Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 9 (5): 978-982, 2009
ISSN 1812-5654

Dissolution of *Philosamia ricini* Silk Film: Properties and Functions in Different Solutions

Y. Srisuwan and P. Srihanam
Department of Chemistry, Faculty of Science, Mahasarakham University, 44150, Thailand

Abstract: In this study, dissolution of Eri (*Philosamia ricini*) cocoons in different solutions was investigated and compared with Nang-Lai variety (*Bombyx mori*) cocoons. The Lithium Bromide (LiBr), calcium nitrate ($Ca(NO_3)_2$), Zinc chloride ($ZnCl_2$), Lithium thiocyanate ($LiSCN{\cdot}xH_2O$), 85% phosphate and mixture (calcium chloride ($CaCl_2$)/Ethanol/H_2O; 1:2:8) solution systems were used. Efficiency of the dissolving solution was examined by measuring the percentage of dissolved silk. It was found that the Nang-Lai silk was completely dissolved in all solutions, whereas, Eri silk was slightly dissolved, except for 85% phosphate solution. The Fourier transform infrared spectroscopy (FTIR) spectra of the Eri silk film composed of β-sheet form than Nang Lai silk film. With thermogravimetric analysis, the Eri silk film showed two stages of thermal decompositions while the Nang-Lai silk was a single stage. In conclusion, thermal stability of the Eri silk was higher than the Nang-Lai silk.

Key words: Silk, structure, solution, thermal decomposition

INTRODUCTION

Silk is a natural fiber, produced from some animals including silkworm (Jin *et al*., 2002). Generally, silk is divided into mulberry (domesticated or cultivated) and non-mulberry (wild) silks. Silks differ wildly in composition, structure and properties which were from both different source and amino acid contents. The major of silk application is textile. However, silk has been used as material sutures for long time (Rossitch *et al*., 1987). Earlier report has shown of many differences between domestic: *Bombyx mori* (*B. mori*) and wild silk. The different properties of both types indicated such on their structure or powder (Wang *et al*., 2000).

Miyazawa *et al*. (2002) has been drawn about characteristic different of domesticated silkworm (*Bombyx mori*) and wild silkworm (*Samia cynthia ricini*, *Antheraea yamamai*, *Caligula japonica*), by using FT-IR method. Furthermore, interesting in wild silk is high increase by Asakura *et al*. (2004), Monti *et al*. (2004), Dash *et al*. (2006), Tao *et al*. (2007) and Hashimoto *et al*. (2008). The repeated of GAGAGS amino acid motifs in the crystalline regions of fibroin make dissolution of such silk a formidable task (Phillips *et al*., 2004). Different methods have been applied for dissolution of silk fibroin including $CaCl_2$/ethanol/water solution (Ajisawa, 1998), solutions of LiBr, LiSCN, $Ca(NO_3)_2$-MeOH-H_2O or LiBr-EtOH (Chen *et al*., 2001) as well as ionic liquids (Phillips *et al*., 2004). The most study on wild silk is Genus *Antheraea* (Freddi *et al*., 1994; Kweon *et al*., 2000, 2001; Dash *et al*., 2007), but less for *Philosamia ricini*. Thus, this study tries to dissolve the wild (*Philosamia ricini*) silk. We chosen many common systems: LiBr, $Ca(NO_3)_2$, $ZnCl_2$, LiSCN, 85% phosphate and 1:2:8 mole ratios of $CaCl_2$/Ethanol/H_2O. The best system was chosen for dissolution of wide silk to make the silk film. The differences in some properties were compared to the domesticated silk, *B. mori*.

MATERIALS AND METHODS

Materials: Both Nang Lai variety (*B. mori*) and Eri (*Philosamia ricini*) silk cocoons were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. The cocoons were strips and then degummed twice using 0.5 g mL^{-1} Na_2CO_3 and thoroughly rinsed 2 times in warm distilled water. They were then dried at room temperature before dissolving. All of dissolving solution including LiBr, $Ca(NO_3)_2$, $ZnCl_2$, LiSCN, 85% phosphate, $CaCl_2$ and ethanol in analytical grade were used.

