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Structural and thermal characteristics of *Antheraea pernyi* silk fibroin/chitosan blend film

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Abstract

Antheraea pernyi silk fibroin (SF)/chitosan blend films were prepared by mixing aqueous solution of *A. pernyi* SF and acetic acid solution of chitosan. The conformation of *A. pernyi* SF in blend films was revealed to be a β-sheet structure, mainly due to the effect of acetic acid used as a mixing solvent. According to the Fourier transform infrared (FTIR) spectra, NH groups of SF and C=O and NH_2 groups of chitosan might have participated in a specific intermolecular interaction among themselves. The exotherm of SF was not exhibited in blend films due to the precrystallization of SF induced by acetic acid. The blend films showed two distinct maximum decomposition temperatures at around 294 (chitosan component) and 369°C (*A. pernyi* component), which could be indirect evidences of a phase separation. Scanning electron microscopy (SEM) results confirmed that the phase separation occurred in *A. pernyi* SF/chitosan blend film. Blending with *A. pernyi* SF can enhance the thermal decomposition stability of chitosan. © 2001 Published by Elsevier Science Ltd.

Keywords: *Antheraea pernyi* silk fibroin/chitosan blend film; Conformation

1. Introduction

Recently, natural polymers have been studied as biotechnological and biomedical resources due to their unique properties including nontoxicity, biodegradability, and biological compatibility. However, natural homopolymer by itself is inadequate to meet the diversity of demands for biomaterials. In order to improve the performance of the individual natural polymer, many blend films such as domestic silk fibroin (SF)/chitosan [1,2], poly(vinyl alcohol)/SF [3], chitosan/cellulose [4], Poly(vinyl alcohol)/chitosan [5,6], among others, have been prepared using a solution blend method.

SF, one of the typical natural protein polymers, is classified into domestic (*Bombyx mori*) and wild type (*Antheraea pernyi*, etc.). SF spun by a wild silkworm *A. pernyi* is considered as a potential biotechnological and biomedical material. A few types of amino acid residues have been characterized for the chemical composition of *A. pernyi* SF possessing small side chain such as alanine, glycine, serine, and several basic amino acids. In particular, *A. pernyi* SF contains tripeptide sequence of arg-gly-asp, known as a cell adhesive site for mammalian cell culture. Therefore, it has been investigated as a potential biomaterials such as a matrix for the enzyme immobilization [7] and mammalian fibroblast cell culture [8].

Chitosan, a deacetylated product of chitin, has been widely used in the field of biomedical applications [9,10], particularly as a biomaterial for wound dressing and artificial skin due to its unique properties such as acceleration of wound healing and attainment of a smooth healing skin surface [11].

This research was, thus, aimed at determining the structural characteristics and thermal properties of blend films composed of *A. pernyi* SF and chitosan. Mixtures of aqueous *A. pernyi* SF solution and 2% acetic acid chitosan solution in various blend ratios were prepared for the blend films. The structural characteristics, thermal properties, and morphology of the blend films were examined through Fourier transform infrared (FTIR) spectroscopy, X-ray diffractometry (XRD), thermogravimetry, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM).

2. Experimental

2.1. Materials

A. pernyi silk fibers were degummed using an enzymatic degumming method and dissolved in a calcium

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nitrate solution. They were first treated with degumming solution (Alcalase 2.5 l from Novo Industri Company 1 g/l, sodium bicarbonate 5 g/l, and nonionic surfactant 1 g/l) at 55°C for 60 min. The degummed fibers were washed in a mixture solution of nonionic surfactant (2 g/l) and sodium hydrosulfite (5% on the weight of fiber) and thoroughly rinsed in warm distilled water. They were then dried at room temperature and stored in a desiccator prior to dissolution.

The dissolution of degummed fibers were carried out in the melt of calcium nitrate·4 hydrate for 5 h at 105°C. The solution was then dialyzed in a cellulose tube (molecular cutoff: 3500) against distilled water for 4 days at room temperature. The regenerated *A. pernyi* SF solution was freeze-dried and stored in a refrigerator prior to use.

Chitosan, with degree of deacetylation calculated to be 75%, was purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and used without further purification.

