

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Study on Silk Sericin and Chitosan Blend Film: Morphology and Secondary Structure Characterizations

¹P. Srihanam, ²W. Simcheur and ³Y. Srisuwan

¹Department of Chemistry,

²Central Instrument,

³Division of Secretary and Department of Chemistry,

Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand

Abstract: This study aimed to prepare and characterize silk sericin and chitosan blend film as well as the native silk sericin and chitosan films. The films were observed their morphology using Scanning Electron Microscope (SEM). The secondary structures of the films were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Transparency of the films was investigated with UV-visible spectroscopy. The results found that all of silk films were smooth throughout the film surfaces, including blend film. This showed that silk sericin and chitosan very well compatible. However, phase separation is also being observed. It is show that the interaction between two materials might be miscible together. The FTIR results indicated that the most of films were composed both in random coil and β -sheet forms which predominantly of the random coil structures. The results suggesting the blend film between sericin and chitosan did not change the intramolecular structure when compared to the native films. The silk sericin and blend films were slightly yellowish color and were higher transparent than chitosan film. However, % transmittance at λ_{max} of 660 nm showed that all of films have similar values. The result suggested that the transparency of the film did not change even blend together. It is a promising that both silk sericin and chitosan would be blended into many forms for applications in specifically fields.

Key words: Characteristics, chitosan, film, silk sericin

INTRODUCTION

Natural polymers have been focused as bioresources in both technological and biomedical applications (Kweon *et al.*, 2001). The various types of them were also reported including collagen, gelatin, chitosan and silk. However, they still have limited their uses from high cost and questionable purity (Cheung *et al.*, 2008). Moreover, natural homopolymer demands are not sufficient for biomaterial applications. Therefore, blend polymers have been studied and reported (Fan *et al.*, 2008).

Silk is a fibrous protein and has been long history used in surgery (Acharya *et al.*, 2009). Each silk fiber contain of two main proteins; fibroin and sericin. Silk Fibroin (SF) is an insoluble fibrous, while sericin is a glue-like protein which was soluble very well in hot water. The SF is one of the candidate materials for biomedical applications (Min *et al.*, 2004). However, Silk Sericin (SS) has also reported on its excellent properties (Cho *et al.*, 2003), protein based materials (Dash *et al.*, 2007). The secondary structure of sericin is a random coil with some β -sheet structure (Huang *et al.*, 2003; Teramoto *et al.*, 2006).

Chitosan (CS), a derivative of chitin, is obtained from those of shrimp shells, crabs, lobsters (Zhou *et al.*, 2009), as well as other insects such as silkworms (Paulino *et al.*, 2006). It shows excellent feature for various applications (Mao *et al.*, 2001; Kong *et al.*, 2008; Du *et al.*, 2009), according to its properties such as hydrophilicity, biocompatibility, biodegradability, antibacterial and bio-adsorbent (Lei *et al.*, 2009). Due to its cationic polymer, it can be cross-linked by various reagents (Yang and Shao, 2000) or blend with other natural polymers (She *et al.*, 2008).

With some excellent properties both Silk Sericin (SS) and chitosan (CS), in the present study, we prepared the blend film of Silk Sericin (SS) and chitosan (CS) by casting on the polystyrene plate using evaporation method. Then, the SS/CS was studied their morphology and cross-section under SEM. The intermolecular interaction between SS and CS was analyzed by FT-IR spectroscopy. UV-visible spectroscopy was used to observe transparency of film. The results would be used as basic knowledge to understand about the natural polymer used and led to be applied in various fields, especially food and pharmaceutical applications.

MATERIALS AND METHODS

This study was performed for 4 months from June 1, 2009 to October 1, 2009. All of experiment and transparency observation were done on the Department of Chemistry, Faculty of Science. The SEM micrographs and secondary structure analysis were carried out at the Central Instrument, Faculty of Science, Mahasarakham University, Thailand.

Materials: *Bombyx mori* (*B. mori*) cocoons were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. They were boiled in water at 98-100°C for 30 min to obtain silk sericin solution. The obtained sericin was then filtered and concentrated to give 1% (w/w).

