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Preparation of Flexible Silk Fibroin Films Plasticized with Glucose

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Abstract: Aim of this study was to prepare flexible silk fibroin films by blending with glucose. The silk fibroin films from the silkworm, *Bombyx mori*, were prepared by solution evaporation technique. Solution blending was used to blend glucose into silk fibroin films to enhance film flexibility and wettability. Intermolecular bonding between silk fibroin and glucose can be observed from spectra of Fourier transform infrared spectroscopy. Images of scanning electron microscopy showed that the film morphology was homogeneous throughout the film matrices. Flexibility of silk fibroin films were improved by blending glucose. Elongations at break of the silk fibroin/glucose blended films increased and tensile strengths decreased as the glucose ratio increased. These flexible silk fibroin films provide potential new biomaterials for use in biomedical, pharmaceutical and packaging applications.

Key words: *Bombyx mori*, biodegradable films, plasticization, mechanical properties

INTRODUCTION

Silk fibroin (SF) is a biodegradable and biocompatible natural protein polymer created by the *Bombyx mori* silkworm (Altman *et al*., 2003) and has recently been extensively investigated as a biomaterial such as matrix for cell culture substrate (Inouye *et al*., 1998) and drug delivery system (Hofmann *et al*., 2006; Wang *et al*., 2007). The minimal inflammatory reactions *in vitro* and *in vivo* of SF film have been reported by Meinel *et al*. (2005). However, the SF films were limited for practical use due to their very brittle in the dry state. Blending is probably the best alternative for its convenience and effectiveness. Blended films of SF/poly(ethylene oxide) (Jin *et al*., 2004), SF/chitosan (Kweon *et al*., 2001) and SF/nylon (Liu *et al*., 2004) have been studied before and some blended films even showed satisfactory properties. However, blending of SF with the other macromolecules exhibited severe phase separation. Then, the preparation of blending of SF with small molecules was interested in this research to prevent phase separation.

Conformation transition of SF from random coil form to β-sheet form can be induced by treatments such as heating, stretching and/or immersion in polar solvents. This transition makes SF attractive as a biomaterial because SF with a β-sheet structure is resistant to water and has good mechanical properties. In the present study, the flexibility of SF film was improved by blending with glucose. The blended films were analyzed by FTIR spectroscopy, scanning electron microscopy (SEM) and tensile testing. Film transparency and moisture uptake of the films were also determined.

MATERIALS AND METHODS

Materials

Silk Fibroin (SF) solution was prepared as described in follows. Cocoons from *B. mori* were degummed by boiling twice in 0.5% Na_2CO_3 solution at 95°C for 30 min to remove sericin, then rinsed

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with distilled water and dried at room temperature. Degummed SF fibers were dissolved in the ternary solvent, $CaCl_2$-ethanol-water (mole ratio = 1:2:8), by stirring at 80°C for 2 h. The resulting SF solution was then dialyzed in cellulose tube for 3 days against distilled water. The final concentration after dialysis was adjusted to 1% (w/v) against distilled water. All solvents and non-solvents were of analytical grade. Glucose (Ajax, Australia) was used as without further purification.

Methods

Preparation of SF/Glucose Blended Films

The SF/glucose blended films were prepared by dissolving the appropriate amount of glucose in SF solution before film casting on polystyrene Petri-dishes. The films with SF/glucose blended ratios of 8/0, 8/1, 8/2, 8/4 and 8/8 (w/w) were investigated. Each blended film was fabricated from 20 mL of 1% (w/v) SF solution. The films were dried at 40°C for 48 h and *in vacuo* at room temperature for a week before characterization.

Characterization of SF/Glucose Blended Films

FT-IR spectra were collected by Fourier transform infrared (FT-IR) spectroscopy using Perkin-Elmer Spectrum GX FTIR spectrophotometer with air as the reference. The resolution of 4 cm^{-1} and 32 scans were chosen in this study.

Film morphology was investigated by Scanning Electron Microscopy (SEM) using JEOL JSM-6460LV SEM. The film was fractured in liquid nitrogen and coated with gold for enhancing the surface conductivity before scan.

Mechanical properties, elongation at break, tensile strength at break and Young's modulus were preformed by tensile tester using Instron Model 4301 Universal Testing Machine with 50±5% Relative Humidity (RH). The films with 10×25 mm in size were tested with the speed of 50 mm min^{-1} and 1 kN load cell.

Film transparency was determined by measuring the percent transmittance at 660 nm using UV-Visible spectrophotometer (Lamda 25, Perkin-Elmer Instrument) earlierdescribed (Srisuwan *et al*., 2008).