2.2. Preparation of blend film

The casting solution of blend film was prepared by blending the aqueous SF and acetic acid chitosan solutions. *A. pernyi* SF (0.06–0.30 g) was dissolved in 50 ml of deionized water and chitosan (0.06–0.30 g) was dissolved in 40 ml of 2% acetic acid solution. The two solutions were then mixed for 2 h with stirring and poured onto a polystyrene plate. The solvent was subsequently evaporated at room temperature for 2 days. In order to neutralize acetic acid, the blend film was immersed in 1 N NaOH for 1 day and then washed repeatedly with distilled water to remove NaOH thoroughly.

2.3. Measurements

FTIR spectra were obtained using a Madic M series FTIR spectrometer (USA) in the spectral region of 1400–700 cm^{-1}. X-ray diffraction patterns were obtained using a D-MAX-3 Diffractometer (Rigaku Company, Japan), and CuKα radiation with a wavelength of 1.54 Å was used. The scanning speed was 0.5°/min, and the measurement range was $2\Theta = 5–35°$ under 30 kV and 20 mA.

DSC measurements were performed with a Thermal Analysis Instrument (TA 2910, USA) at a heating rate of 10°C/min and a nitrogen gas flow rate of 50 ml/min. Thermogravimetric analysis (TGA) was run under the flow of nitrogen gas at a scanning speed of 20°C/min using Rheometric Scientific TGA 1000 (USA).

Surface morphology was observed through a scanning electron microscope (JSM 5410LV, JEOL, Japan) after gold coating. The fractured cross surfaces of blend films were achieved by cooling in liquid nitrogen.

3. Results and discussion

3.1. Fourier transform infrared (FTIR) spectra

Many researchers often investigate the conformation of SF and its blend using IR spectroscopy since the IR spectrum represents typical absorption bands sensitive to the molecular conformation of SF. The IR spectra of *A. pernyi* SF/chitosan blend films are shown in Fig. 1. *A. pernyi* SF (Fig. 1(a)) showed strong absorption bands at 1630 (amide I), 1520 (amide II), 1220 (amide III), 965 (amide IV), and 700 cm^{-1} (amide V), attributed to the β-sheet conformation, and 620 cm^{-1} (amide V), attributed to the α-helix conformation [12,13]. On the other hand, the chitosan film (Fig. 1(e)) showed absorption bands at 1647 and 1589 cm^{-1}, attributed to the C=O and $-NH_2$ stretching, respectively. The absorption band at 1151 cm^{-1} was assigned to the anti-symmetric stretching of C–O–C bridge, and 1074 and 1032 cm^{-1} were assigned to the skeletal vibrations involving the C–O stretching [14].

The IR spectra of blend films revealed that the amide II band of SF at 1520 cm^{-1} shifted to around 1530 cm^{-1} and

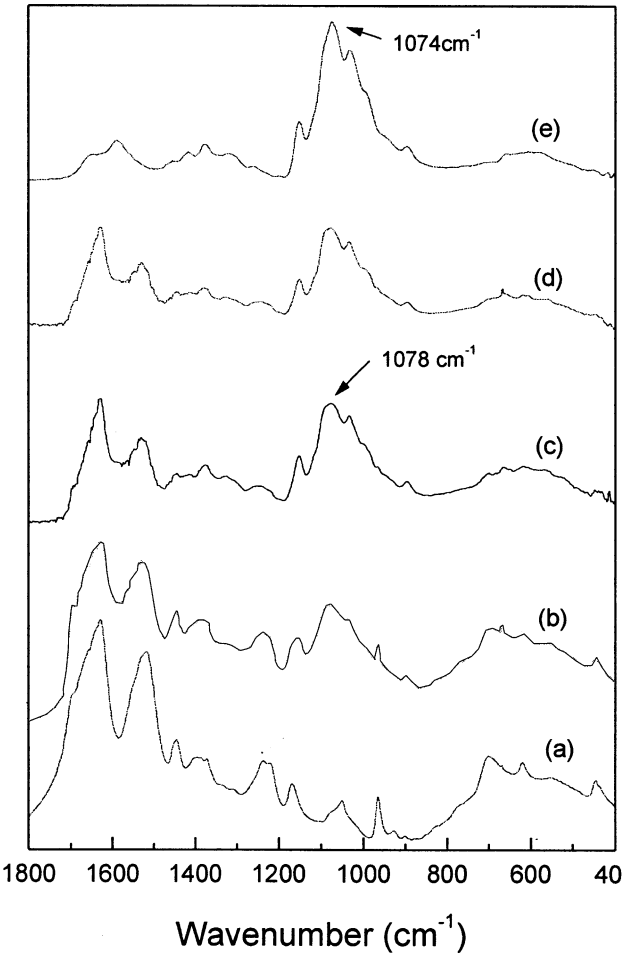


Fig. 1. IR spectra of *A. pernyi* SF/chitosan blend films; (a) 10:0, (b) 8:2, (c) 6:4, (d) 4:6, and (e) 0:10.