Chitosans (CS) with 90% deacetylation and molecular weights of 15 kDa was purchased from Seafresh Chitosan Lab Co., Ltd. (Thailand) and without further purification. The 1% acetic acid aqueous solution was used as a solvent for dissolution chitosan solution and adjusted to 1% (w/w) in final. All solvents used were analytical grade.

Methods

Preparation of SS/CS blend films: The SS/CS blend films at 1:1 (w/w) ratio were prepared by mixing of 10 mL 1% (w/v) SS solution and 10 mL 1% (w/v) CS solution, stirred for 1 h before casting on 5 cm polystyrene plates. The plates were dried at 40°C for 48 h to obtain the blend film. They were then kept under vacuum at room temperature for a week before characterization. The native SS and CS films were also prepared by the same method for using as control.

Characterization of SS/CS blend films: All of films were analyzed their secondary structure by FT-IR spectroscopy (Perkin-Elmer Spectrum GX). In this study used air as the reference. The resolution of 4 cm⁻¹ and 32 scans were chosen for analysis.

The morphology of the films was investigated by scanning electron microscope (SEM) (JEOL JSM-6460LV). The films were fractured in liquid nitrogen and coated with gold for enhanced surface conductivity before investigation.

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

RESULTS AND DISCUSSION

FT-IR spectra: Figure 1a showed the absorption bands of native SS film at 1684 cm⁻¹ (amide I), 1559 cm⁻¹ (amide II),

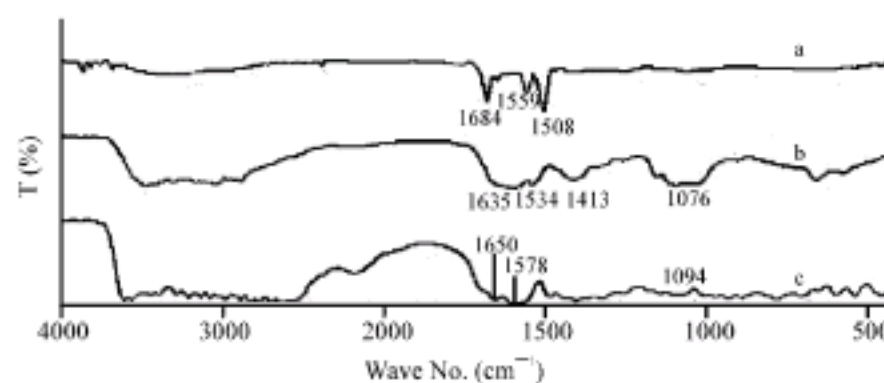


Fig. 1: FT-IR spectra of (a) SS, (b) blend SS/CS and (c) CS films

while the blend SS/CS film showed widely absorption bands at about 1640 cm⁻¹ (amide I), 1534 cm⁻¹ (amide II) and at 1100-1080 cm⁻¹ (Fig. 1b). For native CS film FT-IR spectrum showed absorption band at 1650 cm⁻¹ with shoulder band at 1578 and 1103 cm⁻¹. Moreover, the bands at about 1094 and 1000 cm⁻¹ were also appeared (Fig. 1c). All of results indicated that the blend film composed of both SS and CS characteristics since the amide I band appeared in the range of the carbonyl group.

Morphology observation: Surface analysis of films was determined by Scanning Electron Microscope (SEM). SEM micrographs of native SS and CS films cross-section showed smooth throughout area of the films (Fig. 2a, c). In contrast, SEM micrograph revealed that the surface of the blend film cross-section was dense and quite rough skin layer (Fig. 2b). The blend film exhibited sheet-like morphology cover the surface area. Indeed, SS film showed highly smooth surface than those of CS and blend films.

Transparency investigation: All of films were light transparency with thin layer. The native SS and blend films were higher transparency compared to native CS film. They were slight yellowish color. The % transmittance at λ_{max} of 660 nm (%T₆₆₀) in all of SS, CS and blend films were similar values at about 84-87% as shown in Table 1.

