

Current
CHEMICAL RESEARCH

Received: 10/08/2010
Accepted: 22/12/2010

The effect of methanol treatment on the thermal properties and secondary structure of Eri *Samia ricini* silk fibroin/chitosan blend films

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Abstract

This study was aimed to prepare Eri silk fibroin (SF)/chitosan (CS) blend films with different blend ratios by a solvent evaporation method in comparison with that of both native SF and CS films. The blend films were used as subject material for study the effect of methanol treatment. The properties of the blend films including morphology, secondary structure and thermal decomposition were investigated by using scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectrometer and thermogravimetric analysis (TA), respectively. The native SF and CS films were appeared homogeneous throughout the film surface and matrix without phase separation. All SF/CS blend films showed both SF and CS characteristics. FTIR results indicated that the SF and blend films were composed of a higher proportion of β -sheet structure after methanol treatment. The results of thermal decomposition obtained from TA instrument suggested that SF and CS were formed of intermolecular bonds, especially H-bonds. Those of films showed that increasing SF contents resulted in an increase in β -sheet structure. In addition, it was shown that the thermal stability of the SF/CS blend films could be affected by blend ratio as well as methanol treatment. The results in this study implied that the SF/CS blend films treatment with methanol would be used for various fields including drug controlled release, cell culture or medical applications.

Keywords

Blend films; Chitosan; Chemical structures; Eri silk fibroin; Thermal stability.

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INTRODUCTION

Natural polymers such as collagen, gelatin, hyaluronic acid, chitosan, and silk fibroin are important renewable biodegradable polymers for several applications. However, high cost and questionable purity have prevented them from being used more extensively^[1]. In addition, the properties of natural homopolymer often do not meet the specifications required of biomaterials. Therefore, blend polymers have been widely studied to improve the performance of the individual natural polymer^[2,3].

A derivative of chitin, chitosan (CS), is the second most abundant polymer in nature. It is extracted from the shells of crustaceans such as shrimp, crabs, lobsters^[4], as well as insects like the silkworm^[5]. Chitosan is a polysaccharide with many useful properties including hydrophilicity, biocompatibility, biodegradability, antibacterial activity and bioadsorbance^[4,6]. Chitosan has been used in biotechnology^[7], biomedicine^[8-10], food ingredients^[11], and also cosmetics^[12]. Chitosan is composed of many NH₂ residues which caused the charges that make it a cationic polymer. It can also be cross-linked by

various reagents^[13] or blended with other natural polymers^[14].

Silk is a fibrous protein produced by some Lepidoptera, especially the silkworm. Different silks vary greatly in compositions and properties and this is influenced by their specific source^[15]. Each silk fiber consists of at least two types of proteins fibroin and sericin. Silk fibroin (SF) is an insoluble fibrous component while sericin is a glue-like protein which has good solubility in hot water and some organic solvents. The SF is a kind of attractive materials and has been applied in various fields including cosmetics, medical materials, and food additives^[16]. Generally, silk is divided into two categories: mulberry and non-mulberry silk (or silk from wild silkworms)^[17]. The majority of studies have been on mulberry (*Bombyx mori*) rather than wild silk, especially Eri (*Samia ricini*) silk.

In this study, Eri SF and CS blend films were prepared by film casting of Eri SF/CS blend solution. Influences of blend ratio and post alcohol treatment on film morphology, secondary structure and thermal properties were investigated and discussed.

EXPERIMENTAL

Materials

Chitosan (CS) with 90% deacetylation and molecular weight of 80 kDa was purchased from Seafresh Chitosan Lab Co., Ltd., (Thailand). The 1% (w/v) chitosan solution was prepared by dissolving the chitosan powder with 2% (w/v) acetic acid aqueous solution. The Eri (*S. ricini*) silk cocoons were kindly supplied by the Silk Innovation Center (SIC), Mahasarakham University, Thailand. The cocoons were stripped and degummed twice using 0.5% (w/v) Na₂CO₃ solution then thoroughly rinsed twice in warm distilled water. They were then dried at room temperature before dissolving. The Eri silk fibroin (SF) was dissolved with 9 M Ca(NO₃)₂ solution with SF/Ca(NO₃)₂ solution ratio of 1/10 (w/v) at 95-100 °C^[18]. The SF hydrolysate was filtered, and then dialyzed in cellulose tube (molecular weight cut off 7500 Da) against distilled water for 3 days at room temperature. The SF solution obtained after dialysis was approximately 0.5% (w/v).

