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Influence of *Philosamia ricini* Silk Fibroin Components on Morphology, Secondary Structure and Thermal Properties of Chitosan Biopolymer Film

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Abstract: This study aimed to prepare Eri (*Philosamia ricini*) Silk Fibroin (SF)/chitosan (CS) blend films by a solvent evaporation method and to compare the blend films with both native SF and CS films. Influence of SF ratios on the morphology, secondary structure and thermal decomposition of the CS blend films were investigated. The native SF and CS films were uniform and homogeneous without phase separation. For the blend films, the uniform can be found less than 60% of SF composition. All of SF/CS blend films showed both SF and CS characteristics. FT-IR results showed that the blend films composed of both random coil and β -sheet with predominant of β -sheet form. Interaction of intermolecular between SF and CS have occurred which were measured by thermogravimetric thermograms. Increasing of SF contents was leading to the increase of β -sheet structures which were enhanced the thermal stability of the CS blend films.

Key words: Biopolymer, chitosan, silk fibroin, secondary structure, thermal stability

INTRODUCTION

Natural polymers have been focused as bioresources in both technological and biomedical applications (Kweon *et al.*, 2001). The various attractive properties of the natural polymers such as non-toxicity, biodegradability and biocompatibility were also reported. Collagen, gelatin, chitosan, silk and hyaluronic acid are natural polymers which have been studied and applied in various applications. However, their high cost and questionable purity have limited their uses (Cheung *et al.*, 2008). While natural homopolymer demands are not sufficient for biomaterial products, therefore, blend polymers have been studied to improve the performance of the individual natural polymer (Fan *et al.*, 2008).

Silks are fibrous protein polymers that are produced by some Lepidoptera larvae. Their compositions and properties differ widely by the specific sources (Altman *et al.*, 2003). Each silk fiber consists of two types of proteins; fibroin and sericin. Silk Fibroin (SF) is an insoluble fibrous component, whereas sericin is a glue-like protein that is well soluble in hot water or some organic solvents. The SF is one of the candidate materials for biomedical applications. Recently, SF has been applied in various fields including cosmetics, medical materials and food additives (Min *et al.*, 2004). Generally, silks are

divided into two categories: mulberry and non-mulberry or wild silkworms (Acharya *et al.*, 2009). There are more studies on mulberry (*Bombyx mori*) silk, especially Eri (*Philosamia ricini*) silk than wild silk.

Chitosan (CS), a derivative of chitin, is the second most abundant in nature. It 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 is a polysaccharide and shows excellent features for application such as hydrophilicity, biocompatibility, biodegradability, antibacterial and bio-adsorbent (Lei *et al.*, 2009). Chitosan has been used in various applications such as biotechnology (Mao *et al.*, 2001), biomedicine (Du *et al.*, 2009; Illuni, 1998; Kong *et al.*, 2008), food ingredients (Chien *et al.*, 2007) and cosmetics (Ravi-Kumraer *et al.*, 2004). 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).

So, far, the influence of Eri SF components on the properties of CS film has limited available information. In this study, we prepared the blends of SF/CS with different components and studied the effect of the SF on morphology, secondary structure and thermal behavior of the CS. The goal of the study is to explore the properties of SF as a reinforcing material for enhancing the CS

properties. To clarify the influence of the Eri SF on CS properties, native Eri SF and CS films were also carried out in this study.

MATERIALS AND METHODS

This study was constructed for 4 months from April 1, 2009 to August 1, 2009. The experiments were carried out on Department of Chemistry, Faculty of Science. The characterization of secondary structure was done at the Central Instrument, Faculty of Science, Maharakham University, Thailand.

Materials: Chitosan (CS) with 90% deacetylation and molecular weight of 80 kDa was purchased from Seafresh Chitosan Lab Co., Ltd., (Thailand). It was used without further purification. The 1% (w/v) of chitosan solution was prepared by dissolving the chitosan powder with 1% (w/v) acetic acid aqueous solution. The Eri (*P. ricini*) silk cocoons were kindly supplied from Silk Innovation Center (SIC) Maharakham University, Thailand.

