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Effect of Different Organic Solvents and Treatment Times on Secondary Structure and Thermal Properties of Silk Fibroin Films

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Abstract: The aims of this study are to deal with the secondary structures and thermal properties of the Silk Fibroin (SF) films after treatment with different organic solvents; ethanol, ethyl acetate and methanol at various times. Influence of the organic solvents on secondary structure and thermal behavior of the SF films were investigated using Fourier transform infrared (FT-IR) spectroscopy. The results showed the secondary structures of SF films were changed from random coil into high strength, β-sheet structure. With TG, DTG and Heat flow results, the SF films increased their thermal stability after treatment with the organic solvents. However, the stability of SF films did not affect by treatment times. Therefore, a treatment SF film with organic solvent for 30 min is suitable condition. Comparison among organic solvents used, methanol is the best solvent to induce strength and thermal properties of SF films. This study indicated that various organic solvent could be used to improve SF properties with short time treatment.

Key words: Film, silk fibroin, secondary structure, spectroscopy, thermal behavior

INTRODUCTION

In last decade, natural polymers have been discovered and were used as bioresources in both technological and biomedical applications (Kweon *et al*., 2001). Various excellent properties of natural polymers such as non-toxicity, biodegradability and biocompatibility were attracted to study. Therefore, many kinds of natural polymers were reported. However, some limitations such as their high cost and questionable purity have occurred (Cheung *et al*., 2008). Moreover, 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).

Silk is a fibrous polymer that is produced by some Lepidoptera larvae including *Bombyx mori* silkworm (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).

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Many factors such as environmental temperature and humidity at spinning, food and healthy condition of silkworm are often significant affects on the SF properties (Zhao *et al*., 2007). SF films often require chemical treatments in order to enhance their stability and mechanical properties (Tasukada *et al*., 1995). Methanol is one of frequently used to improve that stability and mechanical properties. However, treatment of SF with methanol can induce high β-sheet structure ratios, resulting of brittle materials and also occur instantly in an uncontrollable crystallization (Jeong *et al*., 2006). In addition, other solvents and reagents have also used for improving the properties of the films including KCl, NaCl (Vepari and Kaplan, 2007), hexane (Valluzzi *et al*., 1999), heat (Freddi *et al*., 1997). Here, we report the effect of different solvents on SF films after submersion in different time points. All SF films were then investigated their secondary structures and thermal behavior. We also attempt to compare present results in order to find the most suitable solvent for enhancing SF film properties.

MATERIALS AND METHODS

This study was constructed for 3 months from May 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, Mahasarakham University, Thailand.

Materials

The *Bombyx mori* silk cocoons were kindly supplied from Silk Innovation Centre (SIC) Mahasarakham University, Thailand. All used chemical reagents were analytical grade.

Sample Preparation

The silk cocoons were boiled twice with 1.5% (w/v) Na_2CO_3 to obtain silk fibroin (SF). The SF was dissolved with tertiary solvent system of $CaCl_2$:ethanol:H_2O (1:2:8 by mol). The solution was firstly warmed to about 90 °C, then gradually added SF into the solution and stirred with controlled temperature at 95-100°C until silk already dissolved (approximately 60 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 Films Preparation

Ten milliliter of 1% SF solution was cast on 5 cm diameter of polystyrene plates. The plates were left at 40°C in oven for 3 days. The SF films with approximately 10-15 μm thickness were obtained.

Organic Solvents Submersion

The SF films were immersed in different kinds of organic solvents: 80% ethanol, 80% ethyl acetate and 80% methanol. The treatment times were assigned for 30, 60 and 90 min. After finish in each time point, the SF films were left in air-dried and transferred into vacuum oven to make sure the films were completely dried.

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 ~2000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

Thermal Behavior Analysis

Eight to ten milligrams were prepared and 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 to 1000°C at a heating rate of 10°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 analysis of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS

FT-IR Investigation

The SF films treated with 80% ethanol showed strong absorption bands at 1,635 cm^{-1} (amide I), 1,539 cm^{-1} (amide II) and 1,242 cm^{-1} (amide III) in all of time points (Fig. 1) while SF films treated with ethyl acetate showed strong absorption bands at 1,630 cm^{-1} (amide I), 1,525 cm^{-1} (amide II) and 1,230 cm^{-1} (amide III) in all of time points (Fig. 2). On the other hand, SF films treated with methanol showed strong absorption bands at 1,630 cm^{-1} (amide I), 1,523 cm^{-1} (amide II) and 1,240 cm^{-1} (amide III) in all of time points (Fig. 3).