Preparation of SF/CS blend films

The SF/CS blend films were prepared by solution blending of SF and CS solutions before casting on polystyrene plates with 5 cm in diameter. The SF/CS

blends ratios of 8/2, 6/4 and 4/6 (w/w) were investigated. Both native SF and CS films were prepared as control by the same method. The blend solutions were dried at 40 °C for 3 days to obtain blend films with approximately 10-15 μm in thickness. All of the films were treated with 80% (v/v) methanol solution for 90 min until use.

Morphological observation

Morphology of the films was determined by scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The films were sputter coated with gold for enhancing surface conductivity before scan. A voltage of 15 kV was used.

FTIR analysis

The chemical structure of the films was analyzed using a Fourier transform infrared (FTIR) spectrometer (Perkin Elmer-Spectrum GX, USA). Analysis was performed in the spectral region of ~4000-400 cm⁻¹ at resolution of 4 cm⁻¹ and 32 scans.

Thermal properties investigation

The thermogravimetric analysis (TGA) was performed using TA instruments, SDT Q600 (Luken's drive, New Castle, DE). Each film (~10 mg) was loaded in a platinum crucible. The samples were non-isothermal heated from 50 °C to 1000 °C at a heating rate of 20 °C/min. The TGA was carried out in nitrogen with a flow rate of 100 ml/min. The TG and heat flow data were recorded with TA instrument's Q series explorer software. The analyses of the data were performed using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS AND DISCUSSION

Morphology

The properties of SF/CS blend films are known to be influenced by several factors including composition, interfacial tension, processing condition and preparation techniques^[19]. Figure 1 shows SEM micrographs of the blend film surfaces of Eri SF, CS and SF/CS blends. Both native films (Figures 1a and 1e) have a uniform microstructure. The Eri SF/CS blend films (Figures 1b, 1c and 1d) also exhibited a generally homogeneous microstructure. However, phase separation of their texture was also observed. The higher phase separations increased with increases in SF and CS contents. It might be suggested that the intermolecular interactions between SF and CS molecules were incompletely formed. One possible reason for this is that

the ratio of SF and CS did not balance to form bonds together. The smoothness of the films surfaces increased following exposure to methanol. It is well known that alcohol reacts more readily with water than SF, and this is likely to have caused dehydration of the SF films. It would be expected that the hydrogen bonding of SF molecules was stronger due to closer molecules after methanol treatment. The resulting films were also more brittle and fragile though^[2]. The interactions of SF molecules could be affecting on the secondary structures of the components.

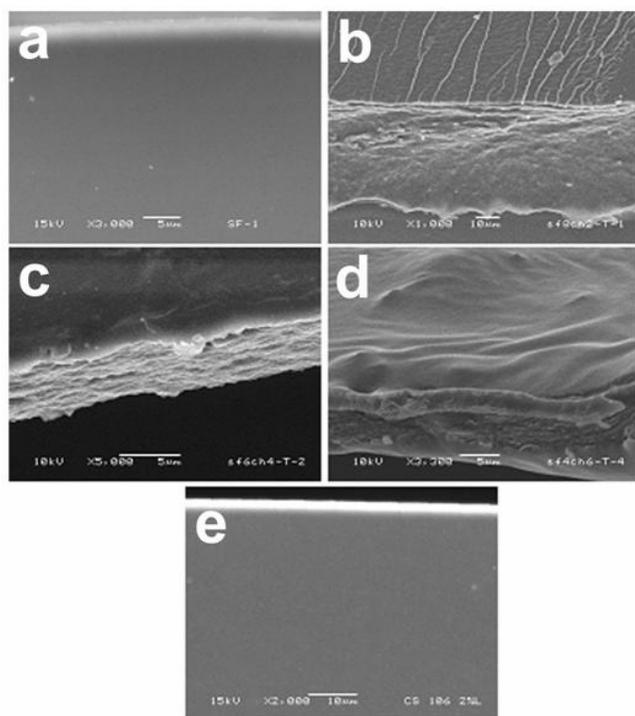


Figure 1 : SEM micrographs of methanol-treated blend films with SF/CS ratios of (a) 10:0, (b) 8:2, (c) 6:4, (d) 4:6 and (e) 0:10 (w/w).