Sample preparation: The cocoons were stripped and then degummed twice using 0.5% (w/v) Na_2CO_3 and thoroughly rinsed 2 times in warm distilled water. They were then dried at room temperature before dissolving. The Eri SF was dissolved with 9 M $\text{Ca}(\text{NO}_3)_2$ solution which applied method from Tao *et al.* (2007). Briefly, dried Eri SF was mixed at the ratio of 1 g fibroin to 10 mL of 9 M $\text{Ca}(\text{NO}_3)_2$ solution. The solution was firstly warmed to about 90°C, then gradually added SF into the solution and stirred with controlled temperature at 100-105°C until silk already dissolved (approximately 45 min). The SF hydrolysate was filtrated and then dialyzed in cellulose tube against distilled water for 3 days at room temperature. The final concentration after dialysis was adjusted to 1% (w/v) with distilled water.

Silk fibroin/chitosan blend films preparation: The blend of 10 mL SF/CS solution at various compositions was prepared by casting on the polystyrene plates, with a diameter of 5 cm. The ratios of SF/CS were 10:0, 8:2, 6:4, 4:6 and 0:10, respectively. The plates were left at 40°C in oven for 3 days. The blend films with approximately 10-15 μm thickness were obtained.

Morphological characterization: All of SF/CS blends including native SF and CS (controls) were observed under the Scanning Electron Microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The samples were sputter coated with gold for enhancing surface conductivity.

Secondary structure analysis: The native SF, CS and SF/CS blend films were analyzed for their secondary structure using FT-IR spectrometer (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~4000-400 at 4 cm^{-1} spectral resolution and 32 scans.

Thermal behavior measurement: The films cut into 8-10 mg were loaded in a platinum crucible. The thermogravimetric analysis (TGA) was then performed using TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The samples were non-isothermal heated from 50°C to 1000°C at a heating rate of $10^\circ\text{C min}^{-1}$. The TGA was carried out in nitrogen with the flow rate of 100 mL min^{-1} . The TG and heat flow were recorded with TA instrument's Q series explorer software. The analyses of the data were done using TA Instrument's Universal Analysis 2000 Software (version 3.3B).

RESULTS AND DISCUSSION

SEM observation: Figure 1a-e shows the SEM micrographs of the blend film surfaces for the Eri SF and CS in various ratios. The native Eri SF film (Fig. 1a) showed a uniform microstructure similarly to the film prepared from native CS (Fig. 1b). The Eri SF/CS blend film containing 20% CS also exhibited a uniform microstructure. The brittle particles which could be affected to the phase separation were found by the increase in the CS contents.

FT-IR investigation: Figure 2a-e showed the FTIR spectra of the native Eri SF, SF/CH blend films. Figure 2a and 2e illustrated the morphological texture of native SF and CS films. Figure 2b-d showed the FT-IR spectra of SF/CS blend films. These findings indicated that all of blend films illustrated both SF and CS characteristics.

Thermal behavior analysis: Thermal decomposition of native Eri SF, CS and SF/CS blend films was analyzed from the thermogravimetric (TG) curves as shown in Fig. 3.

Both native SF and CS films did not completely decomposed even at 1000°C. At the temperature of 200-400°C, the rapid weight loss was observed. The native CS film showed more rapid thermal decomposition than that of native SF and SF/CS blend films. Clearly evidence of thermal decomposition was supported by Differential TG (DTG) curves as shown in Fig. 4. The SF/CS blend films took place in at least two stages: the first step, from 250 to 300°C and the second, from 300 to 400°C. Figure 5 showed heat flow thermograms of the films measured at the temperature range of 50 to 1000°C. Those of all films showed thermal stability until above 200°C. The