Thermal Behavior Analysis

Thermogravimetric (TG) curves showed that all of SF films did not completely decompose even at 1000°C. TG curves of SF films did not much different when treated with ethanol even increasing of time treatment (Fig. 4). This result was similar as gave from ethyl acetate (Fig. 5) and methanol (Fig. 6). The detail of those decomposition peaks were clearly evidenced by differential thermogravimetric (DTG) curves. For ethanol treated SF films, the

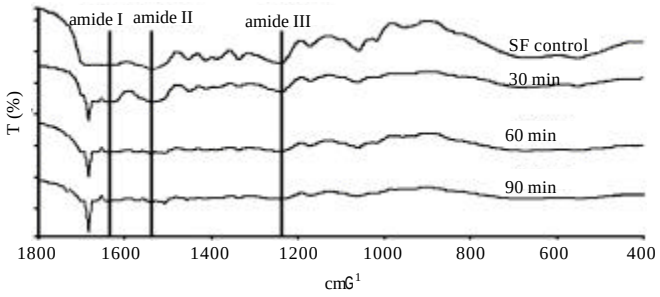

Fig. 1: FT-IR spectra of SF films treated with ethanol at different time point

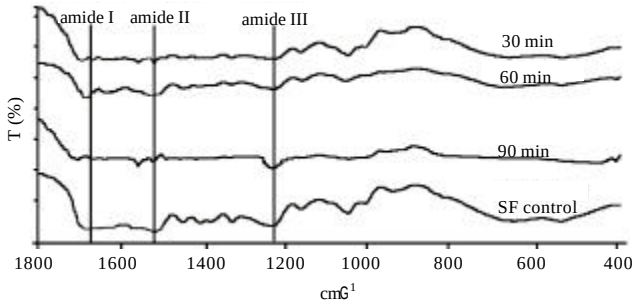

Fig. 2: FT-IR spectra of SF films treated with ethyl acetate at different time point

