IJIE Int. J. Indust. Entomol. 40(1) 28-32 (2020)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2020.40.1.28

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)
[Note]

Effect of addition of methanol on rheological properties of silk formic acid solution

Yu Jeong Bae and In Chul Um*

Department of Biofibers and Biomaterials Science, Kyungpook National University, Daegu 41566, Republic of Korea

Abstract

Recently, many studies have been undertaken on the wet spinning and electrospinning of silk because wet-spun fibers and electrospun webs of silk can be applied in the biomedical and cosmetic fields owing to the good biocompatibility of silk. The rheological properties of silk solution are important because they strongly affect the spinning performance of the silk solution and the structures of resultant fibrous materials. Therefore, as a preliminary study on the effect of solvent composition on the rheological properties of silk fibroin (SF) solution and structure of the resultant film, in the reported work, methanol was added to the SF formic acid solution. A small amount of methanol (i.e. 2%) added to the SF formic acid solution significantly altered the rheological properties of the solution: its shear viscosity increased by 10 folds at low shear and decreased on increasing the shear rate, demonstrating shear thinning behavior of the SF solution. Dynamic tests for the SF solution indicated that the addition of 2% methanol altered the viscous state of the SF formic acid solution to elastic. However, the molecular conformation (i.e. β-sheet conformation) of the regenerated SF film cast from formic acid remained unchanged on the addition of 2% methanol.

Int. J. Indust. Entomol. 40(1), 28-32 (2020)

Received : 12 Feb 2020
Revised : 3 Mar 2020
Accepted : 10 Mar 2020

Keywords:
silk fibroin,
rheological properties,
solvent composition,
molecular conformation

Introduction

Silk has attracted the attention of researchers in biomedical and cosmetic applications because silk offers useful properties as a biomaterial such as blood compatibility (Sakabe *et al.*, 1989; Um *et al.*, 2002), good cell adhesion and proliferation (Minoura *et al.*, 1995), and low inflammatory reaction in a body (Meinel *et al.*, 2005).

To apply silk in the biomedical and cosmetic fields, silk is tailored into various forms. Therefore, recently, studies on electrospun silk webs and wet-spun silk have been conducted extensively because fibrous and porous silk materials can be fabricated by wet-spinning and electrospinning (Cho *et al.*, 2012; Chung and Um, 2014; Ha *et al.*, 2005; Ko *et al.*, 2018; Park and Um, 2018).

Rheological properties of silk solution are among the important factors that affect the wet- and electro-spinning performance of silk. For instance, it has been reported that the fiber diameter, porosity, and electrospinning rate of electrospun silk web are strongly dependent on the viscosity of silk dope (Park and Um, 2018; Jang and Um, 2015; Kim and Um, 2014; Park and Um, 2017; Yoon *et al.*, 2013). This suggests that we can control the

---

***Corresponding author.**
In Chul Um (Ph.D.)
Department of Biofibers and Biomaterials Science, Kyungpook National University, Daegu 41566, Republic of Korea
Tel: +82-53-950-7757 / FAX: +82-53-950-6744
E-mail: icum@knu.ac.kr

wet- and electro-spinning performance of silk solution if we can alter the rheological properties of the silk solution. The solvent is one of the crucial factors affecting the rheological properties of the silk solution. Cho *et al.* (2012) and Jo and Um (2015) reported that the rheological properties of silk solution in water and formic acid are quite different.

In the reported study, as a preliminary investigation of the effect of solvent composition on the rheological properties of silk solution, methanol was added to a silk fibroin (SF) formic acid solution and the rheological properties of the regenerated SF solution and molecular conformation of the resultant SF film were examined.

Materials and Methods

Preparation

The regenerated SF was prepared by using a method reported previously (Cho *et al.*, 2012; Chung *et al.*, 2015; Park and Um, 2018). Briefly, *Bombyx mori* silk cocoons were degummed with a sodium oleate (0.3% (w/v)) and sodium carbonate (0.2% (w/v)) solution at 100 °C for 1 h and the liquor ratio was set at 1:25. After the degumming process, the cocoons were rinsed thoroughly with purified water and dried at 105 °C. The purified water was obtained using a water purification system (RO50, Hana Science, South Korea) with a reverse osmosis membrane.