Percent moisture uptake of the films was determined by the method as described as follows (Khamhan *et al*., 2008). The sample films with 20×20 mm in size were dried in vacuum at room temperature for a week. After weighing, they were kept in a desiccator with 90±5% RH maintained with a saturated sodium chloride solution at 30±2°C. The sample films were weighed again after kept in the desiccator for a week. The percent moisture uptake was calculated from Eq. 1. The moisture (%) uptakes are the average of three different measurements.

$$\text{Moisture uptake (\%)} = \frac{\text{Mf-Mi}}{\text{Mi}} \times 100 \quad (1)$$

where, M_i and M_f are the initial and final weights (g) of the films before and after moisture uptake, respectively.

RESULTS AND DISCUSSION

FT-IR Spectra

Intermolecular interactions between SF film matrices and glucose were determined from FTIR spectra. The positions of absorption bands especially amide I and II bands indicate the conformation of SF. Figure 1 shows FTIR spectra of the blended films with different SF/glucose ratios. The absorption bands of SF film in Fig. 1a at 1654 cm^{-1} (amide I), 1558 cm^{-1} (amide II) and 1239 cm^{-1} (amide III) were assigned to predominantly random coil structure of SF film (Kweon *et al*., 2001; Jin *et al*., 2004), while the band at 1533 cm^{-1} attributed to β-sheet conformation. The shifting of these

bands was found after the blending glucose indicated the SF conformation transition was occurred. The amide I and II bands were shifted to 1634 and 1543 cm^{-1}, respectively, in accordance with the structural changes to predominantly β-sheet conformation. The amide I band at 1669 cm^{-1} also attributed to β-sheet form (Jin *et al*., 2004).

In addition, the amide III bands clearly shifted to higher wave number after glucose blending supported the SF conformation transition from random coil structure to β-sheet form (Kweon *et al*., 2001). Thus it can be concluded that the blending glucose can induce conformation changes of SF from random coil to β-sheet form. This may be expected due to the intermolecular bonds between hydroxyl groups of glucose and carbonyl/free amino groups of SF film matrices.

Film Morphology

Thicknesses of the SF and its blended films were determined from SEM images. The film thicknesses increased as the glucose blended ratio increased because the increasing film mass with constant film area (Table 1). Morphology of all blended films was homogeneous and continuous phase as shown in Fig. 2 and 3 for the film cross sections and surfaces, respectively. The phase separation between SF film matrices and glucose did not occur for all blended ratios. From the morphological results, it can be concluded that the film properties were consistent throughout the film matrices.

Mechanical Properties

Mechanical properties of the films were investigated by tensile testing. Stress-strain curves of the blend films with different SF/glucose blended ratios are shown in Fig. 4. The results of mechanical properties are also shown in Table 1. The pure SF film displayed the typical behavior of brittle

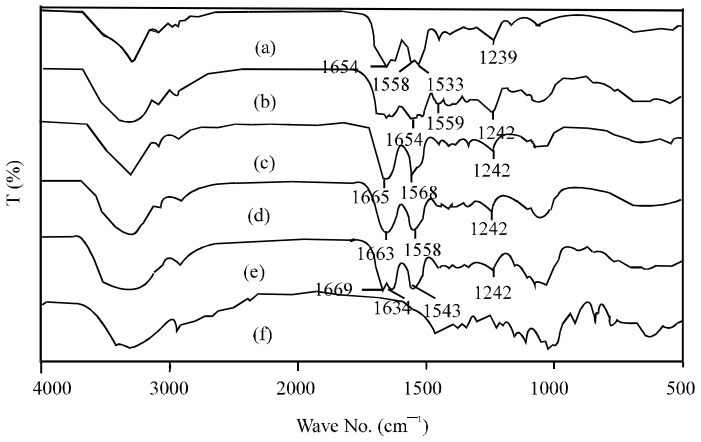

Fig. 1: FT-IR spectra of the blended films with SF/glucose ratios of (a) 8/0, (b) 8/1, (c) 8/2, (d) 8/4, (e) 8/8 (w/w) and (f) glucose

Table 1: Film thicknesses and mechanical properties of the SF/glucose blend films

SF/glucose blend films (w/w)	Film thickness[a] (μm)	Elongation at break[a] (%)	Tensile strength at break[a] (MPa)	Young's modulus[a] (MPa)
8/0	55±10	1.2±0.5	8.2±1.1	4.98±1.45
8/1	60±11	1.5±0.6	6.5±1.2	4.19±1.15
8/2	74±9	4.2±1.0	3.8±0.5	0.71±0.24
8/4	87±10	5.3±0.9	2.6±0.4	0.27±0.10
8/8	95±12	20.0±1.4	0.5±0.2	0.04±0.02

