

CHAPTER IV
SYNTHESIS AND STUDY OF SERICIN-G-PLA:
COMPARISON BETWEEN NANG NOI, NANG LAI, DOK BUA AND
LUANG PAIROTE SPECIES

4.1 ABSTRACT

In this study we present an experiment for bulk synthesis of the sericin-g-PLA by using Sn(Oct)₂ as catalyst and study the effect of Thai silk cocoon species (Dok Bua (DB), Luang Pirote (LP), Nang Noi (NN), and Nang Lai (NL)) 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 peaks at 1188 and 1215 cm⁻¹ that assigned to the symmetric C–O–C stretching modes of the ester group. The methyl rocking stretching and C–CH₃ vibration of polylactide appeared at 1130 and 1045 cm⁻¹, respectively. The broad peak around 3600 cm⁻¹ belonged to the hydroxyl group and the amino group of sericin which became less after polymerized with lactide. These evidences suggested that the lactide was reacted with sericin. Also, the molecular weight of the grafted copolymers was in range from 5.2 to 6.1 kg/mole. And NL-g-PLA showed the highest grafting percentage of the grafted copolymers.

Keywords: Sericin; Poly(lactic acid); Grafted copolymer; Grafting percentage

4.2 INTRODUCTION

Graft copolymerization is a useful technique for modifying the properties of synthetic and natural polymers, and graft copolymers have been used as a stabilizer of emulsion and dispersion, sorption agents and biomedical materials. Silk sericin (SS) is a natural macromolecular protein derived from the silkworm *Bombyx mori*, and is characterized by unusually high serine content (Zhang, 2002). Recently, SS has been widely used in enzyme immobilization, biomaterials, cosmetic,

membrane material, and functional fibers. Although silk sericin has many good properties, it has some drawbacks such as instability in water, poor solubility in organic solvents, and low strength against microbial attack. Chemical modification of SS is a useful method to improve these drawbacks, and meanwhile allows the acquisition of additional properties of modified SS. The graft copolymerization of various monomers onto SS has been reported, such as vinyl acetate, acrylic acid, acrylamide, and styrene (Song *et al.*, 2006).

Biodegradable polyesters, such as poly(lactic acid) (polylactide, PLA) and its copolymers, have become of more interest as food packaging and film wrap and for other consumer products due to their good biodegradability, biocompatibility, high mechanical strength and excellent shaping and molding properties. However, it is strongly hydrophobic, rigid and brittle (Auras *et al.*, 2004, Weber *et al.*, 2002). It would be a significant breakthrough to prepare hybrid graft copolymers of SS and PLA to combine the advantageous properties of both PLA and SS.

In this study, the characteristic of sericin-g-PLA with different species of Thai silk sericin; Nang Noi, Nang Lai, Dok Bua and Luang Pirote, were investigated. Different species of silk cocoon have different of properties of sericin depending on its amino acid composition and amount of each amino acid. Sericin-g-PLA was synthesized by bulk polymerization with stannous octoate as the catalyst. The grafted copolymers were determined the functional groups 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.

4.3 EXPERIMENTAL

4.3.1 Materials

Four different species of Thai silk cocoon (*Bombyx Mori*); Nang Noi, Nang Lai, Dok Bua and Luang Pirote were 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.

4.3.2 Extraction of Silk Sericin

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

4.3.3 Preparation of Sericin-g-PLA

Stannous (II) octoate ($\text{Sn}(\text{Oct})_2$) (0.2 wt%) was added into sericin powder (SS) (2 wt%). Then, they were mixed with lactide monomer (LA) (98 wt%) in glass reactor and stirred at 400 rpm, $140 \text{ }^\circ\text{C}$ for 10 hr. After 10 hr of mixing, the mixture was cooled down to the room temperature to get the sericin-g-PLA. Then, sericin-g-PLA was grinded into powder.