To prepare regenerated SF samples, the degummed silk was dissolved in a ternary solvent containing $CaCl_2/H_2O/EtOH$ (1/8/2 molar ratio) at 85 °C for 3 min. For dissolution, a liquor ratio of 1:20 was used. After dissolution, the SF solutions were dialyzed with a cellulose tube (molecular weight cut off = 12,000–14,000) against circulating purified water for 4 days at room temperature. The regenerated SF solutions were then dried to obtain the regenerated SF powders.

To prepare a 9% (w/w) regenerated SF formic acid solution, the regenerated SF powders were dissolved in 98% formic acid at 250 rpm for 3 h. A 9% (w/w) regenerated SF formic acid/methanol (98/2) solution was prepared by adding methanol to the regenerated SF formic acid solution.

20 mL aliquots of 9% (w/w) regenerated SF solutions (100/0 and 98/2 formic acid/methanol) were poured into petri-dishes in fume hoods to prepare regenerated SF films.

Measurement and characterization

9% regenerated SF solutions with different solvent compositions were used for the rheological measurements. The shear viscosity was measured by a rheometer (MARS III, Hakke, Germany) using a cone and plate geometry with a shear rate of 0.1–100 s^{-1} at 20 °C. The radius and angle of the cone were 60 mm and 1°, respectively. Frequency sweep tests were conducted to evaluate the complex viscosity of the SF solutions. The angular frequency ranged from 0.1 to 100 rad/s at 20 °C with a strain of 0.1% (Jo and Um, 2015; Park and Um, 2015).

A Fourier transform infrared spectrometer (FTIR, Nicolet 380, Thermo Fisher Scientific, USA) was operated in the attenuated total reflection mode to examine the molecular conformation and crystallinity index of the regenerated SF films (Park *et al.*, 2019a, 2019b; Park and Um, 2018). The crystallinity index was calculated from the FTIR spectrum as the ratio of the intensity of the peaks at 1260 and 1235 cm^{-1} using Eq. 1.

$$\text{Crystallinity index(\%)} = \frac{A_{1260\,cm^{-1}}}{A_{1235\,cm^{-1}}} \times 100 \qquad (1)$$

where $A_{1235cm^{-1}}$ is the absorbance at 1235 cm^{-1} and $A_{1260cm^{-1}}$ is the absorbance at 1260 cm^{-1}.

Results and Discussion

Rheological properties of SF solution

Fig. 1 shows the steady-state flow of the regenerated SF solution with different solvent compositions. The SF formic acid solution (i.e. 100/0 formic acid/methanol) showed almost Newtonian flow behavior consistent with previous reports (Chung and Um, 2014; Park and Um, 2015; Jang and Um, 2015). In the case of the 98/2 formic acid/methanol SF solution, the shear viscosity at 0.1 s^{-1} increased remarkably (i.e., 10 fold) and decreased on increasing the shear rate, showing shear thinning behavior. As can be seen in Fig. 2, the complex viscosity of regenerated SF solutions according to the angular frequency showed a very similar trend as the shear viscosity of the SF solution. This indicates that a dynamic test can be utilized to examine the steady-state flow of SF solutions.

It is interesting to note that addition of only 2% methanol in formic acid results in a significant change in the steady-state flow of the SF formic acid solution. Formic acid has been

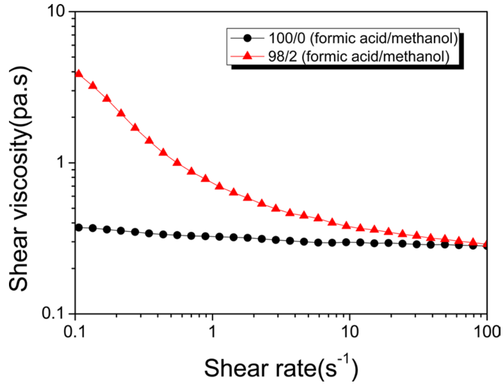

Fig. 1. Effect of methanol addition on steady state flow of 9% (w/w) SF formic acid solution.

