ISSN 1996-5052

Current Research in Chemistry

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

Current Research in Chemistry 2 (2): 50-56, 2010
ISSN 1996-5052 / DOI: 10.3923/crc.2009.8.14

Effect of Chitosan Molecular Weights on Characteristics of Silk Fibroin/Chitosan Blend Films*

Y. Baimark, P. Srihanam and Y. Srisuwan
Center of Excellence for Innovation, Department of Chemistry,
Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand

Abstract: The aim of this study was to prepare silk fibroin (SF)/chitosan (CS) blend films by a solvent evaporation technique. Influence of CS molecular weights on SF conformation transition and thermal stability of the blend films was investigated. SF conformation in the pure SF film was predominantly random coil form, whereas the SF/CS blend films showed co-existed between random coil and β-sheet form with predominantly β-sheet form. Intermolecular bonds between SF and CS in the blend films can be detected from FTIR spectra and thermogravimetric thermograms resulted in the increase of β-sheet form of SF components and thermal stability of CS components, respectively. The SF conformation changes from random coil to β-sheet forms and the CS thermal stabilities in the blend films increased as the CS molecular weight increased. The all blend film matrices were uniform and homogeneous without phase separation. Different CS molecular weights did not affect film transparency of the blend films.

Key words: Biodegradable films, *Bombyx mori*, polymer blends, conformation transitions, thermal stability

INTRODUCTION

Silk Fibroin (SF) is a biodegradable and biocompatible natural protein polymer created by the *Bombyx mori* silkworm (Altman *et al*., 2003) and has recently been extensively investigated as a biomaterial such as matrix for cell culture substrate (Kim *et al*., 2005) and drug delivery system (Hofmann *et al*., 2006; Wang *et al*., 2007). The minimal inflammatory reactions *in vitro* and *in vivo* of SF film have been reported by Meinel *et al*. (2005). However, the applications of SF films were limited due to their very brittle in the dry state. The poor mechanical properties of SF film could be improved by blending it with other polymers.

Chitosan (CS) is a natural polysaccharide that has received great attention in a variety of applications such as wound dressing, drug delivery systems and food packaging due to its biodegradability, biocompatibility and its excellent film-forming property (Ravi-Kumaer *et al*., 2004; Muzzarelli and Muzzarelli, 2005). Although, a few works have been reported on the preparation and characterization of SF/CS blend films (Park *et al*., 1999; Kweon *et al*., 2001) but the SF/CS blend films prepared from different CS molecular weights have not been published.

In the present study, the SF conformation changes and the thermal stabilities of SF/CS blend films as a function of CS molecular weights were investigated. The film morphology and transparency of SF/CS blend films were also determined.

Corresponding Author: Yodthong Baimark, Department of Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand
Tel: +66-43-754246 Fax: +66-43-754246

*Originally Published in Current Research in Chemistry, 2009

MATERIALS AND METHODS

Materials

Silk fibroin (SF) solution was prepared as follows. Cocoons from *B. mori* were degummed by boiling twice in 0.5% Na_2CO_3 solution at 95°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, $CaCl_2$-ethanol-water (mole ratio = 1:2:8), by stirring at 80°C for 2 h. The resulting SF solution was then dialyzed in cellulose tube for 3 days against distilled water. The final concentration after dialysis was adjusted to 1% (w/v) with distilled water. Chitosans (CS) with 90% deacetylation and molecular weights of 15,000, 100,000 and 740,000 Dalton designed as CS15000, CS100000 and CS740000, respectively were purchased from Seafresh Chitosan Lab Co., Ltd., (Thailand) and used without further purification. The 1% (v/v) acetic acid aqueous solution was used as a solvent for preparation the 1% (w/v) chitosan solution. All solvents used were analytical reagent grade.

Methods

Preparation of SF/CS Blend Films

The SF/CS blend films with 1:1 (w/w) were prepared by mixing 10 mL of 1% (w/v) SF and 10 mL of 1% (w/v) CS solutions, stirred for 1 h before film casting on polystyrene Petri-dishes. The films were dried at 40°C for 48 h under vacuum at room temperature for a week before characterization. The pure SF and CS films were also prepared by the same method as controls.

Characterization of SF/CS Blend Films

FT-IR spectra were obtained by Fourier transform-infrared (FT-IR) spectrometer using Perkin-Elmer Spectrum GX FTIR spectrometer. The resolution of 4 cm^{-1} and 32 scans were chosen in this study.

