericin-g-PLA

Solubility of the extracted copolymers in chloroform and in water is shown in Table 5.3. Sericin-g-PLA copolymers with 2 and 4 wt% of NL sericin contents show the closed value of solubility. The solubility of sericin-g-PLA in water significantly increased when sericin contents increase from 4 wt% to 8 wt%. The solubility related to the hydrophilicity of the materials. The various solubilities of sericin-g-PLA depended on the copolymer component since the sericin (NL) component is hydrophilic and PLA is hydrophobic (Hu *et al.*, 2011). This indicates that raising the sericin content results in the increase of hydrophilic part leading to greater water solubility.



**Table 5.3** Solubility of extracted sericin-g-PLA in chloroform and in water

<b>Mass ratio (sericin:PLA)</b>	<b>%Solubility (in chloroform)</b>	<b>%Solubility (in water)</b>
2:98	56.57	8.96
4:96	54.80	8.10
6:94	39.73	12.83
8:92	27.88	19.72

## 5.5 CONCLUSIONS

Sericin-g-PLA from Nang Lai species of Thai silk cocoon was synthesized by bulk polymerization with stannous octoate as the catalyst. The characteristics of the copolymers were exposed. FTIR spectra confirmed that PLA has been successfully grafted onto silk sericin (NL). Moreover, the broad absorption band of the -OH group of sericin was increased with the increase of sericin content. The highest grafting percentage and solubility in water were found in the copolymer with 8 wt% of NL content. These are due to raising the sericin content results in the increase of hydroxyl group and hydrophilic part. In contrast, it showed the lowest molecular weight compared with other NL contents of the grafted copolymers.

## 5.6 ACKNOWLEDGEMENTS

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