Methods

Dissolution of silk: The Eri silk was tested with several systems: 9M LiBr, 9M $Ca(NO_3)_2$, 9M $ZnCl_2$, 9M LiSCN, 85% phosphate and 1:2:8 mole ratios of $CaCl_2$/Ethanol/H_2O (mixture solution). Time for dissolving of the silk was determined and compared between each solution. All silk hydrolysate were then centrifuged at 1000 g for 30 min. The silk solution was then separated and the precipitate part was taken in air-dried, calculated silk remaining (%) as follow equation;

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

$$\text{Silk remaining}(\%) = \frac{\text{Weight after desolving}}{\text{Weight before desolving}} \times 100$$

Finally, the best solution was chosen to dissolve Eri silk for film preparation step.

Eri silk was dissolved with the 85% phosphate follow the modified of earlier reported by Wang *et al*. (2000). Briefly, dried Eri silk fibroin and the solution were mixed at the rate of 1 g fibroin to 10 mL of solution. The solution was firstly warmed to about 100°C, then gradually added fibroin into the solution and stirred with controlled temperature at 100-105°C until silk already hydrolyzed. The hydrolysate was neutralized, filtrated, dialyzed in cellulose tube against distilled water for 3 days at room temperature and concentrated.

Nang Lai variety: The Nang Lai variety was dissolved by using the mixture solution of $CaCl_2$/Ethanol/H_2O (1:2:8 mole ratios) follow with earlier reported by Ajisawa (1998). In addition, the solution used for dissolving of Eri silk was also used.

Preparation of silk film: The silk solution was stirred and then 10 mL solution was cast on the polystyrene plates. The plates were left air dried at room temperature for about 2-3 days. Finally, the films with a thickness of 20-30 μm were removed.

Investigation of silk films: The silk films were analyzed for their structure with fourier transform infrared spectroscopy (FTIR, Perkin Elmer-Spectrum Gx) in the spectral range of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans. FTIR was used to measure the absorption bands which represented the silk structure. A TA-Instrument TG SDT Q600 thermogravimetric analyzer was used to determine of the thermal decomposition pattern of the silk films. The analysis condition was 50-100°C for heating sample at 20°C min^{-1} rates under nitrogen atmosphere.

Peptide bond measurements: To confirm the hydrolysis of silk, biuret reaction was performed as earlier reported by Wang *et al*. (2000). In brief, 1 mL of 20% of NaOH was added into 1 mL of 1% silk solution, then added 0.5% $CuSO_4$. The solution was then mixed until color became violet. The depth of color was correlated with the number of peptide bonds in the reaction. After placing at 37°C for about 10 min, the solution was cooled at room temperature and measured at 540 nm. In the same time, ninhydrin reaction was also tested. Briefly, 1 mL of silk solution was diluted with phosphate buffer pH 7.0 to obtain 5 dilutions at 10 folds for each. One milliliter of ninhydrin solution was added, mixed together and then boiled at 100°C for 10 min. The color became blue-violet, then observed and measured at 540 nm.

RESULTS AND DISCUSSION

Solubility of the wild silk in the different systems: As shown in the Table 1 % silk remaining was highest when used calcium nitrate, but lowest and fastest for 85% phosphate solution. Thus, the suitable solution for dissolving Eri silk was 85% phosphate. It quite similar with earlier reported by Wang *et al*. (2000) that Eri silk was dissolved by this solution but different in time used.

Nang Lai variety was dissolved by using $CaCl_2$/Ethanol/H_2O (1:2:8 mole ratios) and quite different when compared to the Eri silk. However, the solution from 85% phosphate was similar both physical appearance and biuret reaction as shown in Table 2. However, both biuret and ninhydrin reactions were supported that the current method is validity.

