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Conformation Transition and Thermal Properties Study of Silk Fibroin and Poly (ϵ -Caprolactone) Blends

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Abstract: In this study, influence of intermolecular interactions between Silk Fibroin (SF) and Poly (ϵ -Caprolactone) (PCL) in homogeneous blend form on SF conformation changes and thermal properties of the blends was investigated and discussed. The SF/PCL blends were prepared by solution blending and precipitating method, respectively. Dimethylsulfoxide and isopropanol were used as a solvent and a non-solvent, respectively. The blends were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermo Gravimetry (TG) and Scanning Electron Microscopy (SEM). Conformation transition of SF component from β -sheet to random coil forms can be induced by blending with PCL. Melting temperature and heat of melting of the PCL decreased as increasing the SF ratio. Thermal stability of the SF can be enhanced by blending with PCL. FTIR, DSC and TG results suggested that intermolecular hydrogen bonds were formed between SF and PCL molecules in the blends. Homogeneous morphology of blends was illustrated by SEM micrographs.

Key words: Biodegradable polymers, polymer blends, hydrogen bonds, conformation change, thermal stability

INTRODUCTION

Silk Fibroin (SF), one of the typical protein polymers spun by *Bombyx mori* silkworm, is a potential biomaterial for wide use in biological and biomedical fields due to its good biological compatibility as well as biodegradability (Altman *et al.*, 2003; Chiarini *et al.*, 2003). Many researchers have been reported on the SF properties and applications in various forms such as fiber, powder, film and coated (Cai *et al.*, 2002; Chen *et al.*, 2004; Wang *et al.*, 2004; Meinel *et al.*, 2005; Bayraktar *et al.*, 2005; Wu *et al.*, 2006; Hofmann *et al.*, 2006). However, the properties of SF are invariably only partially matched with the requirements of the application. Consequently, attention has turned in recent years to blending as a convenient means of adjusting polymer properties to meet specific requirements through the appropriate molecular interactions. Conformational changes of SF from random coil to β -sheet forms were obtained by blending with chitosan results in an increase of crystallinity and an enhancement of the mechanical properties of SF (Chen *et al.*, 1997; Park *et al.*, 1999). Increasing hydrophilicity of SF was obtained by blending with poly(ethylene glycol) (Jin *et al.*, 2004). The molecular interactions of SF and poly(vinyl alcohol) blends have been also investigated (Tanaka *et al.*, 1998).

Most of the earlier study have concentrated on the preparation of SF blends by blending with the hydrophilic polymers. Although, blends of SF and biodegradable hydrophobic poly(ϵ -caprolactone-co-D,L-lactide) (Kesenci *et al.*, 2001) have been reported as the polymer composites, the molecular interactions between SF and hydrophobic biodegradable polyesters may not clear as in the case of homogeneous blends.

This study reported on the preparation and characterization of the silk fibroin/poly (ϵ -caprolactone) (SF/PCL) blends. Blending can not be carried out in the melt state because SF is unable to melt readily. On the other hand, PCL is a hydrophobic polyester. Thus finding a proper solvent is the key for proper SF/PCL blending. In order to study the SF/PCL interactions at the molecular level, we prepared homogeneous blends composed of the two macromolecules. The chosen strategy was to mix the two components together in solution before co-precipitating. Using this approach it was possible to control the relative amounts of the two macromolecular components. Having maximized the interface between the two components by dissolution in the same solvent, it was possible to monitor their molecular interactions by Fourier Transform Infrared (FTIR) spectroscopy. This research could provide the evidence that indicates a

certain strong interaction between SF and PCL, which greatly affects SF's thermal transition and decomposition behaviors.