Thermal stability of the films was determined by thermogravimetry (TG) using a TA-Instrument TG SDT Q600 thermogravimetric analyzer. For TG analysis, 10 - 20 mg sample were heated from 50 to 1,000°C at the heating rate of 20°C min^{-1} under nitrogen atmosphere.

Film morphology was investigated by scanning electron microscopy (SEM) using JEOL JSM-6460LV SEM. The film was cut by paper-scissors and coated with gold for enhancing the surface conductivity before scan.

Film transparency was determined by measuring the percent transmittance at 660 nm using UV-Visible spectrophotometer (Lamda 25, Perkin-Elmer Instrument) as described by Khamhan *et al.* (2008).

RESULTS AND DISCUSSION

FT-IR Spectra

Functional groups of SF and CS components and their intermolecular interactions are determined from FTIR spectra of their pure films and the blend films as shown in Fig. 1 and 2, respectively. The positions of absorption bands, especially amide I and II bands indicate the conformation of SF. The FTIR spectrum of pure SF film (Fig. 1a) showed absorption bands at 1660 cm^{-1} (amide I), 1551 cm^{-1} (amide II) and 1242 cm^{-1} (amide III), assigned to the SF with random coil conformation (Park *et al.*, 1999), whereas FTIR spectra of CS films (Fig. 1b-d) showed absorption bands at 1635-1644 cm^{-1} (amide groups) and 1565-1590 cm^{-1} (free amino groups). The absorption bands at 1153-1155 and 890-898 cm^{-1} attributed to saccharide structure of CS.

Figure 2 shows the FTIR spectra of blend films. It was found that the blend films illustrated the both SF and CS characteristics. It can be observed that amide I and II bands of SF were separated into

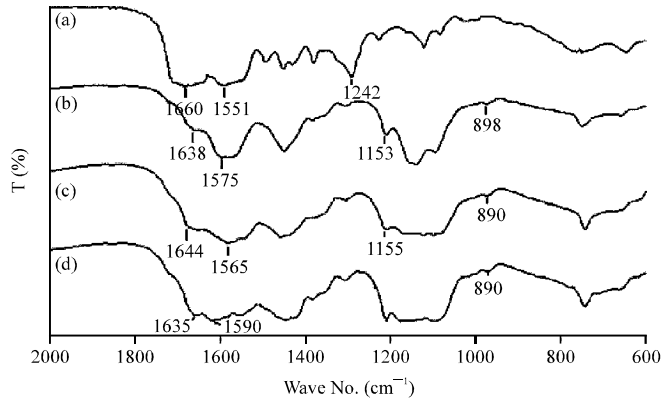

Fig. 1: FTIR spectra of (a) SF, (b) CS15000, (c) CS100000 and (d) CS740000 films

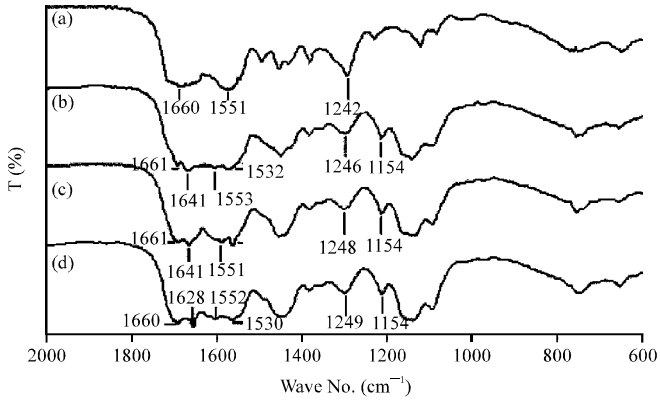

Fig. 2: FTIR spectra of (a) SF, (b) SF-CS15000 blend, (c) SF-CS100000 blend and (d) SF-CS740000 blend films

two bands. The lower wave number bands of amide I (1628-1645 cm^{-1}) and II (1530-1532 cm^{-1}) of SF in the blend films were assigned to β-sheet conformation (Kweon *et al*., 2001) suggesting that the SF in blend films were co-existed of random coil and β-sheet form with predominantly β-sheet form. In addition the amide III bands were shifted to higher wave number supporting that SF conformation changed from random coil to β-sheet form. The separating and shifting of conformationally sensitive bands (amide I, II and III bands) of SF, suggesting that the blending of CS induced structural transitions and the intermolecular interactions between SF and CS had occurred. This was expected as the intermolecular bonds between carbonyl groups of SF and free amino groups of CS (Chen *et al*., 1997). The results may indicate that the rigid CS molecules could act as the mold plate to stretch the SF molecules causing a changed from random coil to β-sheet conformation. The SF conformation of blend films was predominantly β-sheet form.