Secondary structure

The amide functional groups of SF protein in FTIR spectra have previously been used to investigate SF conformations; amide I ($1700\text{--}1600\text{ cm}^{-1}$), amide II ($1600\text{--}1500\text{ cm}^{-1}$), and amide III ($1300\text{--}1200\text{ cm}^{-1}$) bands^[20,22]. The FTIR spectra of the native Eri SF, CS and blend films from the current study are shown in Figure 2, and their positions of amide bands were summarized in TABLE 1. The FTIR spectrum of the native SF film (Figure 2a) showed absorption bands at 1630 cm^{-1} (amide I), 1525 cm^{-1} (amide II), 1230 cm^{-1} (amide III), 945 cm^{-1} (amide IV), and 655 cm^{-1} (amide V), indicating a β -sheet structure^[20,21]. For the CS film (Figure 2e), the FTIR spectrum showed intense absorption at 1675 cm^{-1} (amide groups), 1590 cm^{-1} (free amino groups), and 1100 cm^{-1} (saccharide structure)^[14,23]. Moreover, the bands around 1070 cm^{-1} and 1030 cm^{-1} were assigned to the skeletal vibrations of C-O stretching^[2]. The blend

films (Figures 2b, 2c and 2d) indicated that the amide I bands of SF were shifted from 1630 cm^{-1} to 1625 cm^{-1} after blending and almost all bands of SF shifted to somewhat higher wave number. In the case of CS, the C=O and NH_2 bands slightly shifted to lower wave number. The results suggested that CS component could induce β -sheet structure conformation of the methanol-treated native SF film^[14]. It was expected that the intermolecular interaction between NH groups of SF and carbonyl NH_2 groups of CS were appeared^[2]. It is concluded that methanol-treated SF/CS blend films showed greater proportion of β -sheet SF structure than the methanol-treated native SF film. The finding that Eri SF appears to reinforce the strength of CS film by bonding formation is very promising. It has been reported that crystallization of SF film does not appear when treated with absolute methanol. Therefore, 80% methanol was selected for use as a treatment in the current study. The results revealed that methanol significantly affects on the structural changes in the films since absorption bands decreased after exposure to methanol, an observation attributed to an increase in β -sheet structure^[20,24].

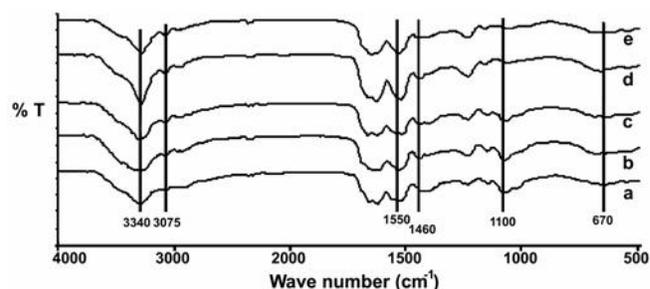


Figure 2 : FTIR spectra of methanol-treated blend films with different SF/CS ratios.

TABLE 1 : Amide absorption bands of methanol-treated SF/CS blend films.

List	Ratio SF/CH	Amide I (cm ⁻¹)	Amide II (cm ⁻¹)	Amide III (cm ⁻¹)	Amide IV (cm ⁻¹)	Amide V (cm ⁻¹)
1	10/0	1630	1525	1230	945	655
2	8/2	1625	1530	1235	1020	670
3	6/4	1625	1530	1240	1030	670
4	4/6	1625	1550	1250	1040	690

Thermal properties analysis

Thermal decomposition of native Eri SF, CS and SF/CS blend films was analyzed from the thermogravimetric (TG) curves (Figure 3). The initial weight loss which occurred below 100°C was due to moisture evaporation^[2]. Neither native SF nor CS films incompletely decomposed even at 1000°C . At high temperature, weight loss of SF was continuously observed; this is associated with breakdown of side chain groups of amino acid residues as well as decomposition of pep-

tide bonds^[25]. The native CS film showed more rapid thermal decomposition than that of native SF and SF/CS blend films. This indicated that CS has lower thermal stability than those of other films. The thermal decomposition of CS became gradually prolonged as the SF content increased. Thermal decomposition is indicated by derivative TG (DTG) curves (Figure 4).

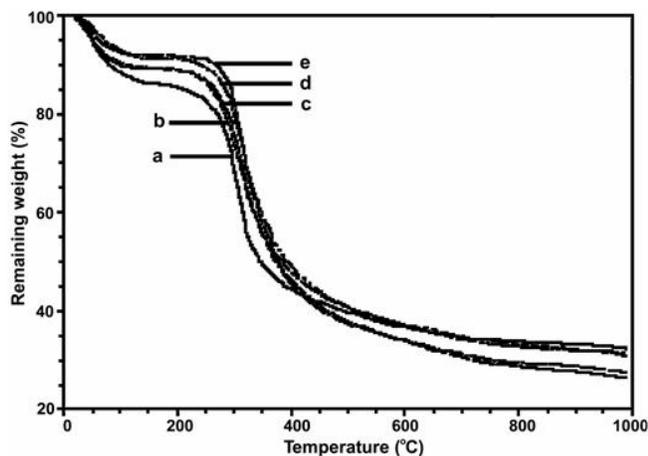


Figure 3 : TG curves of methanol-treated blend films with different SF/CS ratios.