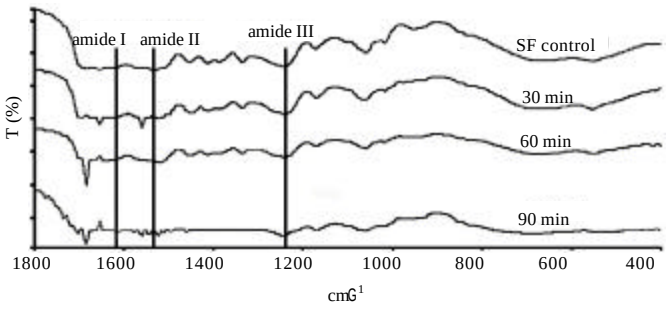

Fig. 3: FT-IR spectra of SF films treated with methanol at different time point

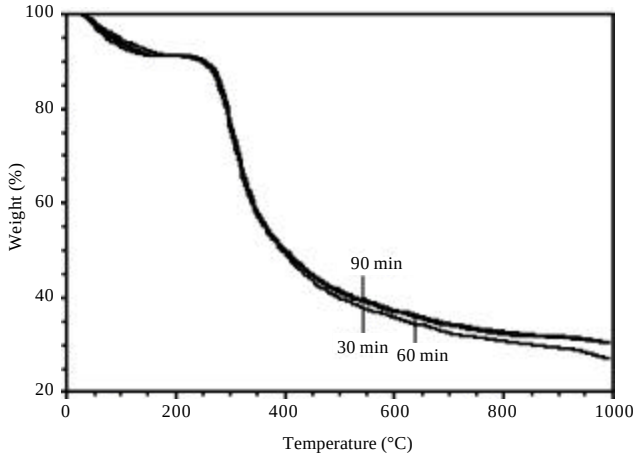

Fig. 4: TG curves of SF films treated with ethanol at different time point

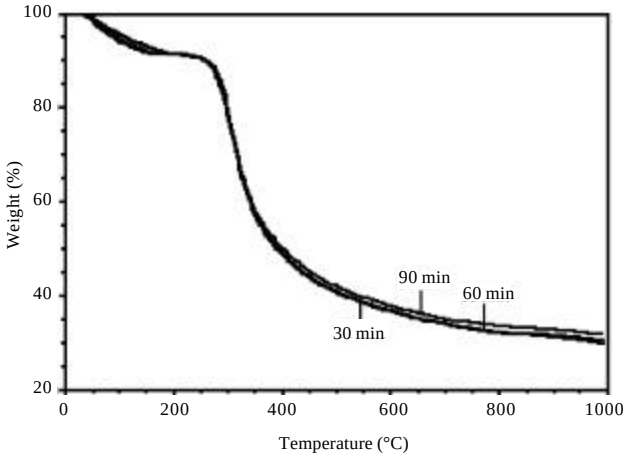

Fig. 5: TG curves of SF films treated with ethyl acetate at different time point

maximum decomposition temperature were 302°C (30 min) and 305°C (60 and 90 min) (Fig. 7). Ethyl acetate treated SF films showed maximum decomposition temperature at 302°C with minor peaks at 305°C in all of time set (Fig. 8). In case of SF films treated methanol, maximum decomposition temperature was approximately 306°C (Fig. 9).

Heat Flow Curves

With heat flow curves, all SF films showed thermal stability until above 200°C. Some different endothermic and exothermic temperatures after treatment SF films with various solvents were observed which were summarized in Table 1. The SF films showed both maximum exothermic and endothermic peaks in all of organic solvents at 60 min. For ethanol, the maximum of exothermic and endothermic peaks occurred at 450°C (Fig. 10), while ethyl acetate was 462°C (Fig. 11). The SF films treated with methanol showed the most stability compared to other solvents at 463 and 471°C of exothermic and endothermic absorptions, respectively (Fig. 12).