It is important to note that the amount of SF with β-sheet form was increased with the CS molecular weight. This can be detected from the shifting to lower wave number of amide I and II bands and to higher wave number of amide III bands of SF β-sheet characteristic bands as shown in Fig. 2b-d. This explained that the higher molecular weight of CS can act as a better mold plate for changing SF conformation from random coil to β-sheet form than the lower CS molecular weight.

Thermal Stability

Thermal stability of SF, CS and blend films was studied from thermogravimetric (TG) thermograms as shown in Fig. 3 and 4 for the CS films and the blend films, respectively. The both SF and CS films did not completely decompose at temperature of 1,000°C. The remaining weights of SF and CS films accorded to the literatures (Um *et al*., 2001; Wu *et al*., 2008). The initial weight loss at around 100°C for SF film is due to loss of moisture. The sharp weight loss took place in the temperature range of 250-400°C associated with the breakdown of side chain groups of amino acid residues as well as the cleavage of peptide bonds (Freddi *et al*., 1999). The SF film showed slower thermal decomposition than the all CS films indicating that the higher thermal stability of SF film. The thermal stabilities of CS films slightly increased with increase in its molecular weight as presented in Fig. 3. The SF/CS blend films with higher CS molecular weight showed slower thermal decomposition as shown in Fig. 4.

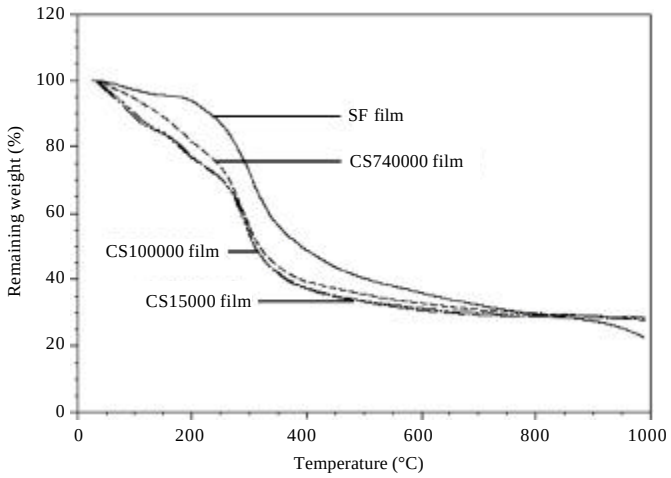

Fig. 3: TG thermograms of SF and CS films

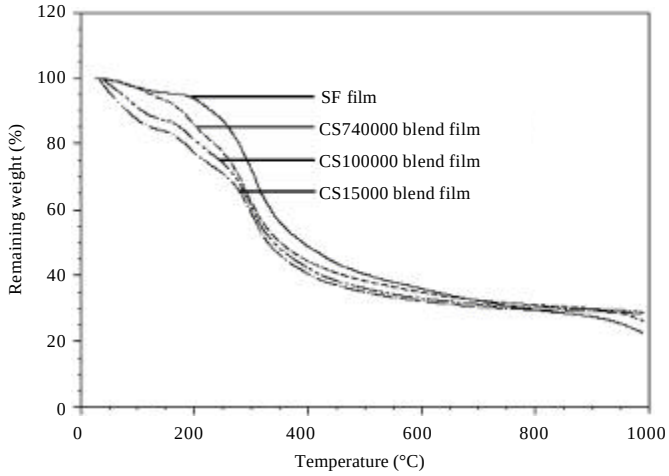

Fig. 4: TG thermograms of SF and SF/CS blend films

However, the thermal stability can be clearly determined from differential TG (DTG) thermograms as shown in Fig. 5 and 6 for the CS films and the blend films, respectively to compare with the SF film. From DTG thermograms, the temperature of maximum decomposition rate ($T_{d, max}$) can be measured and summarized in Table 1. It was found that the $T_{d, max}$ values of the pure films were in order sequence of SF >CS740000>CS100000>CS15000 according to their TG thermograms. The

Table 1: Thermal decomposition properties of SF/CS blend films

SF/CS blend films	$T_{d, max}$ (°C)
SF film	306
CS15000 film	289
CS100000 film	290
CS740000 film	296
SF-CS15000 film	290
SF-CS100000 film	299
SF-CS740000 film	305