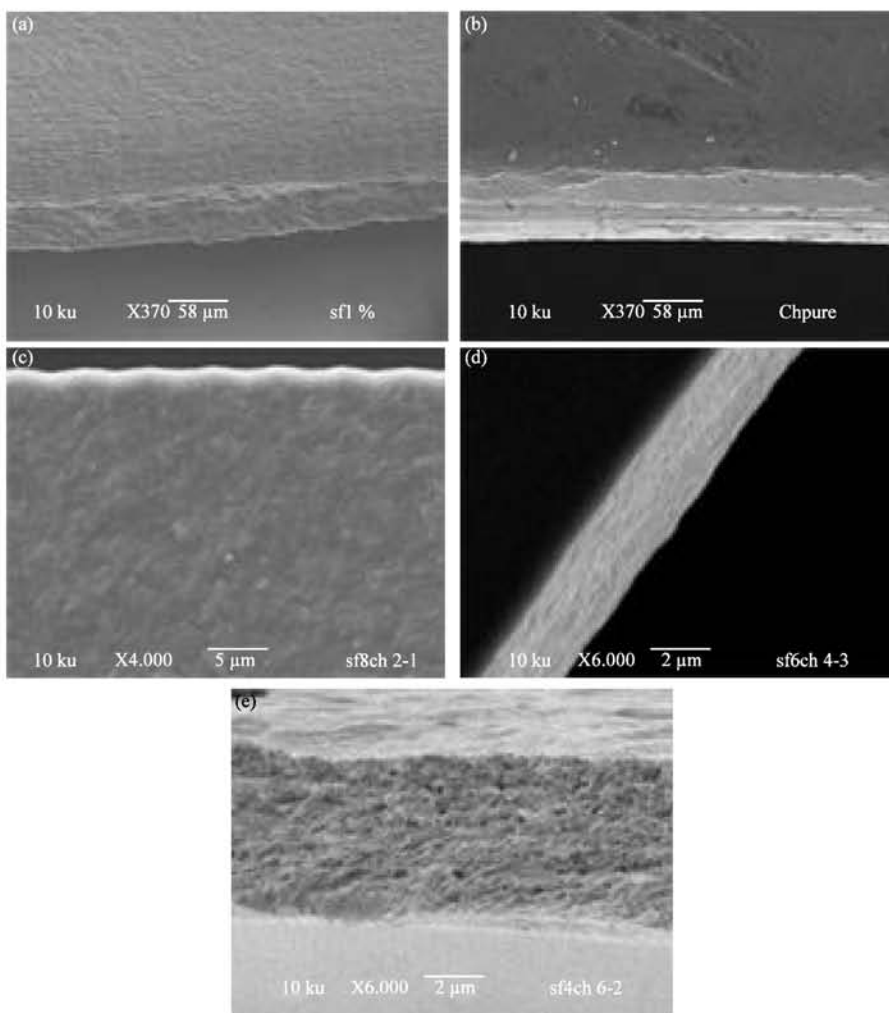


Fig. 1: (a) SEM micrographs of native Eri SF, (b) CS, (c) 4:6 SF/CS, (d) 6:4 SF/CS and (e) 8:2 SF/CS blend films

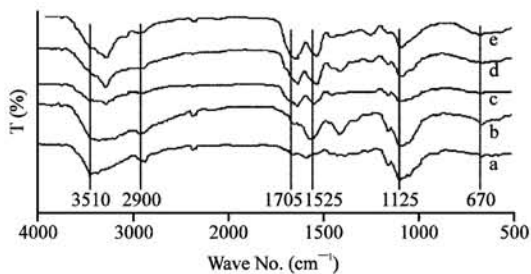


Fig. 2: (a) FT-IR spectra of native CS, (b) 4:6 SF/CS, (c) 6:4 SF/CS, (d) 8:2 SF/CS and (e) native SF films

characteristics of each component were found and different endo/exo peaks. The exo/endo transitions can be summarized in Table 1.

Morphology: It is known that the properties of the SF/CS blend films depend on their phase morphology which is

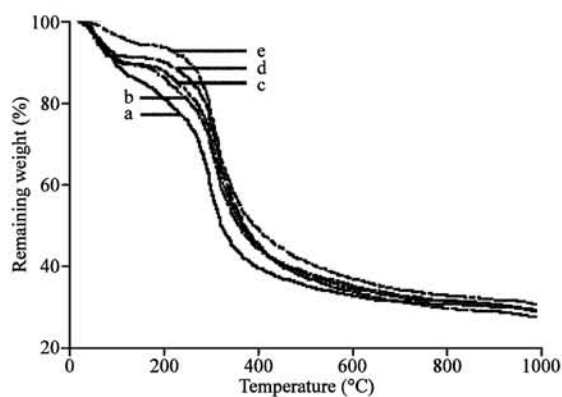


Fig. 3: (a) TG curves of native CS, (b) 4:6 SF/CS, (c) 6:4 SF/CS, (d) 8:2 SF/CS and (e) native SF films

influenced by several factors such as composition, interfacial tension, processing condition as well as