the amide III bands of SF to somewhat higher wave number with decreasing intensity. In the case of absorption bands of chitosan in the blend, the C=O and NH_2 stretching bands disappeared, and the skeletal vibration of C–O–C stretching around 1000–1160 cm^{-1} shifted to slightly higher wave number due to the environmental difference formed through blending process.

The native and regenerated *A. pernyi* SF usually show the characteristic absorption bands at 1660 (amide I), 1550 (amide II), 1270 (amide III), 896 (amide IV), and 620 cm^{-1} (amide V), representing α-helix conformation, and 660 cm^{-1} (amide V), attributed to the random coil conformation. When *A. pernyi* SF was treated with heat and solvent such as aqueous methanol or ethanol, a distinct β-sheet structure could be obtained [13,15–18]. The characteristic absorption bands of *A. pernyi* SF cast from aqueous solution containing acetic acid were consistent with those of SF possessing a β-sheet structure induced by alcohol (Fig. 1(a)). On the other hand, the characteristic bands attributed to the β-sheet structure were not enhanced by increasing chitosan content in blend film. Nevertheless, some characteristic bands of chitosan and SF disappeared or shifted. Though the reason is not yet clear, the above results demonstrate that the NH groups of SF and C=O and NH_2 groups of chitosan might have participated in a specific intermolecular interaction between SF and chitosan.

3.2. X-ray diffraction curve

X-ray diffraction curves of *A. pernyi* SF, *A. pernyi* SF/chitosan blend, and chitosan film are shown in Fig. 2. The *A. pernyi* SF cast from acetic acid solution showed a major diffraction peak at about 20.5° and two minor peaks at about 16.5 and 23.5°, respectively, corresponding to the 4.33, 5.37, and 3.78 Å spacing, which are characteristics of the β-sheet crystalline structure [13,17]. On the other hand, chitosan exhibited two diffraction peaks at 11 and 20°, which are characteristics of the hydrated crystalline structure of chitosan [19,20]. With an increase in the chitosan content of the blends, the intensity of diffraction peak at 11° of chitosan became gradually higher and sharper, while the diffraction angle at 20.5° of *A. pernyi* SF shifted to a lower angle and overlapped with the main peak of chitosan at 20°. The diffraction patterns of *A. pernyi* SF/chitosan blend films showed the curves of both components to overlap each other.

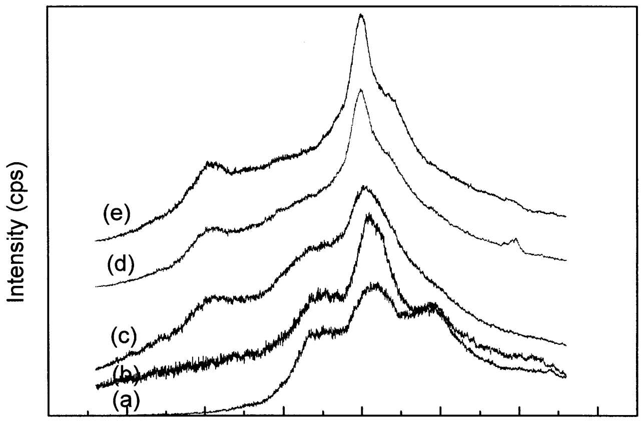


Fig. 2. X-ray diffraction curves of *A. pernyi* SF/chitosan blend films; (a) 10:0, (b) 8:2, (c) 6:4, (d) 2:8, and (e) 0:10.