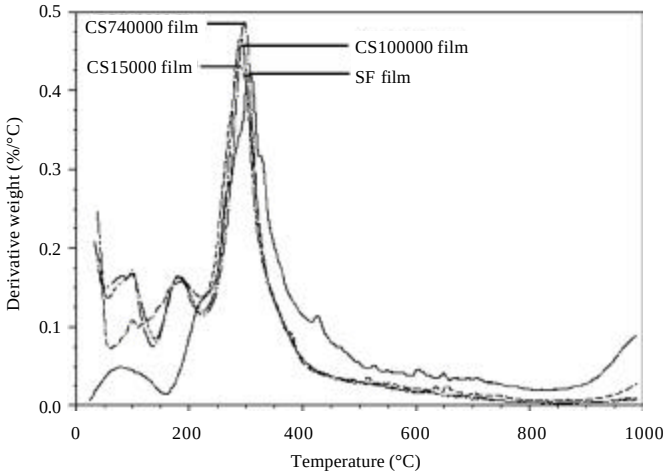

Fig. 5: DTG thermograms of SF and CS films

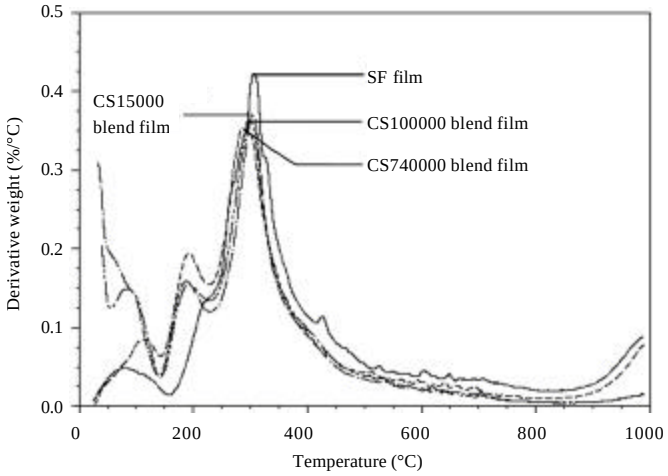

Fig. 6: DTG thermograms of SF and SF/CS blend films

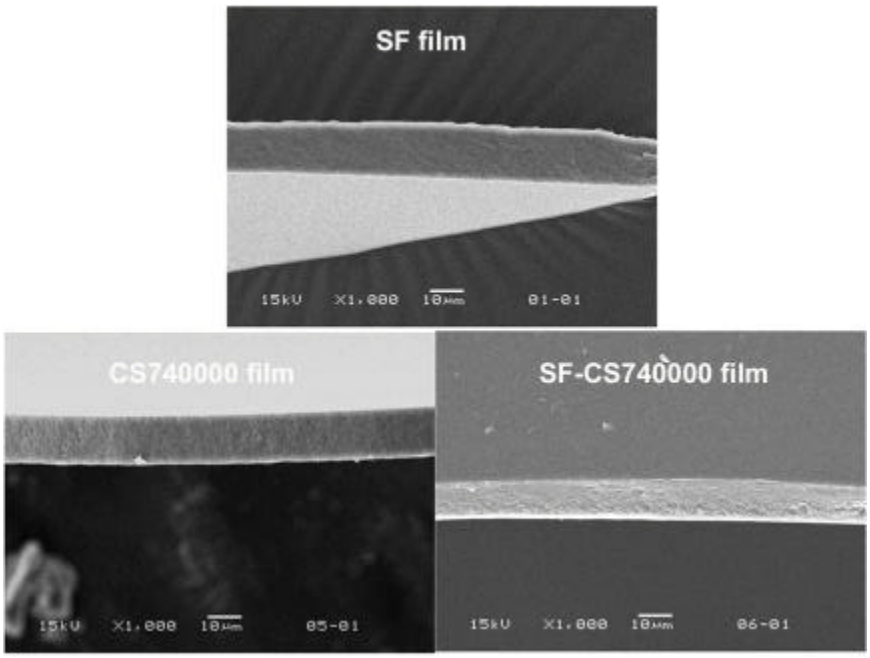

Fig. 7: SEM micrographs of film cross-sections (bar = 10 µm)

blend films had a single main $T_{d, max}$ between SF and CS components suggested the complete miscibility of SF and CS in the blend films. The $T_{d, max}$ values of blend films significantly increased as the CS molecular weight increased. This may be support the increasing intermolecular interactions between SF and CS in the blend films when the CS molecular weight was increased as described in FTIR analysis part.