Results analyzed from Table 1 and 2 showed that the 85% phosphate solution could be hydrolyzed both of silk fibroin with related to the early studied of peptide bond remain (Wang *et al*., 2000). Time used in this study was shorter than earlier reported but result of biuret reaction was the same. In addition, silk fibroin of both silks could be hydrolyzed at the room temperature in the same condition (data not shown). This might be suggesting that the experiment time could be performed as shorter and more safety. However, it might be difference in the film appearances.

FTIR spectra of regenerated silk fibroin films: Generally, analysis of secondary structures of protein are indicated by the amide I (1700-1600 cm^{-1}) amide II (1600-1500 cm^{-1}) and amide III (1300-1200 cm^{-1}) bands (Kweon *et al*., 2000; Hino *et al*., 2003). The pattern of the

Table 1: Effect of salts solution on Eri silk

	Time (h)					
Type of salts	1	2	3	4	5	Silk remaining (%)
9M LiBr	___	___	___	___	1+	85
9M $Ca(NO_3)_2$	___	___	___	___	2+	65
9M $ZnCl_2$	___	___	___	___	1+	82
9M LiSCN	___	___	___	___	2+	70
85% phosphate	4+	4+	5+	5+	5+	5
mixture solution ($CaCl_2$/EtOH/H_2O)	___	___	___	___	1+	87

Remarks: ___ (not dissolve), + (less), 2+ (little), 4+ (good), 5+ (excellent)

Table 2: Characteristics of Eri and Nang Lai silk solution

Silk	Solution	pH	Color	Biuret reaction	Ninhydrin reaction
Eri	85% phosphate	6.9	Light yellow	Light	Deep
Nang Lai	85% phosphate	6.6	Light yellow	Light	Deep
	Mixture solution ($CaCl_2$/EtOH/H_2O)	6.5	Cream yellow	Deep	Light

Table 3: FTIR absorption bands of Eri and Nang Lai silk films

Silk	Amide I (cm^{-1})	Amide II (cm^{-1})
Eri	1629	1533
Nang Lai	1690, 1650, 1620	1445

absorptions in each film was observed. Figure 1 shows the FTIR spectra of the native Eri (A), Nang Lai (B) silk films and blended film of both silk solutions (C), respectively. The amide I (C = O stretching) band of the Eri silk strongly shown at 1629 cm^{-1}, while the Nang Lai variety were 1650 and 1620 cm^{-1} with a shoulder at 1690 cm^{-1} (Table 3). The results suggest that the Eri silk composed of more peptide bonds than the Nang Lai variety since the spectral region at 1700 to 1620 cm^{-1} is the vibration of peptide bond, but the band at wave number below 1620 is amino acid side chain vibration (Wang *et al*., 2000). It is clearly revealed that 85% phosphate solution could be hydrolyzed the Nang Lai variety to obtain smaller amino acids than Eri silk. These results were related to the previous results of biuret reaction.

The amide II (-NH_2 stretching) of Eri silk film was absorbed at 1533 cm^{-1} with small peak at 1448 cm^{-1}, while Nang Lai variety film showed at least 4 peaks: 1575, 1527, 1490 and 1450 cm^{-1}. IR spectra showed that the Eri silk film composed of the higher β-sheet (1533 cm^{-1}) structure than Nang Lai variety film (1575, 1527 cm^{-1}). Amide III of Eri silk film (1238 cm^{-1}) which was β-sheet form contrasted Nang Lai film (1243 cm^{-1}) was irregular structure (Hino *et al*., 2003). Both silk films were similar of C-O stretching (~1068 cm^{-1}) bands (Kweon *et al*., 2001). The absorption band at 3286 cm^{-1} was N-H stretching band of Eri silk film. This band was different for Nang Lai variety film which showed multi-bands at 3386, 3344, 3289 and 3236 cm^{-1}.