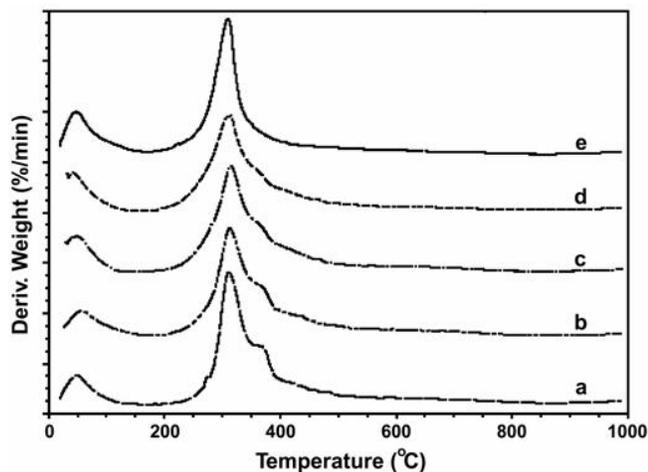


Figure 4 : DTG curves of methanol-treated blend films with different SF/CS ratios.

The temperature of maximum decomposition rate ($T_{d,max}$) of native CS appeared at 325°C, while Eri SF showed a strong thermal decomposition at 325°C with shoulder at 375°C. Peaks are likely to represent decomposition of intermolecular interaction and partial breakage of molecular structure. All of the blend films showed one maximum decomposition temperatures and a shoulder for each component of Eri SF and CS. This indicated that the blend films take two steps of weight loss which represent the characteristics of each component^[2]. Moreover, the $T_{d,max}$ of SF in the blend films gradually decreased in intensity with increasing CS component. Figure 5 shows the heat flow thermograms of the films. The first endothermic peaks oc-

curred at about 100°C: distributions to the dehydration appeared in all samples. All films showed thermal stability until above 200°C. For CS (Figure 5e), one broad exothermic peak around 320°C could be ascribed to a complex process. On the other hand, Eri SF showed a strong endothermic peak at around 308°C and two minor peaks at 235°C and 400°C, attributed to the decomposition of SF^[26]. The endothermic peaks of blend films shifted slightly with increasing CS component. However, the blend films showed mixed characteristics of both components. Higher endothermic peaks were observed with the blend films than native film. This result suggested that CS could reinforce the formation of molecular bonds between each component, resulting in the transition from random coil to β -sheet structure.

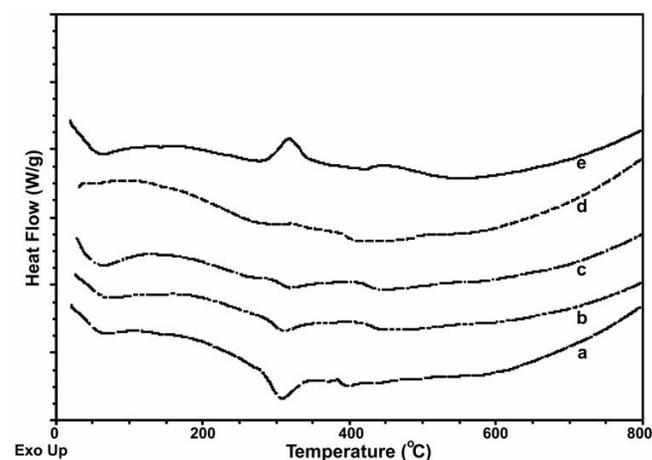


Figure 5 : Heat flow curves of methanol-treated blend films with different SF/CS ratios.

CONCLUSION

The effect of methanol on morphology, secondary structure and thermal properties of the films was investigated. In addition, the ability of SF to enhance CS properties by reinforcement was observed. A uniform microstructure can be achieved for SF/CS blend films without phase separation. FTIR, TG, DTG, and heat flow results indicated the intermolecular interactions between SF and CS of the blend films were formed via NH groups of SF and C=O and NH₂ groups of CS. It is expected that SF induced the changes of secondary structure from random coil to β -sheet, and this increased the thermal stability of the CS films. It is a promising finding that Eri SF can enhance the thermal stability of CS, as this is an important criterion for various applications. In conclusion, Eri SF and methanol treatment are the main factors affecting secondary structure and thermal properties of the CS films.

ACKNOWLEDGEMENTS

We gratefully thank the Division of Research Facilitation and Dissemination, Mahasarakham University and the Center of Excellence for Innovation in Chemistry, Commission on Higher Education, Ministry of Education, Thailand for a partially financial support for this work. Appreciation is also expressed to Dr. Tim Cushnie, Faculty of Medicine, Mahasarakham University for his kind improvement this manuscript.

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