MATERIALS AND METHODS

Materials: Silk Fibroin (SF) was prepared by a chemical degummed method, dissolved and dialysis before lyophilized, respectively. Briefly, cocoons from *Bombyx mori* were degummed by boiling twice in 0.5% (w/v) Na₂CO₃ solution at 98-100 °C for 30 min to remove sericin, then rinsed with distilled water and dried at room temperature. Degummed SF fibers were dissolved in the ternary solvent, CaCl₂-ethanol-water (mole ratio = 1:2:8), by stirring at 80°C for 2 h. The resulting SF solution was then dialyzed (cellulose tube) for 3 days against distilled water. Finally, the dialyzed SF solution was lyophilized to obtain SF powder and kept in desiccator until used.

PCL was synthesized by ring-opening polymerization of ε-caprolactone (CL) monomer (Acros, Germany) at 140°C for 48 h. The 1-dodecanol (Fluka, Germany) and stannous octoate (95%, Sigma, USA) were used as initiating system. Mole ratio of CL: dodecanol was 125:1. The stannous octoate concentration of 0.02 mol% was chosen. As-polymerized PCL was purified by precipitation in n-hexane from chloroform solution before dried until constant weight in vacuum oven at room temperature. Number-average molecular weight and polydispersity of PCL were determined from gel permeation chromatography as 15.9 and 1.8 kDa, respectively. All solvents and non-solvents, the analytical grade were used.

Methods

Preparation of SF/PCL blends: The PCL/SF blend was prepared by solution blending method. Dimethylsulfoxides (DMSO) and isopropanol were used as solvent and non-solvent, respectively. Firstly, the PCL and SF solutions in DMSO were prepared as 2 and 0.5% (w/v), respectively. Then, PCL solution was mixed with SF solution with different volume ratios to obtain SF/PCL blend ratios of 1/0, 2/1, 1/1, 1/2 and 0/1 (w/w). The solution mixture was co-precipitated in isopropanol after being stirred for 10 h at room temperature. The precipitating blends were filtered and then dried in vacuum oven at room temperature for a week before characterization. The final blend ratios of the blends were measured by dissolving it in acetone before centrifugation. Both PCL solution and SF precipitant were dried in vacuum oven at room temperature for a week before then, the SF and PCL weights were determined. The feed and final SF/PCL blend ratios were compared as shown in Table 1. It was found that they are very closely values.

Table 1: Blend ratios and FTIR results of SF/PCL blends

Feed SF/PCL blend ratio (w/w)	Final SF/PCL blend ratio (w/w)	Amide I band (cm ⁻¹)	Amide II band (cm ⁻¹)	Carbonyl band (cm ⁻¹)
1/0	1/0	1626	1522	-
2/1	1.8/1.2	1651, 1626	1539	1729
1/1	0.9/1.1	1653, 1627	1559	1728
1/2	0.8/2.2	1661, 1627	1563	1725
0/1	0/1	-	-	1723

Characterization of SF/PCL blends: FTIR spectra were collected by FTIR spectroscopy using a Perkin-Elmer Spectrum GX FTIR spectrophotometer with air as the reference. The resolution of 4 cm⁻¹ and 32 scans were chosen. Thermal transition and decomposition behaviors of the polymers were characterized by non-isothermal Differential Scanning Calorimetry (DSC) and Thermo Gravimetry (TG), respectively. In this study, a Perkin-Elmer DSC Pyris Diamond differential scanning calorimeter and TA-Instrument TG SDT Q600 thermogravimetric analyzer were used. For DSC analysis, 5-10 mg sample was placed in a sealed aluminum pan and heated at the rate of 10°C min⁻¹ under helium flow. For TG analysis, 10-20 mg sample was heated from 50 to 1,000°C at the rate of 20°C min⁻¹ under nitrogen flow. For morphology study, the blends were fractured in liquid nitrogen and the morphology of their fracture surfaces was observed by Scanning Electron Microscopy (SEM) using JEOL JSM-6460LV SEM after gold coating.