The conformational structure of films has often been studied by FT-IR spectroscopy (Tasukada *et al.*, 1995). As shown in Fig. 1a showed the absorption bands of SS at 1684 cm⁻¹ (amide I), 1559 cm⁻¹ (amide II), assigned to random coil structure (Kweon *et al.*, 2000; Hino *et al.*, 2003). However, the blend films as shown in Fig. 1b peak at about 1635 cm⁻¹ (amide I), 1534 cm⁻¹ (amide II) and at 1100-1080 cm⁻¹ were attributed to β -sheet structures (Kweon *et al.*, 2000). The result indicated that SS and CS have interacted by form bonding together. For native CS film FT-IR spectrum showed absorption band at 1650 cm⁻¹ (amide groups) with shoulder bands at 1578 cm⁻¹ (free amino groups) and 1103 cm⁻¹. Moreover, the bands at about 1094 and 1000 cm⁻¹ were also appeared (Fig. 1c)

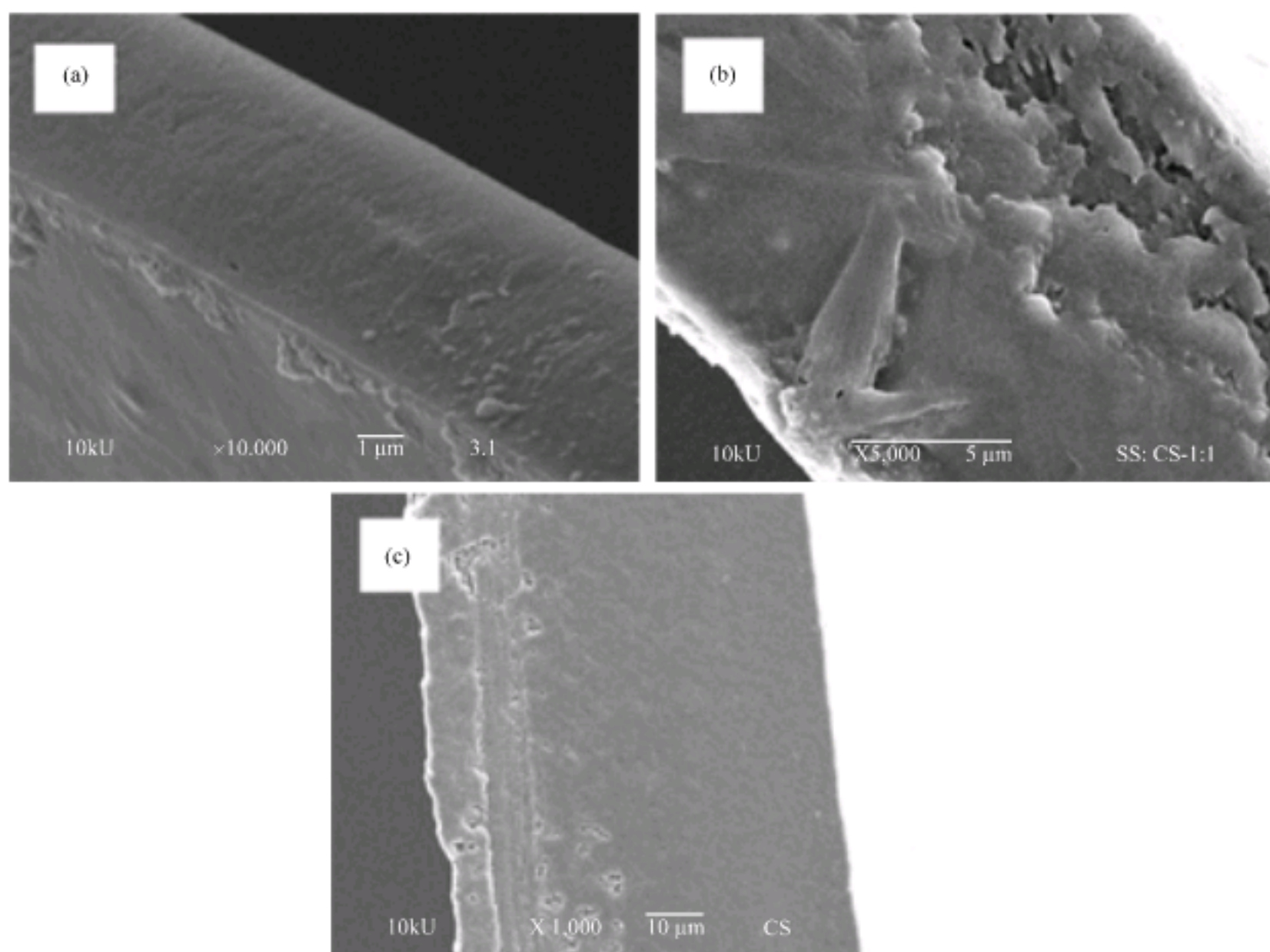


Fig. 2: SEM micrographs of SS (a), blend SS/CS (b) and CS (c) films cross-section

Table 1: Transparency percentage at 660 nm (% T_{660}) of SS, CS and blend SS/CS films

SF/CS ratio (w/w)	SS solution (mL)	CS solution (mL)	T_{660} (%)
2/0	20	0	87±4.5
1/1	10	10	86±7.2
0/2	20	20	84±3.1

(Baimark *et al.*, 2009; Kweon *et al.*, 2001; She *et al.*, 2008). The results indicated that the blend films illustrated both SS and CS characteristics. The appearances of β -sheet in the blend film suggest that SS reinforcing the β -sheet formation. Moreover, the interaction between polysaccharide (CS) and protein (SS) were stronger than physical mixing of two materials. However, the absorption bands of the films by this work were slightly differed comparison to other reported, except the bands of functional groups were the same regions. The reason was according from the differences of sericin sources which were differed by silk variety and environmental (Dash *et al.*, 2006).

The cross-section of blend film was rougher surfaces than both native SS and CS films. The one reason which can be explained this point is phase separation between the material blend. It might be from the suitable ratio used for blending and made incomplete formation bonds. On the other hand, molecular size might be differed and affected to the interaction between SS and CS. However,

all of films showed similar values of % transparency even different color. The results indicating that the transparency of the films did not influenced by blending or phase separation. It is an important point evidenced that both SS and CS excellent compatibility. On the other hand, the acidic CS solution did not change the structure or chemical properties of SS. This was observed by the transparency of the film.

CONCLUSION

In this study, SS/CS blend film as well as native SS and CS were prepared. The secondary structure, morphology and transparency of the film were investigated. The uniform microstructure of the native SF and CS blend films was successfully achieved while blend film was rougher than native under SEM. This point was according form phase separation of the blend films. FT-IR results showed the intermolecular interactions between SS and CS to increase β -sheet structure of the blend films. However, the blend material and secondary structure did not affect to the transparency of the film. It is promising that the blend between SS and CS could be obtained good synergistic properties which were important for further applications such as food and pharmaceuticals.

ACKNOWLEDGMENT

The study was financial supported by the Research Development and Support Unit, Mahasarakham University, Mahasarakham, Thailand.