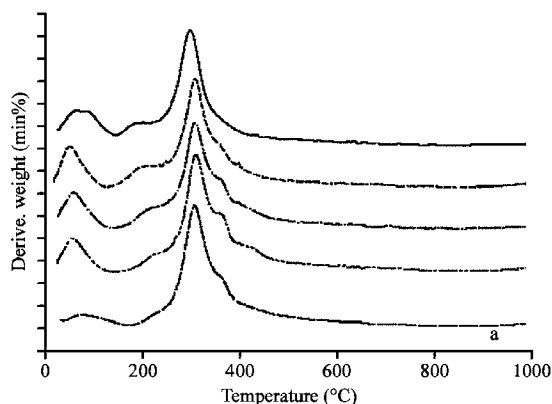


Fig. 4: (a) DTG curves of 4:6 SF/CS, (b) 6:4 SF/CS, (c) 8:2 SF/CS, (d) native SF and (e) native CS films

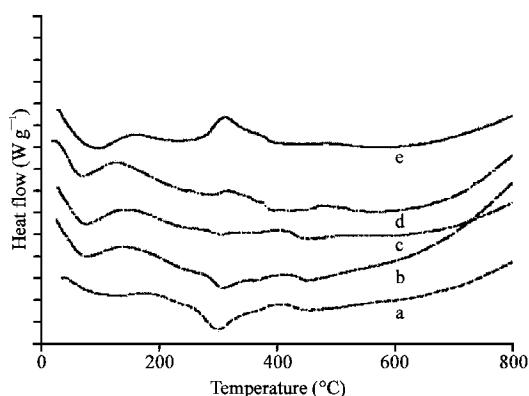


Fig. 5: (a) Heat flow curves of native CS, (b) 4:6 SF/CS, (c) 6:4 SF/CS, (d) 8:2 SF/CS and (e) native SF films

Table 1: Exo/endo transition of the films

| SF/CS | Exothermic (°C) | Endothermic (°C) |
|-------|-----------------|-------------------|
| 10/0 | 170, 310 | 100, 400 |
| 8/2 | 130, 320, 480 | 80, 280, 380, 525 |
| 6/4 | 140, 420 | 80, 305, 450 |
| 4/6 | 140, 420 | 80, 305, 450 |
| 0/10 | 410 | 100, 300, 450 |

preparation techniques (Kayaisang *et al.*, 2009). In this study, the morphology of the Eri SF/CS blend surface was observed under Scanning Electron Microscope (SEM). Generally, the conformation of Eri SF solution was composed of an ordered α -helix and a random coil structure. This would be affected by the sequential alanine residues composed of crystalline parts of Eri SF (Kweon *et al.*, 2001).

Secondary structure: The conformational structure of SF fibers and films has often been studied by FT-IR spectroscopy (Tasukada *et al.*, 1995). The secondary structures of protein are indicated by the amide I ($1700-1600\text{ cm}^{-1}$), amide II ($1600-1500\text{ cm}^{-1}$) and amide III

($1300-1200\text{ cm}^{-1}$) bands (Kweon *et al.*, 2000; Hino *et al.*, 2003). FT-IR spectrum of the native SF showed absorption bands at 1625 cm^{-1} (amide I), 1525 cm^{-1} (amide II) and 1225 cm^{-1} (amide III), indicating the β -sheet structure (Kweon *et al.*, 2000). On the other hand, FT-IR spectrum of the CS film showed intense absorption at 1675 cm^{-1} (amide groups), 1590 cm^{-1} (free amino groups) and $900, 1180\text{ cm}^{-1}$ (saccharide structure) (She *et al.*, 2008). The amide I of native SF in the blend films were strongly absorbed at about 1625 cm^{-1} which was assigned to β -sheet structure (Kweon *et al.*, 2001), whereas amide II were gradually shifted from higher to lower wave number when decreased increased of SF contents. The wave number of contrast amide III bands of SF was gradually decreased when the CS ratios increased. These results suggested that CS blended with Eri SF induced structural interaction from random coil to β -sheet structure (She *et al.*, 2008). The interaction between carbonyl groups of SF and free amino groups of CS was expected and clearly appeared (Chen *et al.*, 1997). It is promising that SF/CS blend films were stronger with β -sheet structure than that of native CS film.