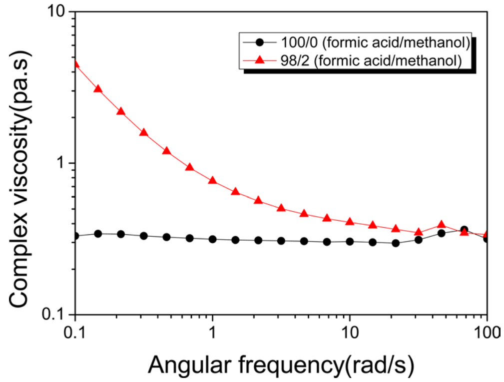

Fig. 2. Effect of methanol addition on complex viscosity of 9% (w/w) SF formic acid solution.

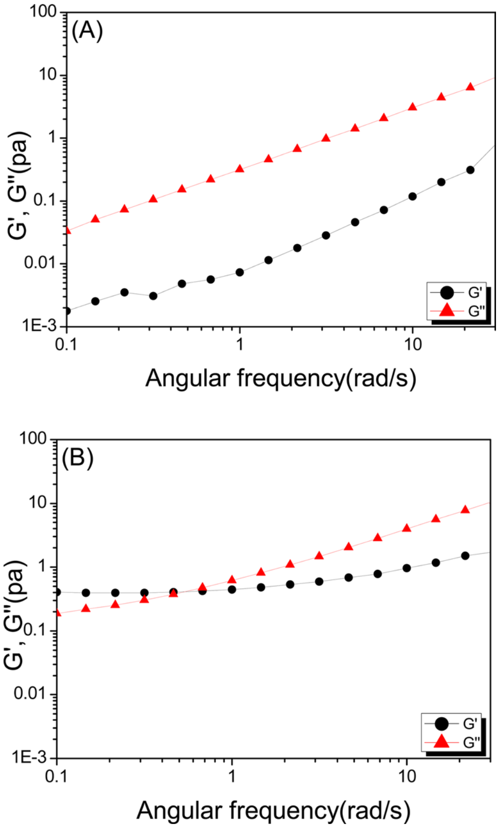

Fig. 3. Effect of methanol addition on dynamic test results of 9% (w/w) SF formic acid solution: (A) 100% formic acid solution and (B) 98/2 formic acid/methanol solution.

used as a good solvent for regenerated SF. Therefore, it has been extensively used in wet-spinning and electro-spinning of regenerated SF solutions (Jang and Um, 2015; Ki *et al.*, 2007; Kim and Um, 2014; Ko *et al.*, 2018). On the other hand, methanol has been used as an effective coagulant for wet-spinning of SF because it is non-solvent for SF (Um *et al.*, 2004; Jang and Um, 2015). Therefore, a significant increase in the SF solution with 2% methanol might be due to the aggregation or flocculation of SF molecules in formic acid. In other words, SF molecules in formic acid becomes aggregated or flocculated owing to methanol raising the viscosity in the low-shear region (i.e. 0.1 s^{-1} or rad/s). As the shear rate is increased, the aggregate or floc of SF molecules induced by methanol becomes disrupted leading to a decreased viscosity. As a result, in the high-shear region (i.e. 100 s^{-1} or rad/s), the shear viscosity of the SF formic acid solution with 2% methanol becomes very similar to the shear viscosity of SF formic acid solution.

To examine the SF solution state depending on the solvent composition in more detail, the viscoelasticity of SF solutions was evaluated by dynamic tests. As can be seen in Fig. 3(A), in the case of the 100% formic acid SF solution, G" was higher than G' in the range of 0.1–30 rad/s. This implies that the viscous state (i.e., liquid phase) was more dominant in this sample. On the other hand, in the case of the 98/2 formic acid/methanol solution, a crossover point appeared at ~0.6 rad/s, i.e., at low shear (<0.6 rad/s), G' was higher than G", indicating an elastic state (i.e., solid state) more evidently for this sample. This result supports the assumption that aggregation or flocculation of SF molecules occur for a 2% methanol content.

The results of the rheological measurements for regenerated SF solutions with different solvent compositions indicate that the viscosity of the SF solution, which is an important parameter in the wet- and electro-spinning of SF, can be changed by varying the solvent composition, even though only 2% methanol addition was tested. It is thought that it is necessary to examine the effect of various solvent compositions on the rheological properties of the SF solution in the subsequent studies.