Film Morphology

Thicknesses of the films measured from SEM micrographs were in the ranges of 12-18 µm. The morphology of all blend films was homogeneous and continuous phase as examples of which are shown in Fig. 7 for the SF, the CS740000 and the SF-CS740000 films. All blend films with different molecular weights of CS show similar evidence. From the morphological results, it can be expected that the film properties were consistent throughout the film matrices.

Film Transparency

The SF and the blend films were highly transparent and slightly yellowish. The % transmittance at λ_{max} of 660 nm ($\%T_{660}$) was used for studying the film transparency. The $\%T_{660}$ of SF, CS and blend films have similar values indicating that the transparency of blend films did not change when blending with different CS molecular weights.

CONCLUSION

The FTIR and TG results showed that the intermolecular interactions between SF and CS of the blend films had occurred. The SF conformation changes from random coil to β-sheet forms and the increasing CS thermal stabilities can be detected by blending of SF and CS. The increasing CS molecular weights can increase interactions between SF and CS in the blend films. The morphology of blend films

observed from the SEM micrographs was uniform and homogeneous throughout the film matrices. All blend films were clear and transparent films. The film transparency of blend films did not change with the CS molecular weights. These SF/CS blend films with different random coil/β-sheet form content and thermal stability might be of interest in biomedical, pharmaceutical and packaging applications.

ACKNOWLEDGMENT

The researchers gratefully acknowledge the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand for financial support.

REFERENCES

Altman, G.H., F. Diaz, C. Jakuba, T. Calabro and R.L. Horan *et al*., 2003. Silk-based biomaterials. Biomaterials, 24: 401-416.

Chen, X., W. Li and T. Yu, 1997. Conformation transition of silk fibroin induced by blending chitosan. J. Polym. Sci. Part B: Polym. Phys., 35: 2293-2296.

Freddi, G., M. Tsukada and S. Beretta, 1999. Structure and physical properties of silk fibroin/polyacrylamide blend films. J. Applied Polym. Sci., 71: 1563-1571.

Hofmann, S., C.T. Wong-Po-Foo, F. Rossetti, M. Textor and G. Vunjak-Novakovic *et al*., 2006. Silk fibroin as an organic polymer for controlled drug delivery. J. Control Release, 111: 219-227.

Khamhan, S., Y. Baimark, S. Chaichanadee, P. Phinyocheep and S. Kittipoom, 2008. Water vapor permeability and mechanical properties of biodegradable chitosan/methoxy poly (ethylene glycol)-*b*-poly(å-caprolactone) nanocomposite films. Int. J. Polym. Anal. Cha., 13: 224-231.

Kim, K.H., L. Jeong, H.N. Park, S.Y. Shin and W.H. Park *et al*., 2005. Biological efficacy of silk fibroin nanofiber membranes for guide bone regeneration. J. Biotechnol., 120: 327-339.

Kweon, H., H.C. Ha, I.C. Um and Y.H. Park, 2001. Physical properties of silk fibroin/chitosan blend films. J. Applied Polym. Sci., 80: 928-934.

Meinel, L., S. Hofmann, V. Karageorgiou, C. Kirker-Head and J. McCool *et al*., 2005. The inflammatory responses to silk films *in vitro* and *in vivo*. Biomaterials, 26: 147-155.

Muzzarelli, R.A.A. and C. Muzzarelli, 2005. Chitosan chemistry: Relevance to the biomedical sciences. Adv. Polym. Sci., 186: 151-209.

Park, S.J., K.Y. Lee, W.S. Ha and S.Y. Park, 1999. Structural changes and their effect on mechanical properties of silk fibroin/chitosan blends. J. Applied Polym. Sci., 74: 2571-2575.

Ravi-Kumaer, M.N.V., R.A.A. Muzzarelli, C. Muzzarelli, H. Sashiwa and A.J. Domb, 2004. Chitosan chemistry and pharmaceutical perspective. Chem. Rev., 104: 6017-6084.

Um, I.C., H.Y. Kweon, Y.H. Park and S. Hudson, 2001. Structural characteristics and properties of the regenerated silk fibroin prepared from formic acid. Int. J. Biol. Macromol., 29: 91-97.

Wang, X., E. Wenk, A. Matsumoto, L. Meinel, C. Li and D.L. Kaplan, 2007. Silk microspheres for encapsulation and controlled release. J. Control Release, 117: 360-370.

Wu, H., Y. Wan, X. Cao and Q. Wu, 2008. Interlocked chitosan/poly (DL-lactide) blends. Mater. Lett., 62: 330-334.