Thermal decomposition analysis: The initial weight loss at below 100°C was due to the moisture evaporation (Kweon *et al.*, 2001). At low temperature, weight was rapidly loss which associated with the breakdown of side chain groups of amino acid residues as well as the decomposition of peptide bonds (Freddi *et al.*, 1997). This finding indicated that CS has lower thermal stability than those of other films. The thermal decomposition of CS gradually increased when the SF content increased. The $T_{d, \max}$ of the films were sequentially arranged of native SF>SF/CS blend>CS films. The SF/CS blend films took place in many stages compared to the native. These results suggested the incomplete miscibility of SF/CS in the blend films. Moreover, the $T_{d, \max}$ values of the blend films were gradually increased as the SF contents increased.

With heat flow curves, the first endothermic peaks at below 100°C distributions to the dehydration appeared in all of samples. In case of CS, one broad endothermic transition occurred at 300°C . This result represented the melting of polysaccharide residues (Marsano *et al.*, 2008) and minor endothermic transition at 450°C due to thermal degradation of free amino groups. The native Eri SF showed broad exothermic peak at 310°C and minor endothermic transition at 400°C and assigned to thermal degradation of fibroin (Li *et al.*, 2003). As higher ratio of SF, the blend film showed thermal stability transition similar to the native SF film, but other blend films were similar to CS film. However, the mixture characteristics of SF and CS were clearly shown. The sharp endothermic

and exothermic peaks at around 200-300°C are due to the decomposition of fibroin molecules with unoriented β -sheet structure (Kweon *et al.*, 2000). Moreover, these regions have been described as the strong molecular motion of α -helix and its transformation into β -sheet structure (Li *et al.*, 2003).

CONCLUSION

In this study, Eri SF/CS blend films were prepared. The influence of SF on morphology, secondary structure and thermal properties of CS biopolymer film was investigated. The uniform microstructure of the SF/CS blend films was successfully achieved. However, phase separation of the blend films can be observed when SF content was used over 60%. FT-IR, TG, DTG and heat flow results showed the intermolecular interactions between SF and CS of the blend films. The SF could be induced the conformation structure of CS from random coil to β -sheet and led to increase thermal stability of the films. As the high ratio of SF, the SF/CS blend films have increased both β -sheet structure and thermal stability compared to native CS. It is promising that the Eri SF could enhance some good properties of the CS up that were important for various applications such as biomedicine and pharmaceuticals.

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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. Biomat.*, 5: 429-437.

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. Polymer Sci. Part B: Polymer Phys.*, 35: 2293-2296.

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.

Chien, P.J., F. Seu and F.H. Yang, 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *J. Food Eng.*, 78: 225-229.

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.

Freddi, G., P. Monti, M. Nagura, Y. Gotoh and M. Tsukada, 1997. Structure and molecular conformation of tussah silk fibroin films: Effect of heat treatment. *J. Polymer Sci. Polymer Phys. Edu.*, 35: 841-847.

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.

Illum, L., 1998. Chitosan and its use as a pharmaceutical excipient. *Pharm. Res.*, 15: 1326-1331.

Kayaisang, S., T. Amornsakchai and S. Saikrasun, 2009. Influence of liquid crystalline polymer and recycled PET as minor blending components on rheological behavior, morphology and thermal properties of thermoplastic blends. *Polymer Adv. Technol.*, 23: 456-458.

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.

Li, M., W. Tao, S. Lu and S. Kuga, 2003. Compliant film of regenerated *Antheraea pernyi* silk fibroin by chemical crosslinking. *Int. J. Biol. Macromol.*, 32: 159-163.

Mao, H.Q., K. Roy, V.L. Troung-Le, K.A. Janes, K.Y. Lin, W. Yan, J.T. August and K.W. Leong, 2001. Chitosan-DNA nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency. *J. Control. Release*, 70: 399-421.

- Marsano, E., P. Corsini, M. Canetti and G. Freddi, 2008. Regenerated cellulose-silk fibroin blends fibers. *Int. J. Biol. Macromol.*, 43: 106-114.
- 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.
- 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.
- 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.
- Tao, W., M. Li and C. Zhao, 2007. Structure and properties of regenerated *Antheraea pernyi* silk fibroin in aqueous solution. *Int. J. Biol. Macromol.*, 40: 472-478.
- 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. Edu.*, 33: 1995-2001.
- 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.