4.3.4 Characterizations

4.3.4.1 *Fourier Transform Infrared Spectroscopy (FTIR)*

The functional groups of freeze-dried silk sericin and 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 $400\text{-}4,000 \text{ cm}^{-1}$ with the resolution of 4 cm^{-1} and the number of scan at 64.

4.3.4.2 *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 $180 \text{ }^\circ\text{C}$ 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. 4.1-4.3, respectively.

$$\text{grafting percentage} = \frac{M_3}{M_1} \times 100 \quad (\text{Eq. 4.1})$$

$$\text{homo PLA percentage} = \frac{M_1 - M_2}{M_1} \times 100 \quad (\text{Eq. 4.2})$$

$$\text{free sericin percentage} = \frac{M_2 - M_3}{M_1} \times 100 \quad (\text{Eq. 4.3})$$

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.

4.3.4.3 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 \times 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 μ 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: $\log M = -27720t + 52681$.

4.3.4.4 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. 4.4.

$$\text{solubility percentage} = \frac{W_1 - W_2}{W_1} \times 100 \quad (\text{Eq. 4.4})$$

4.4 RESULTS AND DISCUSSION

4.4.1 Chemical Analysis of Sericin-g-PLA

The functional group of the copolymer that occurred was confirmed by Fourier Transform Infrared Spectroscopy (FTIR). Figure 4.1 shows the FTIR spectra of PLA and freeze-dried silk sericin (*B. mori*) from four species of Thai silk cocoon; Nang Noi, Nang Lai, Dok Bua and Luang Pilote. The peak positioned 1758 cm^{-1} is assigned to the carbonyl group of the branched polylactide. The 1188 and 1215 cm^{-1} doublets observed are assigned to the symmetric C–O–C stretching modes of the ester group. The methyl rocking stretching and C–CH₃ vibration of polylactide appeared at 1130 and 1045 cm^{-1} , respectively (Liu *et al.*, 2004). All species of Thai silk sericin presented almost similar absorption bands. The spectra showed the characteristic peak of amino acid, composed of N–H stretching band around 3288 – 3291 cm^{-1} (Sarovart *et al.*, 2003), amide I band around 1653 – 1658 cm^{-1} associated with the C=O stretching, amide II band around 1529 – 1535 cm^{-1} associated with NH bending and CN stretching, amide III band around 1242 – 1246 cm^{-1} and amide IV band around 1072 – 1073 cm^{-1} (Srihanam *et al.*, 2009). Moreover, the broad peak around 3600 cm^{-1} corresponded to OH stretching appeared due to the high content of amino acid serine, this characteristic peak appeared nearby the characteristic peak of N–H stretching hence overlapping of this two peaks was occurred.

Figure 4.2 shows the FTIR spectra of extracted sericin-g-PLA. The broad peak around 3600 cm^{-1} is attributed to the –OH group of sericin that became less after polymerized with lactide (Deng *et al.*, 2013). In addition, the peak positioned 1758 cm^{-1} and other two small peaks at 1188 and 1215 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).