Molecular conformation of SF film

Fig. 4 displays the FTIR spectra of regenerated SF films cast from 100% formic acid and 98/2 formic acid/methanol. Regardless of the solvent composition, both SF films showed IR absorption peaks at 1620 cm^{-1} and 1510 cm^{-1}, and a shoulder at 1260 cm^{-1} attributed to β-sheet conformation (Park *et al.*, 2019a, 2019b; Park and Um, 2018). Considering that formic acid and methanol induces β-sheet crystallization of SF (Um *et al.*, 2001), this result is not surprising. A more important issue is the effect of solvent composition on the amount of β-sheet crystallite formation in SF films (i.e., crystallinity of SF film).

Therefore, the crystallinity index was calculated from IR peaks in the amide III band and the results are shown in Fig. 5. As can be seen in figure, it was hard to recognize the difference in the crystallinity indices of the two films. Considering 2% addition of methanol altered remarkably the rheological properties of silk formic acid solution, it is interesting to note that 2% addition of methanol did not change the crystallinity index of regenerated SF film cast from formic acid. This might be due to the different role of methanol and formic acid against solution state of SF and crystallization of SF. That is, as for the solution state of SF, formic acid is a good solvent for SF, whereas methanol is a bad solvent (i.e. non-solvent). Therefore, only 2% addition of methanol results in formation of floc or aggregate of SF molecules. One the other hand, formic acid only does not lead floc or aggregation of SF molecules.

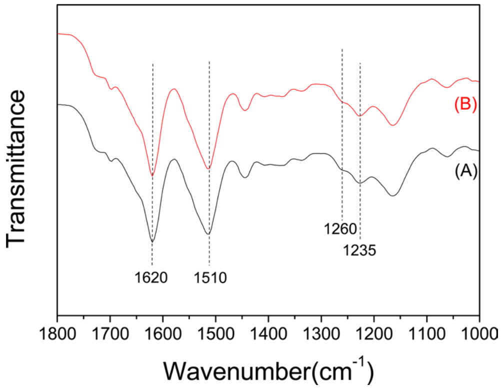

Fig. 4. FT-IR spectra of regenerated SF films cast from (A) 100% formic acid solution and (B) 98/2 formic acid/methanol solution.

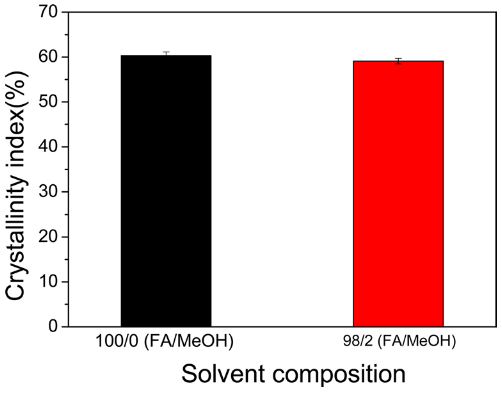

Fig. 5. Crystallinity index of regenerated SF films cast from different solvent compositions.

However, as for the crystallization of SF, it has been reported that formic acid and methanol are effective crystallization agents for SF. Owing to similar crystallization character of formic acid and methanol for SF, 2% addition of methanol does not lead to a change of crystallinity index of SF film cast from formic acid.

Conclusions

In this study, as a preliminary investigation of the effect of solvent composition on the rheological properties of regenerated SF solution was conducted, wherein methanol was added into SF formic acid solutions and the rheological properties were determined and the molecular conformation of resultant SF films was examined.

It was found that the rheological properties of the SF solution changed remarkably on the addition of 2% methanol. On the other hand, the addition of 2% methanol barely affected the molecular conformation of the resultant regenerated SF films.

The results obtained in this study indicate that the rheological properties of the SF solution, which determine the wet- and electro-spinning performance, can be controlled by manipulating the solvent composition. Therefore, more detailed and systematic studies on the effect of solvent composition on the rheological properties of SF solution should be conducted in the future to gain control over the fiber spinnability of SF solutions.

Acknowledgments

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (NRF-2017R1A2B4003655).