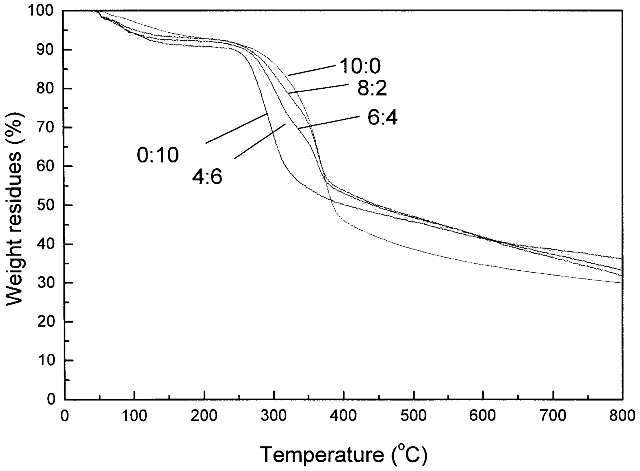


Fig. 3. Thermogravimetric curves of regenerated *A. pernyi* SF, chitosan, and *A. pernyi* SF/chitosan blend films.

3.3. Thermogravimetry

Thermogravimetric curves of regenerated *A. pernyi* SF, *A. pernyi* SF/chitosan blend, and chitosan film are shown in Fig. 3. The initial weight loss at approximately 100°C was due to the evaporation of water. As *A. pernyi* SF content increased, the initial thermal decomposition temperature of the blend film shifted to a higher temperature. In order to examine the thermal behavior of decomposition in detail, differential thermogravimetric (DTG) curves of the blend films were obtained (Fig. 4).

The maximum decomposition temperature of chitosan

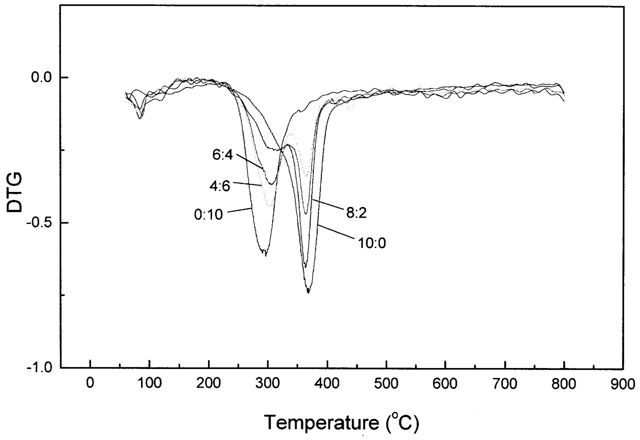


Fig. 4. DTG curves of *A. pernyi* SF/chitosan blend films.

appeared at ca. 294°C, ascribed to a complex process including dehydration of the saccharide rings, and depolymerization and decomposition of the acetylated and deacetylated units of polymer [21,22]. On the other hand, *A. pernyi* SF showed a strong thermal decomposition at 369°C, attributed to the disintegration of intermolecular interaction and the partial breakage of the molecular structure. As expected, the blend films showed two maximum peaks for the decomposition of each component, *A. pernyi* SF and chitosan, indications that the thermal decomposition of this blend takes place through two steps of weight loss with according to the characteristic of each component.

It was also observed that the maximum temperature of decomposition shifted and the intensity of maximum peak varied, depending on the composition of the blend film. In particular, the decomposition temperature of chitosan component in the blend film increased from ca. 294 to 308°C with increasing of *A. pernyi* SF content, indicating the formation of intermolecular interactions between the two components.

3.4. Differential scanning calorimetry

Fig. 5 shows the DSC thermograms of the blend film. The endothermic peak at around 100°C, corresponding to the dehydration of the films, appeared in all blend samples. A strong exothermic peak around 305°C of chitosan could be ascribed to a complex process as revealed through TGA analysis. On the other hand, *A. pernyi* SF cast from acetic acid solution showed a strong endotherm at around 350°C and two minor peaks at around 220 and 280°C, attributed to the decomposition of SF [23]. The DSC thermograms of the blends showed a mixed form of the two components.

It has been reported that a typical DSC thermogram of *A. pernyi* SF cast from aqueous solution exhibits characteristic endothermic and exothermic peaks at 228 and 232°C, respectively [12,23]. The exotherm can be explained through the crystallization of SF, which changed from random coil to β-sheet conformation. *A. pernyi* SF cast from the acetic acid solution in this study showed a different thermal calorimetric curve. The exotherm at around 230°C was not observed, which is due to the precrystallization of *A. pernyi* SF induced by the acetic acid (Fig. 5(a)). When 2% acetic acid solution was added into the aqueous *A. pernyi* SF solution, acetic acid diffused into the SF molecules. Considering the SF is composed of polar amino acids as well as non-polar amino acid residues, assembling of acetic acid and polar amino acid residues induced a hydrophobic interaction between the non-polar amino acid residues. These hydrophobic interactions may have facilitated the aggregation of non-polar amino acid residues, resulting in the formation of β-sheet structure.