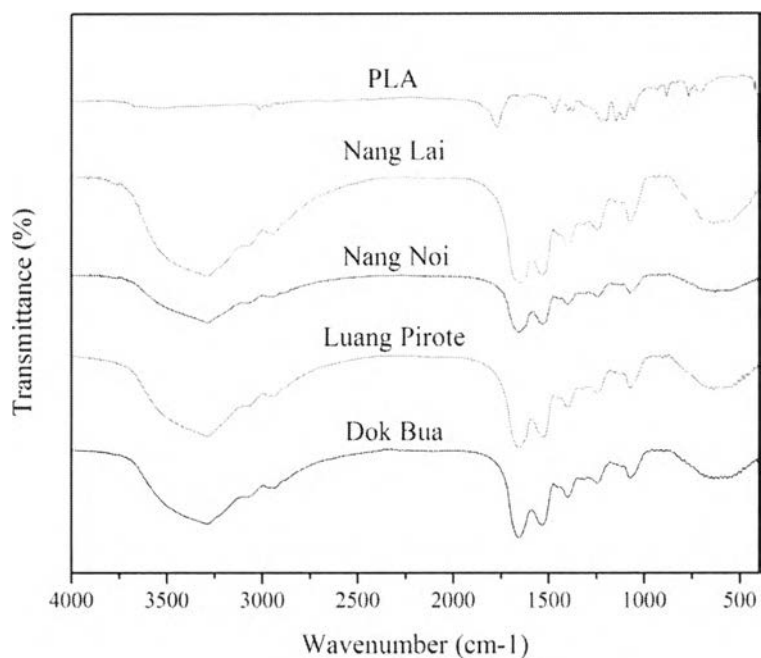


Figure 4.1 FTIR spectra of synthesized PLA and freeze-dried silk sericin extracted from four species of Thai silk cocoon.

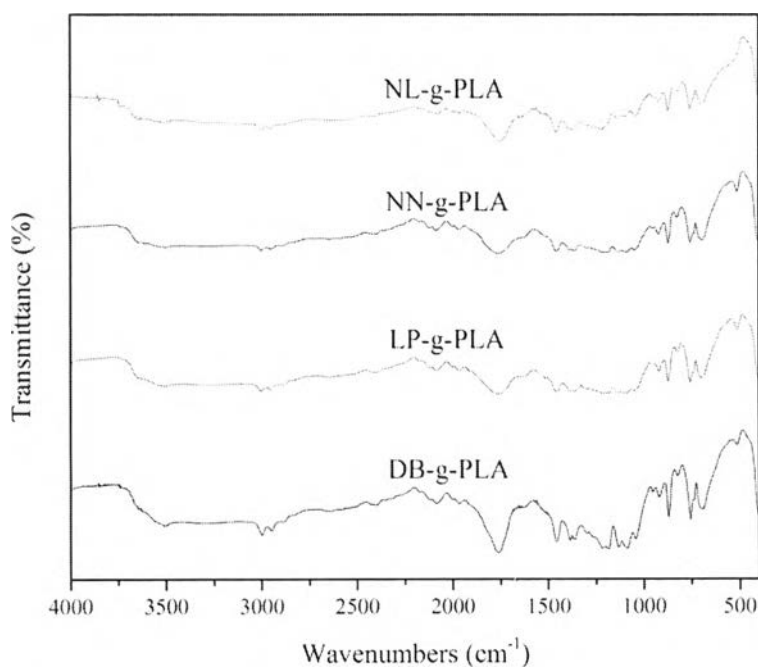


Figure 4.2 FTIR spectra of sericin-g-PLA (DB: Dok Bua, LP: Luang Pirote, NN: Nang Noi, NL: Nang Lai).

The mechanism for graft copolymerization is shown in Figure 4.3. At first, octoate anion-radical abstracts hydrogen from one of the functional groups (i.e., -COOH, -SH, -OH, and -NH₂) in the side chains of the sericin to form corresponding radicals. These radicals initiate partially neutralized PLA grafting onto polypeptide chain of sericin led to a graft copolymer (Hu, 2011).