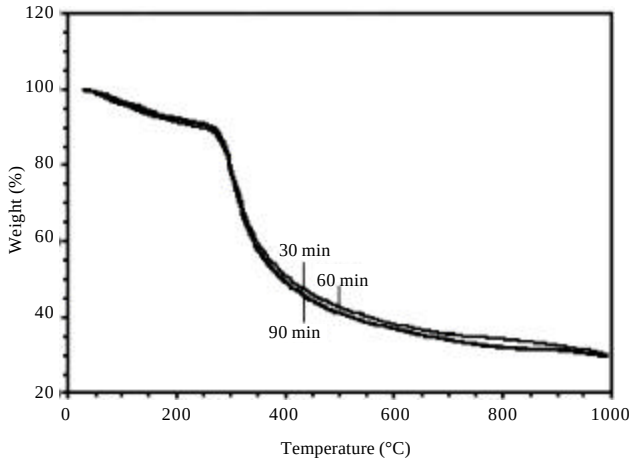

Fig. 6: TG curves of SF films treated with methanol at different time point

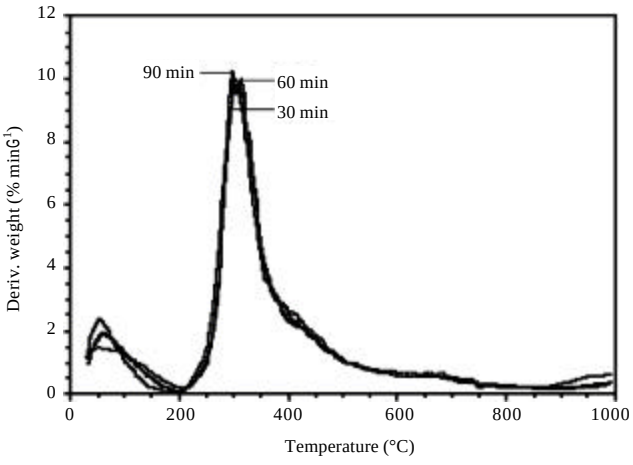

Fig. 7: DTG curves of SF films treated with ethanol at different time point

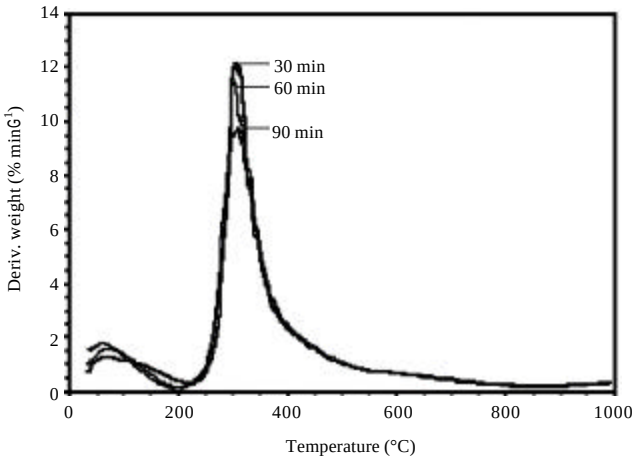

Fig. 8: DTG curves of SF films treated with ethyl acetate at different time point

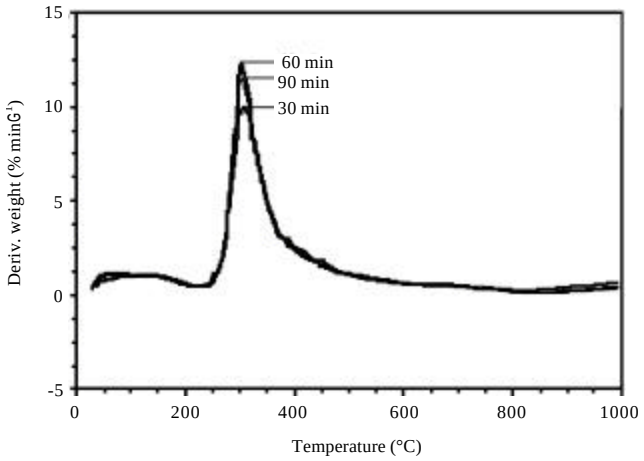

Fig. 9: DTG curves of SF films treated with methanol at different time point

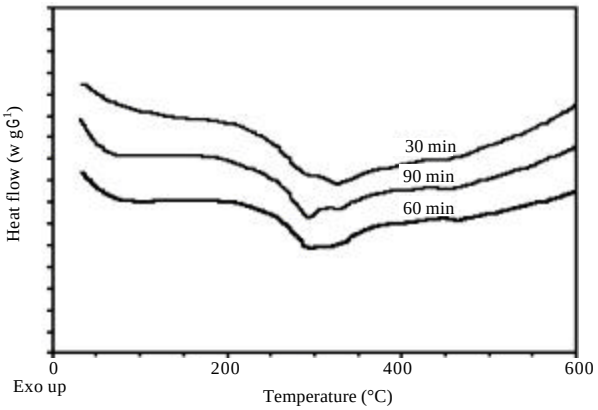

Fig. 10: Heat flow curves of SF films treated with ethanol at different time point

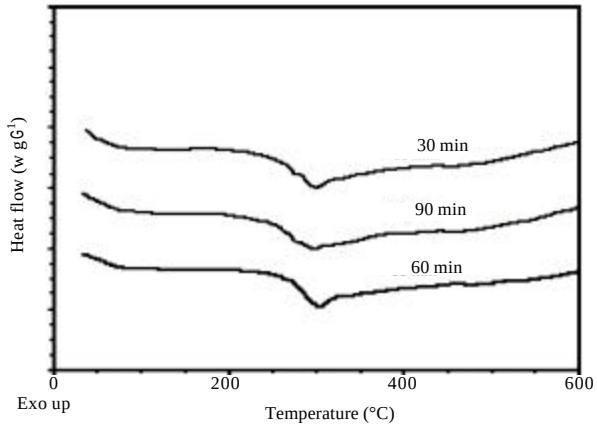

Fig. 11: Heat flow curves of SF films treated with ethyl acetate at different time point

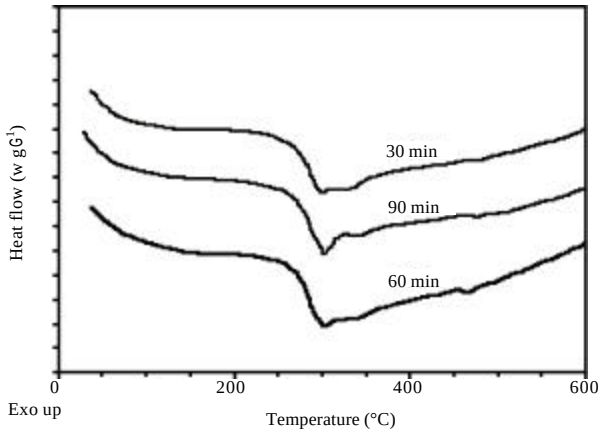

Fig. 12: Heat flow curves of SF films treated with methanol at different time point