The IR spectra revealed that the Eri silk film strongly appeared with stable profiles of absorption bands contrasted to Nang Lai variety film. In addition, the absorption bands of Eri silk was higher indicated of the β-sheet structure than Nang Lai variety which composed of many structures including α-helix, β-sheet and unordered structures. With compare to previously report that fibroin film of wild silks are partly rich in α-helical structure than those of *B. mori* (Kweon and Park, 1999; Li *et al*., 2003). However, some researchers demonstrated that poly (L-alanine) is stable conformations of parallel-chain pleated sheet structure (Moore and Krimm, 1976; Simmond *et al*., 1996). Furthermore, glycine units related to different conformation (Miyazawa *et al*., 2002). Thus, it may be concerned to the structural changes in several fragment of Nang Lai variety fibroin since glycine are dominant amino acids component of *B. mori* silk. These results might be suggested that the Eri silk film stronger than Nang Lai variety film in mechanical properties.

Figure 2 indicated the thermogravimetric curves of the films. The results showed that the Nang Lai variety

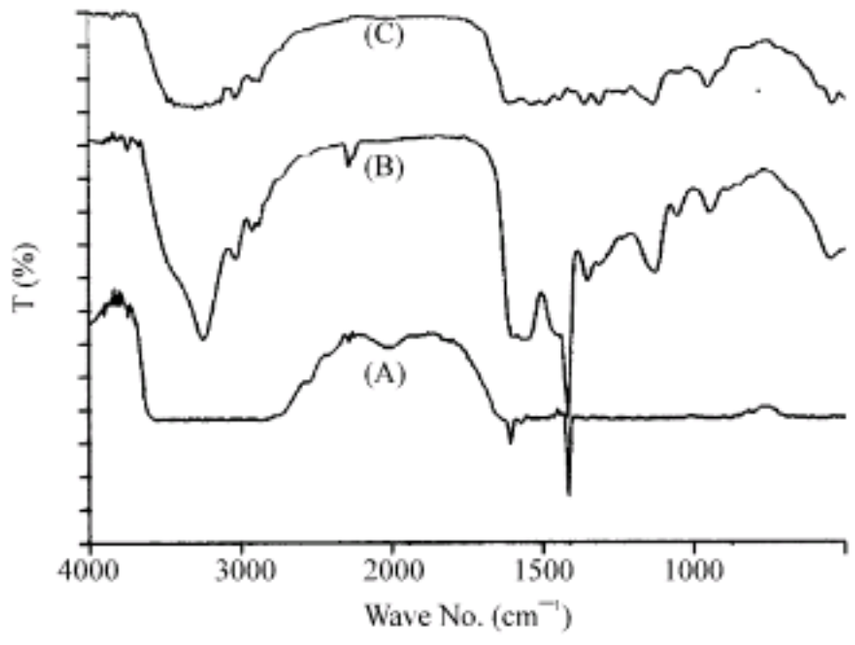

Fig. 1: IR spectra of (A) Eri, (B) Nang Lai and (C) combined of Eri and Nang Lai silk films