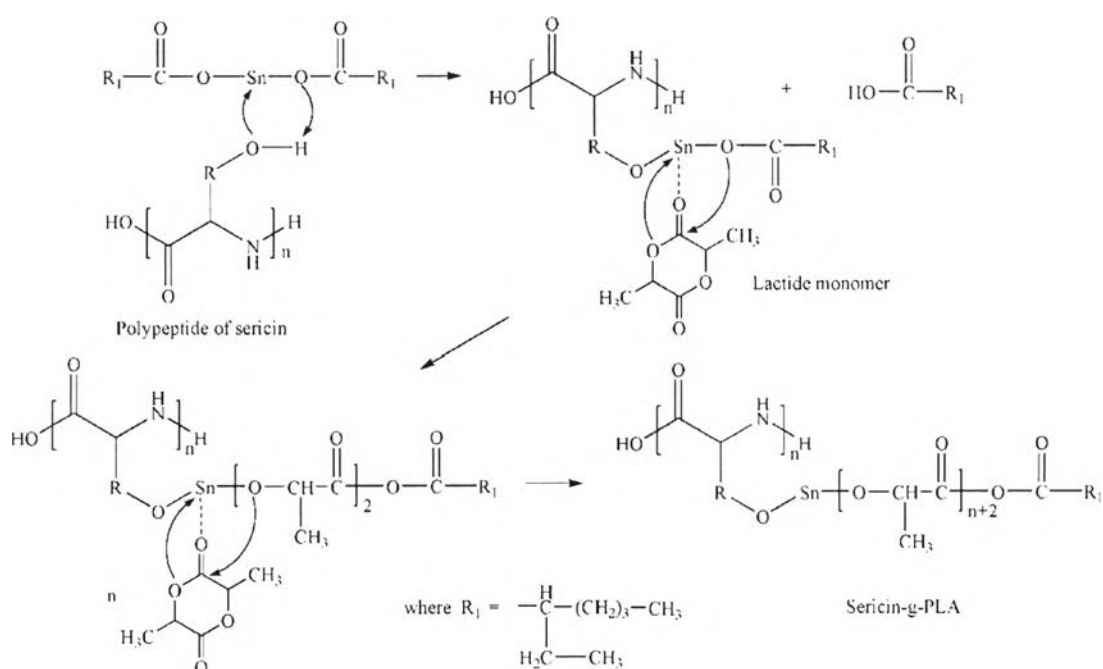


Figure 4.3 Purposed graft copolymerization mechanism between sericin and PLA.

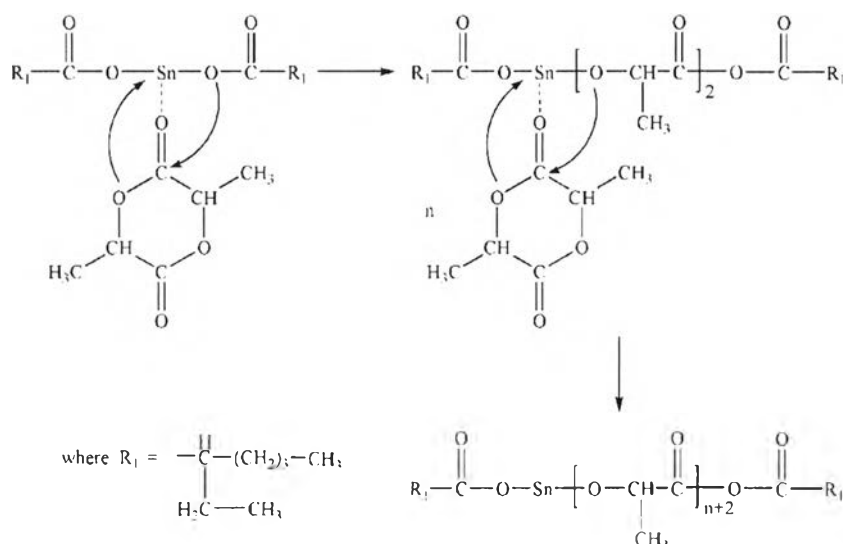


Figure 4.4 Polymerization of PLA initiated by stannous octoate.