RESULTS AND DISCUSSION

FT-IR spectra: SF conformations were often investigated using FTIR spectroscopy since the FTIR spectrum represents typical absorption bands sensitive to the molecular conformation and interaction of SF. The FTIR spectra of SF, PCL and their blends are shown in Fig. 1. The FTIR spectrum of SF showed strong absorption bands of amide I and amide II at 1626 and 1522 cm⁻¹, respectively as shown in Fig. 1a, attributed to β-sheet conformation (Chen *et al.*, 1997; Park *et al.*, 1999; Lee and Ha, 1999; Yang *et al.*, 2002) due to isopropanol treatment. On the other hand, the FTIR spectrum of PCL showed strong absorption band of carbonyl group at 1723 cm⁻¹ as shown in Fig. 1e, attributed to the carbonyl (C=O) stretching. The FTIR spectra of blends with SF/PCL blend ratios of 2/1, 1/1 and 1/2 (w/w) in Fig. 1b-d showed the characteristic bands of both SF and PCL. The positions of absorption bands of amide I, amide II and carbonyl of the blends are shown in Table 1. As expect, intensity of the carbonyl band was increased with increasing the PCL ratio. The amide I band (at 1626 cm⁻¹) of the 2/1 (w/w) SF/PCL blend in Fig. 1b is split into 2 quit distinct bands adjacent to another one (at 1626 and 1651 cm⁻¹) indicated that the SF with random coil and β-sheet forms were coexisted after the blending (Chen *et al.*, 1997; Park *et al.*,

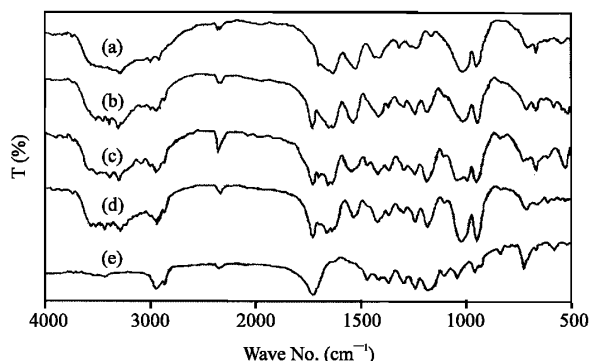


Fig. 1: FTIR spectra of SF/PCL blends; (a) 1/0, (b) 2/1, (c) 1/1, (d) 1/2 and (e) 0/1 (w/w)

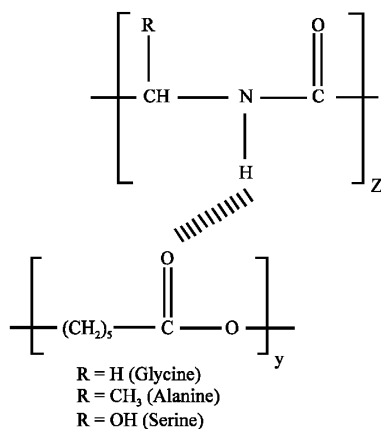


Fig. 2: Intermolecular hydrogen bond between SF and PCL in the blends

1999). This suggests that the intermolecular bonds between SF and PCL were appeared. As PCL ratio increases from 2/1 to 1/2 (w/w) SF/PCL, the amide I band of SF obviously shifts and its wave number increases from 1651 to 1661 cm^{-1} , indicating the interactions between SF and PCL were increased. The amide II bands of SF and carbonyl bands of PCL in the blends were also shifted. The results may be proposed that interaction hydrogen bonds exist between amino and carbonyl groups of the SF and PCL, respectively, as shown in Fig. 2.

Thermal transition: Figure 3 showed DSC curves of SF, PCL and their blends. The DSC results are shown in Table 2. PCL is observed as a semi-crystalline polymer with a melting temperature (T_m) and melting enthalpy or heat of melting (ΔH_m) of 62°C and 106.8 $J g^{-1}$, respectively. The glass transition temperature of the PCL is not observed. This due to the PCL is a highly crystalline polymer that renders detection difficult. The