REFERENCES

- Acharya, C., S.K. Ghosh and S.C. Kundu, 2009. Silk fibroin film from non-mulberry tropical tasar silkworms: A novel substrate for *in vitro* fibroblast culture. *Acta Biomater.*, 5: 429-437.
- Baimark, Y., P. Srihanam and Y. Srisuwan, 2009. Effect of chitosan molecular weights on characteristics of silk fibroin-chitosan blend films. *Curr. Res. Chem.*, 1: 8-14.
- Cheung, H.Y., K.T. Lau, X.M. Tao and D. Hui, 2008. A potential material for tissue engineering silkworm silk/PLA biocomposite. *Comp. Part B Eng.*, 39: 1026-1033.
- Cho, K.Y., J.Y. Moon, Y.W. Lee, K.G. Lee and J.H. Yeo *et al.*, 2003. Preparation of self-assembled silk sericin nanoparticles. *Int. J. Biol. Macromol.*, 32: 36-42.
- Dash, R., S. Mukherjee and S.C. Kundu, 2006. Isolation, purification and characterization of silk protein sericin from cocoon peduncles of tropical tasar silkworm, *Antheraea mylitta*. *Int. J. Biol. Macromol.*, 38: 255-258.
- Dash, R., S.K. Ghosh, D.L. Kaplan and S.C. Kundu, 2007. Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Comp. Biochem. Physiol. Part B*, 147: 129-134.
- Du, W.L., S.S. Niu, Y.L. Xu, Z.R. Xu and C.L. Fan. 2009. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydrate Polymers*, 75: 385-389.
- Fan, H., H. Liu, S.L. Toh and J.C.H. Goh, 2008. Enhanced differentiation of mesenchymal stem cells co-cultured with ligament fibroblasts on gelatin/silk fibroin hybrid scaffold. *Biomaterials*, 29: 1017-1027.
- Hino, T., M. Tanimoto and S. Shimabayashi, 2003. Change in secondary structure of silk fibroin during preparation of its microspheres by spray-drying and exposure to humid atmosphere. *J. Coll. Interf. Sci.*, 266: 68-73.
- Huang, J., R. Valluzzi, E. Bini, B.D. Vernaglia and D.L. Kaplan, 2003. Cloning expression and assembly of sericin-like protein. *J. Biol. Chem.*, 278: 46117-46123.
- 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(α -caprolactone) nanocomposite films. *Int. J. Polym. Anal. Cha.*, 13: 224-231.
- Kong, M., X.G. Chen, C.S. Liu, C.G. Liu, X.H. Meng and L. Yu, 2008. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. *Colloids Surf, B. Biointerfaces*, 65: 197-202.
- Kweon, H.Y., I.C. Um and Y.H. Park, 2000. Thermal behavior of regenerated *Antheraea pernyi* silk fibroin film treated with aqueous methanol. *Polymer*, 41: 7361-7367.
- Kweon, H.Y., I.C. Um and Y.H. Park, 2001. Structural and thermal characteristics of *Antheraea pernyi* silk fibroin-chitosan blend film. *Polymer*, 42: 6651-6656.
- Lei, Z., X. Pang, N. Li, L. Lin and Y. Li, 2009. A novel two-step modifying process for preparation of chitosan-coated Fe₃O₄/SiO₂ microspheres. *J. Mater. Process. Technol.*, 209: 3218-3225.
- Mao, H.Q., K. Roy, V.L. Troung-Le, K.A. Janes and K.Y. Lin *et al.*, 2001. Chitosan-DNA nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency. *J. Control. Release*, 70: 399-421.
- Min, B.M., G. Lee, S.H. Kim, Y.S. Nam, Y.S. Lee and W.H. Park, 2004. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts *in vitro*. *Biomaterials*, 25: 1289-1297.
- Paulino, A.T., J.I. Simionato, J.C. Garcia and J. Nozaki, 2006. Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers*, 64: 98-103.
- She, Z., B. Zhang, C. Jin, Q. Feng and Y. Xu, 2008. Preparation and *in vitro* degradation of porous three-dimensional silk fibroin-chitosan scaffold. *Polymer Degrad. Stab.*, 93: 1316-1322.
- Tasukada, M., G. Freddi, P. Monti, A. Bertoluzza and N. Kasai, 1995. Structure and molecular conformation of tussah silk fibroin films effect of methanol. *J. Polymer Sci. Polymer Phys. Educ.*, 33: 1995-2001.
- Teramoto, H., A. Kakazu and T. Asakura. 2006. Native structure and degradation pattern of silk sericin studied by ¹³C NMR spectroscopy. *Macromolecules*, 39: 6-8.
- Yang, Y.M. and J. Shao, 2000. Synthesis of sulfhydryl chitin and its adsorption properties for heavy metal ions. *J. Applied Polymer Sci.*, 77: 151-155.
- Zhou, L., Y. Wang, Z. Liu and Q. Huang, 2009. Characteristics of equilibrium, kinetics studies for adsorption of Hg(II), Cu(II) and Ni(II) ions by thiourea-modified magnetic chitosan microspheres. *J. Hazard Mater.*, 161: 995-1002.