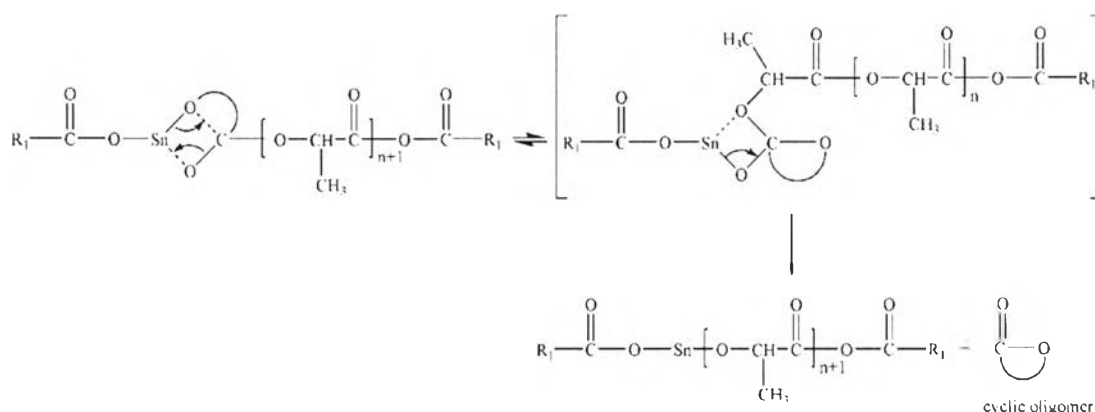


Figure 4.5 Back-biting of PLA catalyzed by stannous octoate.

4.4.2 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 of 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 species of sericin is presented in Table 1. The maximum grafting percentage in the crude polymerization product was obtained in NL/PLA copolymer. The increase of grafting percentage is attributed to the increase of the number of grafting sites on silk sericin backbone (Song *et al.*, 2006). Sericin protein was proposed to graft with the lactide monomer through the hydroxyl groups of a

protein. Thus, the highest grafting percentage related to the highest number of hydroxyl groups in the protein.

Table 4.1 The grafting percentage of sericin/PLA copolymer with different species of sericin

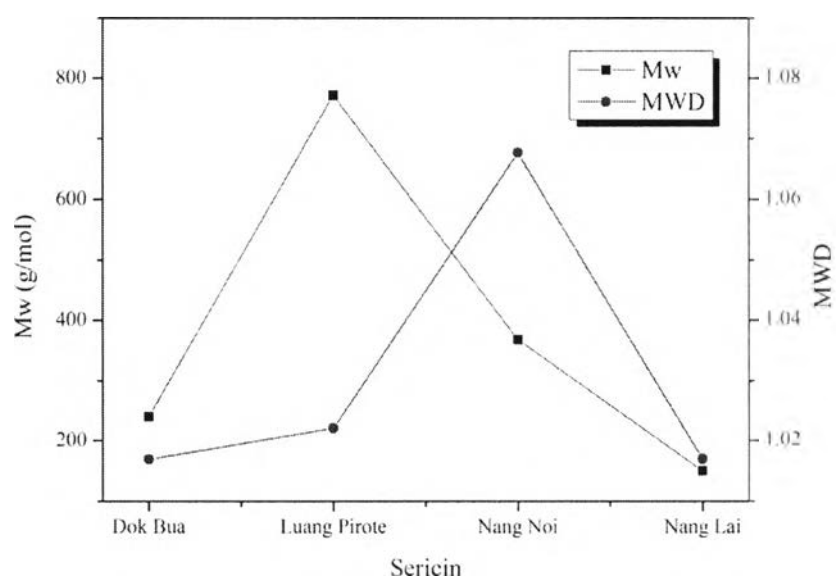
Copolymer	% Homo PLA	%Free sericin	%Grafting
DB/PLA	97.8	1.00	1.20
LP/PLA	97.7	0.53	1.77
NN/PLA	97.5	0.61	1.89
NL/PLA	97.2	0.62	2.18

4.4.3 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 *Bombyx mori* sericin protein produced from silk degumming process are shown in Table 4.2 and Figure 4.6. The average molecular weight of silk sericin was ranged between 150 to 771 g/mol. In addition, molecular weight parameters of sericin-g-PLA copolymers synthesized with different species of sericin at 140 °C for 10 hr are shown the difference of weight average molecular weight (Mw) (Table 4.3 and Figure 4.7). DB-g-PLA was shown the highest of Mw. Moreover, grafting sericin with PLA results in the increase of Mw when compared to sericin protein.