References

Cho HJ, Ki CS, Oh J, Lee KH, Um IC (2012) Molecular weight distribution and solution properties of silk fibroins with different dissolution conditions. Int J Biol Macromol 51, 336-341.

Cho HJ, Yoo YJ, Kim JW, Park YH, Bae DG, Um IC (2012) Effect of molecular weight and storage time on the wet- and electro-spinning of regenerated silk fibroin. Polym Degrad Stabil 97, 1060-1066.

Chung DE, Kim HH, Kim MK, Lee KH, Park YH, Um IC (2015) Effects of different *Bombyx mori* silkworm varieties on the structural characteristics and properties of silk. Int J Biol Macromol 79, 943-951.

Chung DE, Um IC (2014) Effect of molecular weight and concentration on crystallinity and post drawing of wet spun silk fibroin fiber. Fiber Polym 15, 153-160.

Ha SW, Tonelli AE, Hudson SM (2005) Structural studies of *Bombyx mori* silk fibroin during regeneration from solutions and wet fiber spinning. Biomacromolecules 6, 1722-1731.

Jang MJ, Um IC (2015) Effect of different *Bombyx mori* silkworm varieties on the wet spinning of silk fibroin. Int J Indust Entomol 30, 75-80.

Jo YN, Um IC (2015) Effects of solvent on the solution properties, structural characteristics and properties of silk sericin. Int J Biol Macromol 78, 287-295.

Ki CS, Kim JW, Oh HJ, Lee KH, Park YH (2007) The effect of residual silk sericin on the structure and mechanical property of regenerated silk filament. Int J Biol Macromol 41, 346-353.

Kim HJ, Um IC (2014) Relationship between the rheology and electro-spinning performance of regenerated silk fibroin prepared using different degumming methods. Korea-Aust Rheol J 26, 119-125.

Ko JS, Ki CS, Um IC (2018) Effect of sericin content on the structural characteristics and properties of electro-spun regenerated silk. Fiber Polym 19, 507-514.

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

Minoura N, Aiba S, Gotoh Y, Tsukada M, Imai Y (1995) Attachment and growth of cultured fibroblast cells on silk protein matrices. J Biomed Mater Res 29, 1215-1221.

Park BK, Nho SK, Um IC (2019a) Molecular conformation and crystallinity of white colored silkworm cocoons with different silkworm varieties. Int J Indust Entomol 38, 18-23.

Park BK, Nho SK, Um IC (2019b) Crystallinity of yellow colored silkworm variety cocoons. Int J Indust Entomol 38, 51-55.

Park BK, Um IC (2015) Effect of Korean *Bombyx mori* variety on electro-spinning performance of regenerated silk fibroin. Fiber Polym 16, 1935-1940.

Park BK, Um IC (2017) Effects of electric field on the maximum electro-spinning rate of silk fibroin solutions. Int J Biol Macromol 95, 8-13.

Park BK, Um IC (2018) Effect of molecular weight on electro-spinning performance of regenerated silk. Int J Biol Macromol 106, 1166-1172.

Park CJ, Um IC (2018) Effect of heat treatment on the structural characteristics and properties of silk sericin film. Int J Indust Entomol 37, 36-42.

Sakabe H, Ito H, Miyamoto T, Noishiki Y, Ha WS (1989) *In vivo* blood compatibility of regenerated silk fibroin. Sen’i Gakkaishi 45, 487-490.

Um IC, Kweon H, Lee KG, Ihm DW, Lee J, Park YH (2004) Wet spinning of silk polymer: I. Effect of coagulation conditions on the morphological feature of filament. Int J Biol Macromol 34, 89-105.

Um IC, Kweon HY, Hwang CM, Min BG, Park YH (2002) Structural characteristics and properties of silk fibroin/polyurethane blend films. Int J Indust Entomol 5, 163-170.

Um IC, Kweon HY, Park YH, Hudson S (2001) Structural characteristics and properties of the regenerated silk fibroin prepared from formic acid. Int J Biol Macromol 29, 91-97.

Yoon K, Lee HN, Ki CS, Fang D, Hsiao BS, Chu B, *et al.* (2013) Effect of degumming conditions on electro-spinning rate of regenerated silk. Int J Biol Macromol 61, 50-57.