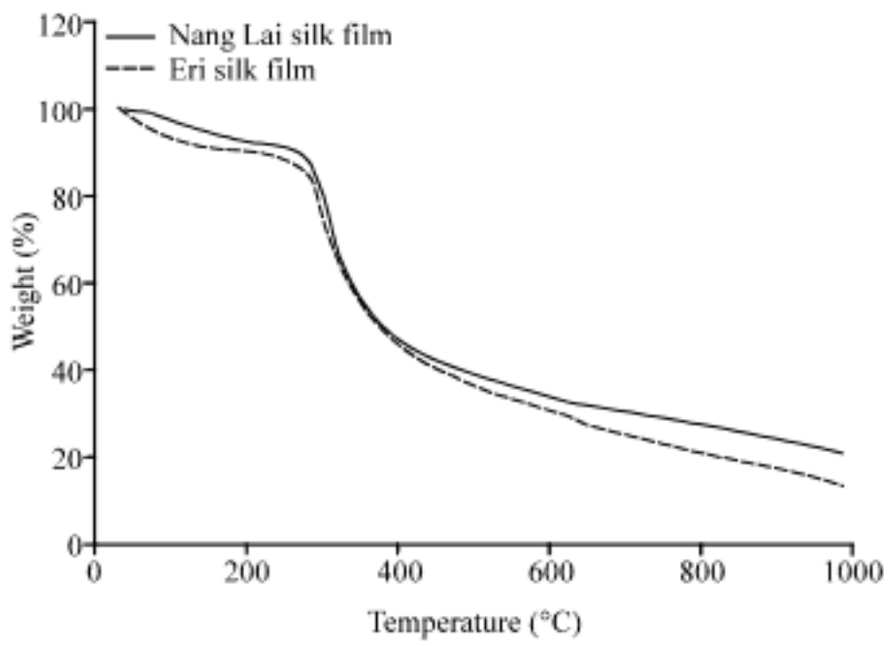

Fig. 2: Thermogravimetric curves of regenerated Eri and Nang Lai silk films

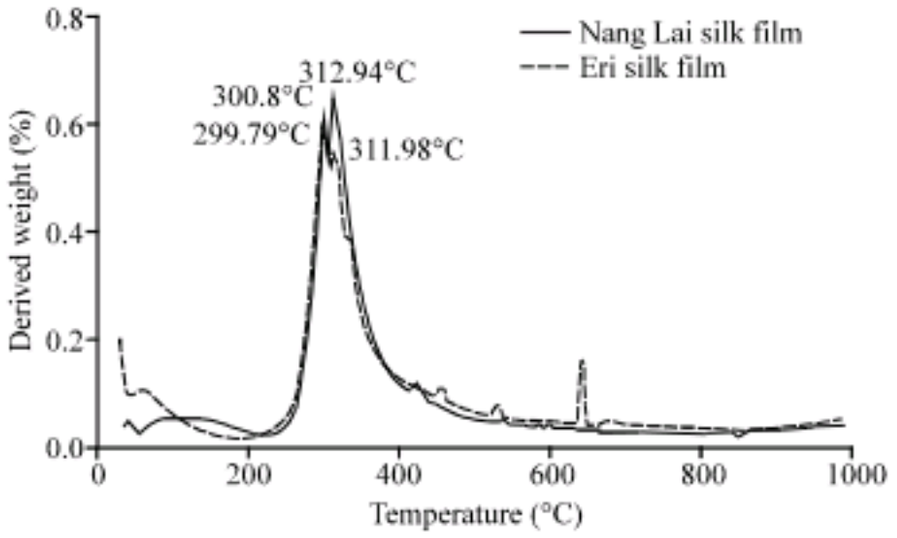

Fig. 3: DTG curves of Eri and Nang Lai silk films

film little stronger than the Eri silk film due to the break point was higher. However, the break points of both silk were closed at about 300°C with reveal by the DTG curves (Fig. 3). More details were the maximum tolerances to the temperature of the Nang Lai variety at about 313°C. The obtained results were arranged value in comparison to earlier report that the maximum tolerance of the

B. mori was 280-308°C (Zhang *et al*., 2002). However, for wild silk (*A. pernyi* and other belonging to the family Saturniidae) thermal decomposition underwent several steps (Kweon *et al*., 2000). Eri silk film found that the thermal decomposition was single step at 312°C. The result suggests that the components of silk were different profiles depend on the types or silk strains. In the cases of the silk film in this study, the compositions of both silk may be the same but different in the compositional ratio.

CONCLUSION

The solution of Eri and Nang Lai variety silks could be prepared by dissolving in 85% phosphate and could then use as samples for regenerating silk films. In general of the both silk films characteristics were similar compared with the earlier reported. Moreover, the thermal decomposition of the prepared films was also closed to the other reports. FTIR results were also confirmed that the method used in this study was practically created for silk solution preparation both domesticated and wild silks.