Table 1: Exothermic and endothermic temperature of the SF films treated with different organic solvents

Solvents	Time (min)	Exothermic (°C)	Endothermic (°C)
Ethanol	30	226, 428	288, 326, 450
	60	217, 450	294, 327, 450
	90	220, 422	290, 327, 450
Ethyl acetate	30	195, 440	300, 330, 460
	60	196, 460	301, 330, 462
	90	195, 442	300, 330, 460
Methanol	30	251, 455	300, 340, 470
	60	253, 463	299, 330, 471
	90	252, 463	300, 340, 471

DISCUSSION

Secondary Structure

The conformational structure of SF has often been studied by FT-IR spectroscopy (Tasukada *et al*., 1995) as the IR spectrum indicates typical absorption bands sensitive to the molecular conformation of SF (Kweon *et al*., 2000). The 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 FT-IR spectrum of SF films treated with organic solvents showed absorption bands shifted into lower wave number, indicating the β-sheet structure (Kweon *et al*., 2000). The FT-IR results also found that treatment times did not much affect on the SF film structures. These results suggested that 30 min is enough for organic solvent treatment. The strength of the SF films after submersion in organic solvents was improved by inducing structural interaction from random coil to β-sheet structure (She *et al*., 2008). The effect of used organic solvents on SF film structures were implied as methanol>ethyl acetate>ethanol, respectively.

Thermal Behavior Analysis

With TG curves, the initial weight loss at below 100°C was due to the moisture evaporation from the films (Kweon *et al*., 2001). At low temperature, weight of the SF film was gradually loss but was rapidly loss upper 200°C 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 SF has high thermal stability. The thermal decomposition of all SF films was sharply showed at around 300-305°C. The $T_{d, max}$ of the SF films treated with organic solvents were sequentially arranged of methanol>ethanol=ethyl acetate. This result suggested that methanol be most suitable solvent for improving SF films stability. With heat flow curves, the first endothermic peaks at below 100°C distributions to the dehydration appeared in all of samples. The SF films treated with methanol appeared sharp endothermic peaks similar patterns at around 300°C for every treatment times as well as SF films treated with ethyl acetate. However, the endothermic peaks were less sharp. The SF films treated with ethanol showed lower of endothermic peaks than other at about 290°C. The sharp endothermic and exothermic peaks at around 290-300°C have been described as the strong molecular motion of α-helix and its transformation into β-sheet structure (Li *et al*., 2003). Moreover, all of SF films showed 2 minor endothermic peaks after treatment with ethanol (327, 450°C), ethyl acetate (340, 460°C) and methanol (340, 475°C), respectively. It is well known that these endothermic are due to the decomposition of fibroin molecules with unoriented β-sheet structure (Kweon *et al*., 2000). Therefore, it is no doubt that the organic solvents used in this study could be helped to increase thermal stability of the SF films by changing the higher ratio of secondary structure from random coil and α-helix into β-sheet structure.

CONCLUSION

The SF films were prepared and used as substrate to treat with organic solvents; ethanol, ethyl acetate. The effects of the solvents and treatment times on morphology, secondary structure and thermal properties of the film were investigated. FT-IR results showed the intermolecular interactions between SF molecules which caused from water dehydration by organic solvents treatments. These results influenced to the secondary structures of SF films which were changed from less strength into high strength, β-sheet structure. With TG, DTG and Heat flow results, the SF films increased their thermal stability after treatment with the organic solvents. However, the stability of SF films did not affect by treatment time. Therefore, treatment SF films with organic solvent for 30 min are enough. Comparison between organic solvents used, methanol is the best solvent to enhance strength and thermal properties of SF films. In conclusion, this study indicated that various

organic solvent could be used to improve SF properties with short time treatment. This might be advantage to modify the SF properties for various applications such as biomedicine and pharmaceutics materials.

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