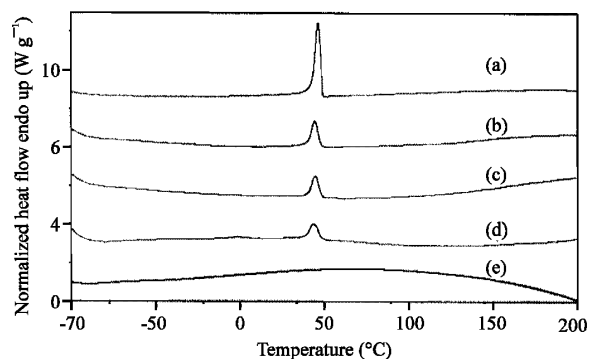


Fig. 3: DSC curves of SF/PCL blends; (a) 0/1, (b) 1/2, (c) 1/1, (d) 2/1 and (e) 1/0 (w/w)

Table 2: Thermal transitions and decomposition behaviors of SF/PCL blends

SF/PCL blend ratio (w/w)	T_m^a (°C)	ΔH_m^a ($J g^{-1}$)	T_{dmax}^b (°C)
1/0	-	-	264
2/1	59	31.9	268, 299
1/1	60	34.1	300
1/2	61	38.9	300
0/1	62	106.7	420

^aObtained from DSC curves, ^bObtained from DTG curves

DSC curve of SF does not exhibit the thermal transition properties in the temperature range of -70 to 200°C. The DSC curves of the blends in Fig. 3b-c showed the T_m and ΔH_m of PCL. However, the T_m and ΔH_m of the blends were lower than the original PCL and decreased as increasing the SF ratio. The ΔH_m of the polymer is closely related to its crystallinity, suggested that the PCL crystallinity was decreased when blending with SF. The results indicated that the blending SF has great influence on the crystallization of crystallisable PCL fractions. The SF molecules can interpenetrated between PCL molecules and interacted with them, then crystallization of PCL was obstructed. This result confirmed that the SF and the PCL have interacted between them by forming hydrogen bonds, especially in the amorphous phase.

Thermal decomposition: Thermo Gravimetric (TG) curves of SF, PCL and their blends are shown in Fig. 4. The TG curve of PCL shows a complete thermal decomposed at about 500°C. The SF did not completely decompose even at 1,000°C. The SF has the main thermal decomposed in the range of 200-300°C. The blends show two stages of thermal decompositions corresponding to each component. However, the temperatures of initial weight loss of each component in the blends show higher than each pure component.

The thermal decomposition behavior can be clearly examined in detail from their differential thermogravimetric (DTG) curves, as shown in Fig. 5. From DTG curves, temperature of maximum decomposition rate ($T_{d,max}$) was

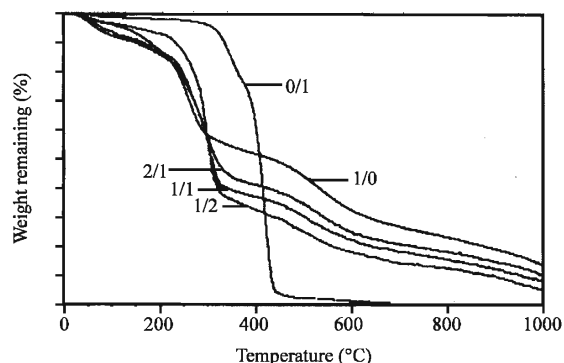


Fig. 4: TG curves of blends with different SF/PCL ratios (w/w)

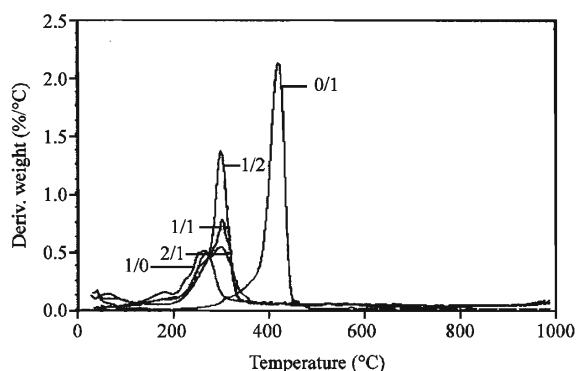


Fig. 5: DTG curves of blends with different SF/PCL ratios (w/w)