ACKNOWLEDGMENTS

We would like to thank Instrumental Center and Department of Chemistry, Faculty of Science and Silk Innovation Center, Mahasarakham University for conveniences analysis facilities.

REFERENCES

Ajisawa, A., 1998. Dissolution of silk fibroin with calcium chloride-ethanol aqueous solution. J. Seric. Sci. Jap., 67: 91-94.

Asakura, T., C. Tanaka, M. Yang, J. Yao and M. Kurokawa, 2004. Production and characterization of a silk-like hybrid protein, based on the polyalanine region of *Samia cynthia ricini* silk fibroin and a cell adhesive region derived from fibronectin. Biomaterials, 25: 617-624.

Chen, X., D.P. Knight, Z. Shao and F. Vollrath, 2001. Regenerated bombyx silk solutions studied with rheometry and FTIR. Polymer, 42: 9969-9974.

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 mylita*. 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.

Freddi, G., Y. Gotoh, T. Mori, I. Tsutsui and M. Tsukada, 1994. Chemical structure and physical properties of *Antheraea assama* silk. J. Applied Polym. Sci., 52: 775-781.

Hashimoto, K., Y. Yamano and I. Morishima, 2008. Induction of tyrosine hydroxylase gene expression by bacteria in the fat body of Eri-silkworm, *Samia cynthia ricini*. Comp. Biochem. Physiol. Part B, 149: 501-506.

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. Colloid Interface Sci., 266: 68-73.

Jin, H.J., S.V. Fridrikh, G.C. Rutledge and D.L. Kaplan, 2002. Electrospinning *Bombyx mori* silk with poly (ethylene oxide). Biomacromolecules, 3: 1233-1239.

Kweon, H.Y. and Y.H. Park, 1999. Structural and conformational changes of regenerated *Antheraea pernyi* silk fibroin films treated with methanol solution. J. Applied Polym. Sci., 73: 2887-2894.

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.

Li, M., W. Tao, S. Kuga and Y. Nishiyama, 2003. Controlling molecular conformation of regenerated wild silk fibroin by aqueous ethanol treatment. Polymer. Adv. Technol., 14: 694-698.

Miyazawa, M., M. Sonoyama and H. Saito, 2002. FT-IR studies on secondary structures of wild silkworm fibroin films. Int. J. Wild Silkmoth Silk, 5: 47-50.

Monti, P., G. Freddi, S. Sampaio, M. Tsukada and P. Taddei, 2004. Structure modifications induced in silk fibroin by enzymatic treatments. A Raman Study. J. Mol. Struct., 744-747: 685-690.

Moore, W.H. and S. Krimm, 1976. Vibrational analysis of peptides, polypeptides and proteins. II. β-poly (L-alanine) and β-poly(L-anaylglycine). Biopolymers, 15: 2465-2483.

Phillips, D.M., L.F. Drummy, D.G. Conrady, D.M. Fox and R.R. Naik *et al*., 2004. Dissolution and regeneration of *Bombyx mori* silk fibroin using ionic liquids. J. Am. Chem. Soc., 126: 14350-14351.

Rossitch, Jr, E., D.E. Bullard and W.J. Oakes, 1987. Delayed foreign-body reaction to silk sutures in pediatric neurosurgical patients. Child's Nerv. Syst., 33: 375-378.

Simmond, A.H., C.A. Michal and L.W. Jelinski, 1996. Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. Science, 271: 84-87.

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.

Wang, X.Y., G.Z. Li, H. Akai, R.Q. Ji, S.L. Shi and W.Y. Zheng, 2000. Production and physical properties of soluble wild silk powder. Int. J. Wild Silkmoth Silk, 5: 57-60.

Zhang, H., J. Magoshi, M. Becker, J.Y. Chen and R. Matsunaga, 2002. Thermal properties of *Bombyx mori* silk fibers. J. Applied Polymer Sci., 86: 1817-1820.