Table 4.2 Mw and MWD of four species of freeze-dried silk sericin

Sericin	Mw (g/mol)	Mn (g/mol)	MWD
Dok Bua	240	236	1.19
Luang Pirote	771	754	1.11
Nang Noi	368	345	1.09
Nang Lai	150	148	1.11

**Figure 4.6** Molecular weight parameter of freeze-dried sericin.**Table 4.3** Mw and MWD of extracted sericin-g-PLA with various species of sericin

Copolymer	Mw (g/mol)	Mn (g/mol)	MWD
DB-g-PLA	6,045	5,074	1.19
LP-g-PLA	5,959	5,356	1.11
NN-g-PLA	5,366	4,716	1.09
NL-g-PLA	5,249	4,941	1.11

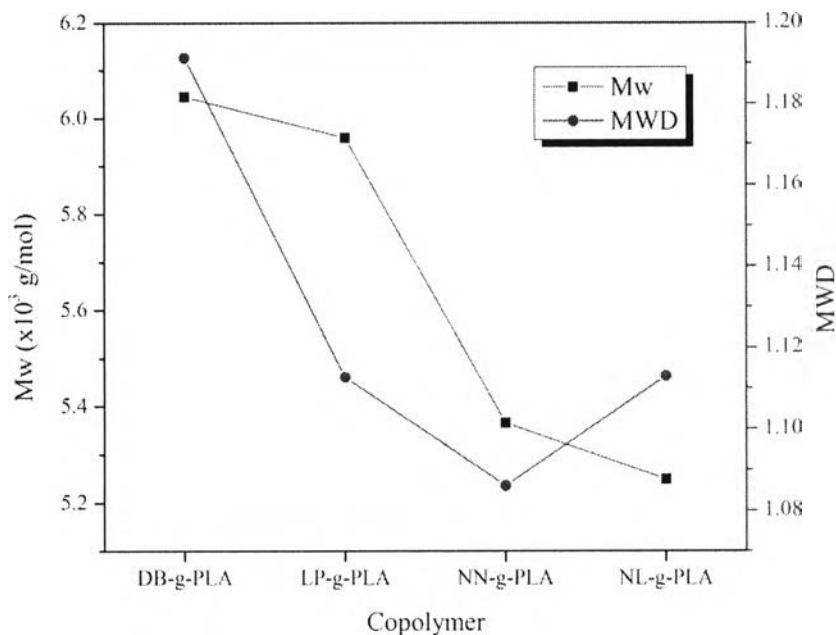


Figure 4.7 Molecular weight parameter of the extracted copolymers.

4.4.4 Solubility of Sericin-g-PLA

Solubility of the extracted copolymers in chloroform and in water is shown in Table 4.3. LP-g-PLA and NN-g-PLA copolymers show the closed value of solubility. The solubility related to the hydrophilicity of the materials. NL-g-PLA shows the highest solubility in water. The various solubilities of sericin-g-PLA depend on the copolymer component since the sericin component is hydrophilic and PLA is hydrophobic (Hu *et al.*, 2011). This indicates that Nang Lai species has higher hydrophilic part than other species leading to greater water solubility.

Table 4.4 Solubility of extracted sericin-g-PLA in chloroform and in water

Copolymer	%Solubility (in chloroform)	%Solubility (in water)
DB-g-PLA	60.89	8.21
LP-g-PLA	52.86	7.55
NN-g-PLA	53.21	7.12
NL-g-PLA	56.57	8.96

4.5 CONCLUSIONS

Sericin-g-PLA from four different species of Thai silk cocoon: Nang Noi, Nang Lai, Dok Bua and Luang Pirote, was synthesized by bulk polymerization with stannous octoate as the catalyst. The dissimilar characteristics in each species to the copolymers were exposed. FTIR spectra confirmed that PLA has been successfully grafted onto silk sericin. The highest grafting percentage and solubility in water were found in NL-g-PLA. These are due to Nang Lai species has higher hydrophilic part than other species. NL-g-PLA showed the lowest molecular weight compared with other grafted copolymers.

4.6 ACKNOWLEDGEMENTS

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