determined and also shown in Table 2. The SF and PCL show $T_{d,max}$ at 264 and 420°C, respectively. The 2/1 (w/w) SF/PCL blend shows two $T_{d,max}$ values of each component suggested that the thermal decomposition of this blend taken place through two steps of weight loss with according to the characteristic of each component. The two $T_{d,max}$ of the 2/1 (w/w) SF/PCL blend at 268 and 299°C (Table 2) are expected to decomposition of SF and PCL components, respectively. However, each $T_{d,max}$ of the 2/1 (w/w) SF/PCL blend is shifted from its original components. Shifting of the $T_{d,max}$ supported the hydrogen bonds between SF and PCL. When the PCL blend ratios increased up to the 1/1 and 1/2 (w/w) SF/PCL, the $T_{d,max}$ of each component were combined together. The TG and DTG results indicated that SF thermal stability can be improved when blending with PCL. In addition, the DSC and TG results indicated that the SF and the PCL can be miscible blended.

Morphology study: Blend morphology was observed from SEM images, as shown in Fig. 6. The SF morphology in Fig. 6a shows particle aggregates. The PCL morphology in Fig. 6d shows plate forms with smooth surface. In SF/PCL blends with ratios of 2/1, 1/1 and 1/2 (w/w), the fracture morphology are homogeneous surfaces, as shown in Fig. 6b-d. The results supported that the homogeneous SF/PCL blends can be prepared. Given the chemical structure of SF and PCL, hydrogen

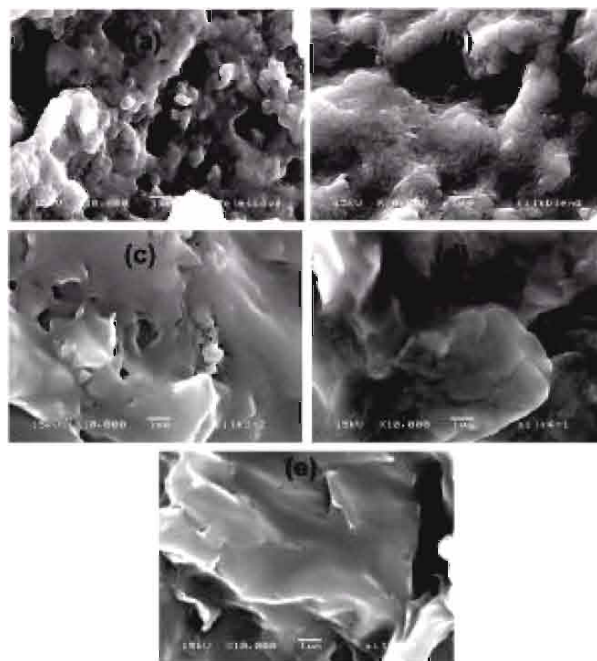


Fig. 6: SEM images of fracture surfaces of SF/PCL blends; (a) 1/0, (b) 2/1, (c) 1/1, (d) 1/2 and (e) 0/1 (w/w), (bar = 1 μ m)

bonds between different molecules were supposed, as shown in Fig. 2, to account for a good miscibility in the blends.

CONCLUSION

The SF/PCL homogeneous blends with different blend ratios were prepared by solution blending and co-precipitating methods using DMSO and isopropanol as a solvent and a non-solvent, respectively. FTIR, DSC and TG results of the blends suggest hydrogen interaction bonds between the SF and PCL were formed, which lead to the miscibility of these components. As proposed that the hydrogen bonds exist between the SF amino groups and the PCL carbonyl groups in the amorphous phase. The conformation transition of SF from β -sheet to random coil forms was induced by blending with PCL. The existence of intermolecular hydrogen bonds between SF and PCL shows largely affect on thermal transitions and decompositions of the blends. These homogeneous blends are promising for use as biodegradable polymers to prepare PCL products containing well dispersed SF fraction by various processes.

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