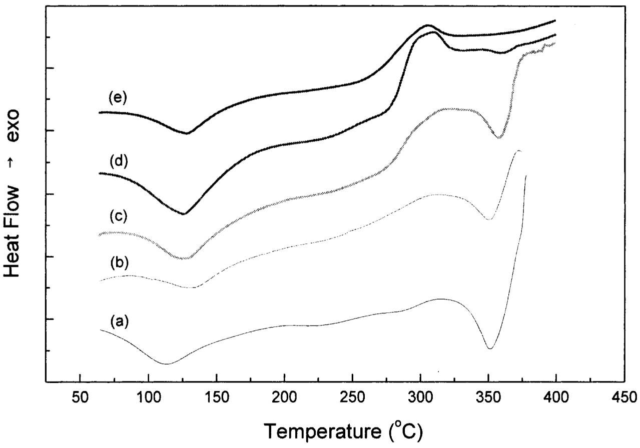


Fig. 5. DSC thermograms of *A. pernyi* SF/chitosan blend films; (a) 10:0, (b) 8:2, (c) 4:6, (d) 2:8, and (d) 0:10.

3.5. Scanning electron microscopy (SEM)

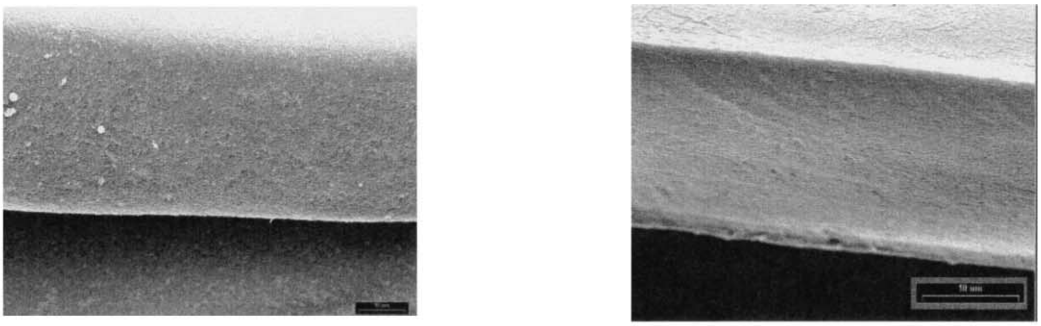
Cryogenically fractured cross surface of *A. pernyi* SF/chitosan blend films are shown in Fig. 6. The pure SF film (Fig. 6(a)) exhibited a dense and uniform microstructure. The blend film containing 20% chitosan also showed a uniform microstructure. On the other hand, the blend film containing 40% chitosan shown as rounder particles immersed in a homogeneous matrix, indicating that the two components of *A. pernyi* SF and chitosan existed in a separated phase.

In our previous report for the morphology of *B. mori* SF/chitosan blend films, a distinct phase separation was not observed with variances in the composition [24]. However, in the case of *A. pernyi* SF/chitosan blend film, phase separation occurred by varying the blending ratio. In an aqueous solution state, the conformation of *A. pernyi* SF was composed of an ordered α-helix and a random coil structure, while *B. mori* SF of random coil structure. Moreover, sequential alanine residues composed of crystalline regions of *A. pernyi* SF could be pact more closely among themselves than alanine–glycine sequence of *B. mori* SF due to their simplicity and homogeneity.

3.6. Discussion

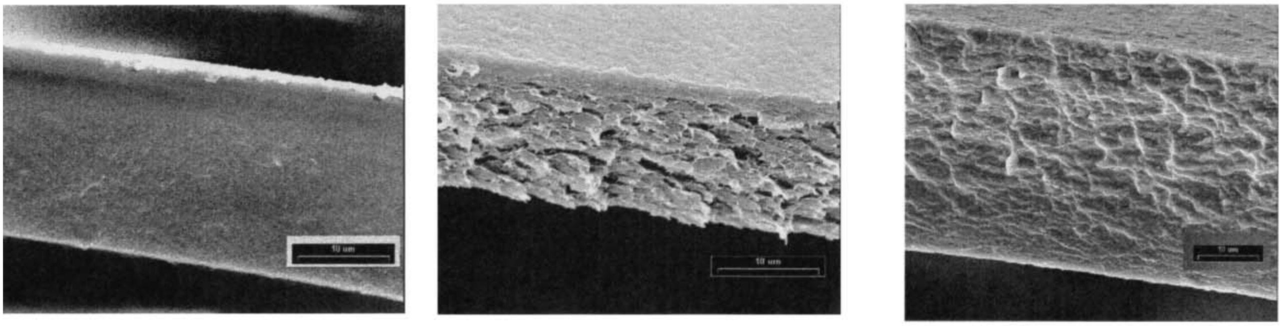
Various SF blend films were prepared through the solution blending technique, and the structural characteristics of SF in the blends have been studied, particularly interactions between SF and the counterpart polymers in the blends. Until now, these structural characteristics have been examined in the viewpoint of the effect of counterpart polymer in the blend. However, the effect of solvent used for dissolving other polymers should be considered on the conformational change of the blend films as well as the polymer component itself because the conformation of SF could be affected by various organic solvents such as aqueous alcohol [13,15–18].

The effect of acetic acid as a solvent for chitosan was examined in the structural changes of *A. pernyi* SF through FTIR spectroscopy (Fig. 1(a)), XRD (Fig. 2(a)), and DSC (Fig. 4(a)). Interestingly, FTIR showed the characteristic absorption bands at 1630, 1520, 1220, 965, and 700 cm^{-1},



A. Pernyi SF film

Chitosan film



A. Pernyi SF:Chitosan=8:2

A. Pernyi SF:Chitosan=6:4

A. Pernyi SF:Chitosan=2:8

Fig. 6. Cryogenically fractured surface of *A. pernyi* SF/chitosan blend films.

attributed to the β-sheet structures of *A. pernyi* SF. XRD also confirmed the results of FTIR. DSC thermograms showed no exotherm, known as the thermal crystallization during heating, at around 230°C. The above results indicate the β-sheet structure as the conformation of *A. pernyi* SF films treated with acetic acid.

On the other hand, the β-sheet structure of *A. pernyi* SF was not enhanced by increasing the chitosan content in the blend films. Therefore, it was concluded that the solvent used for dissolving chitosan rather than chitosan itself mainly affected the conformation of SF in the blend.

The structural characteristics of *B. mori* SF blend have been also investigated in the viewpoint of the relationship between SF and the counterpart polymers. In the case of *B. mori* SF/chitosan blend film formed by mixing aqueous SF and 2% acetic acid chitosan solutions, the β-sheet structure of *B. mori* SF could be enhanced by increasing the chitosan content due to the interaction between SF and chitosan [25,26]. Considering that acetic acid is known as one of the crystallization-inducing organic solvent [27], the conformational changes of *B. mori* SF as well as *A. pernyi* SF blend films may be induced first by acetic acid used as a mixing solvent rather than by chitosan.

However, there are some possibilities that interactions between SF and chitosan exist. According to the results of FTIR (Fig. 1(b)–(d)), TGA (Figs. 3 and 4) and DSC (Fig. 5), some specific interactions between *A. pernyi* SF and chitosan, involving NH groups of SF and C=O and NH_2 groups of chitosan, might have occurred.

4. Conclusions

Structural and thermal characteristics of transparent *A. pernyi* SF/chitosan blend films were investigated through FTIR, XRD, DTG, DSC and SEM. FTIR and XRD results showed the β-sheet structure to be the conformation of *A. pernyi* SF cast from aqueous solution containing acetic acid as one of the blend dope solution. This result was also confirmed through DSC, in which no exotherm was shown in the thermogram. The β-sheet conformation of SF in the blend is mainly due to the acetic acid used for dissolving chitosan rather than the interaction with the chitosan component. However, FTIR spectra showed that the characteristic absorption bands shifted or disappeared and thermal decomposition temperature of chitosan component in blend film increased with an increase of *A. pernyi* SF content, indication of some intermolecular interactions between SF and chitosan. Nevertheless, cryogenically fractured surface of blend films shown as rounder particles immersed in a homogeneous matrix in the case of blend film containing over 40% chitosan, indication of phase separation between two polymers.

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