[a]Average values measured from at least three samples

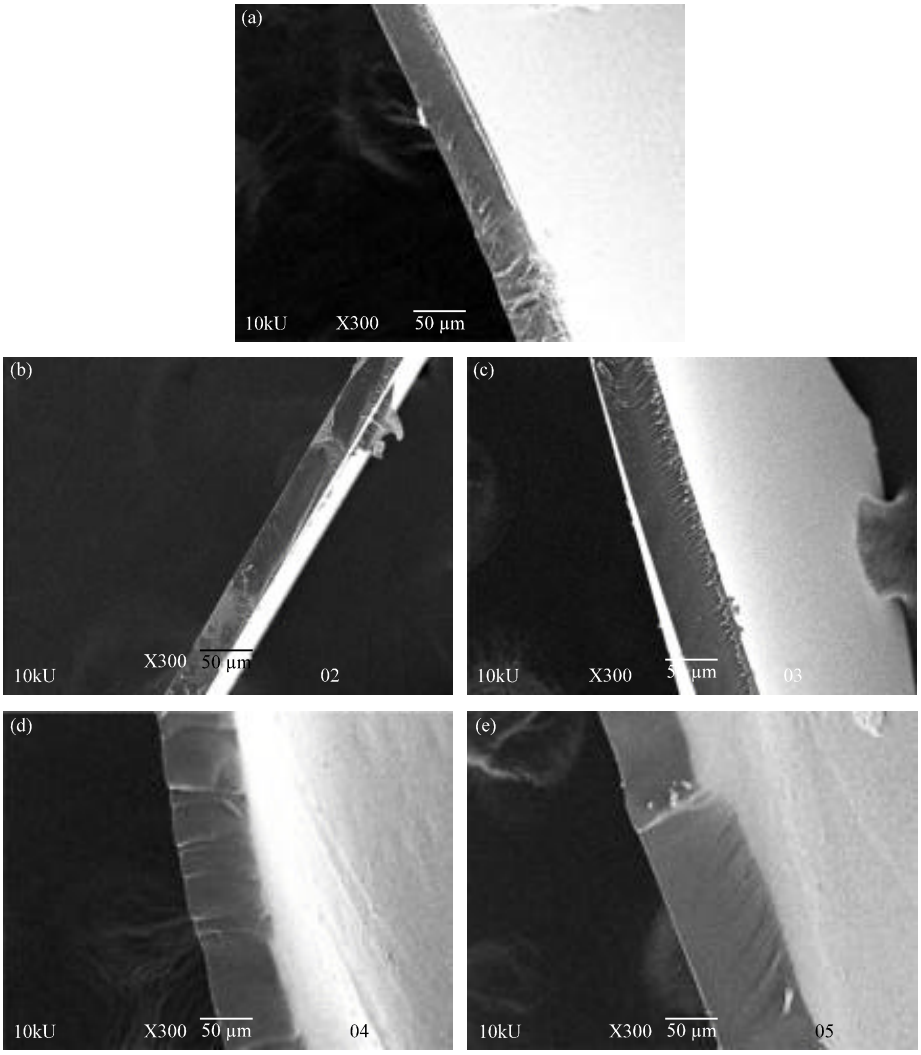

Fig. 2: SEM images of the film cross-sections with SF/glucose ratios of (a) 8/0, (b) 8/1, (c) 8/2, (d) 8/4 and (e) 8/8 (w/w) (bar = 50 µm)

films, with high tensile strength (8.2 MPa) and low elongation (1.2%) values. Table 1 shows that the elongation at break of the SF film increased, while the tensile strength at break and the Young's modulus decreased, indicating that the flexible SF films were obtained. The blended SF films with 8/8 blended ratio show highest elongation as 20%. The results suggested that the glucose shows potential for use as a biocompatible plasticizer for improving the flexibility of SF film. The interactions between SF film matrices and interpenetrated glucose have described in above FTIR results. In addition, the elongation at break of blended films also increased when the glucose ratio was increased. Thus, the SF film flexibility increased with the glucose blended ratio.

Freddi *et al.* (1999) reported that the elongation of SF increased by blending with polyacrylamide. However, when the polyacrylamide ratio was increased up to 25% (w/w), the elongation of blended films decreased due to the phase separation. This is a limit of blending SF with macromolecule. Whereas the phase separation of 8/8 (w/w) SF/glucose blended films observed from

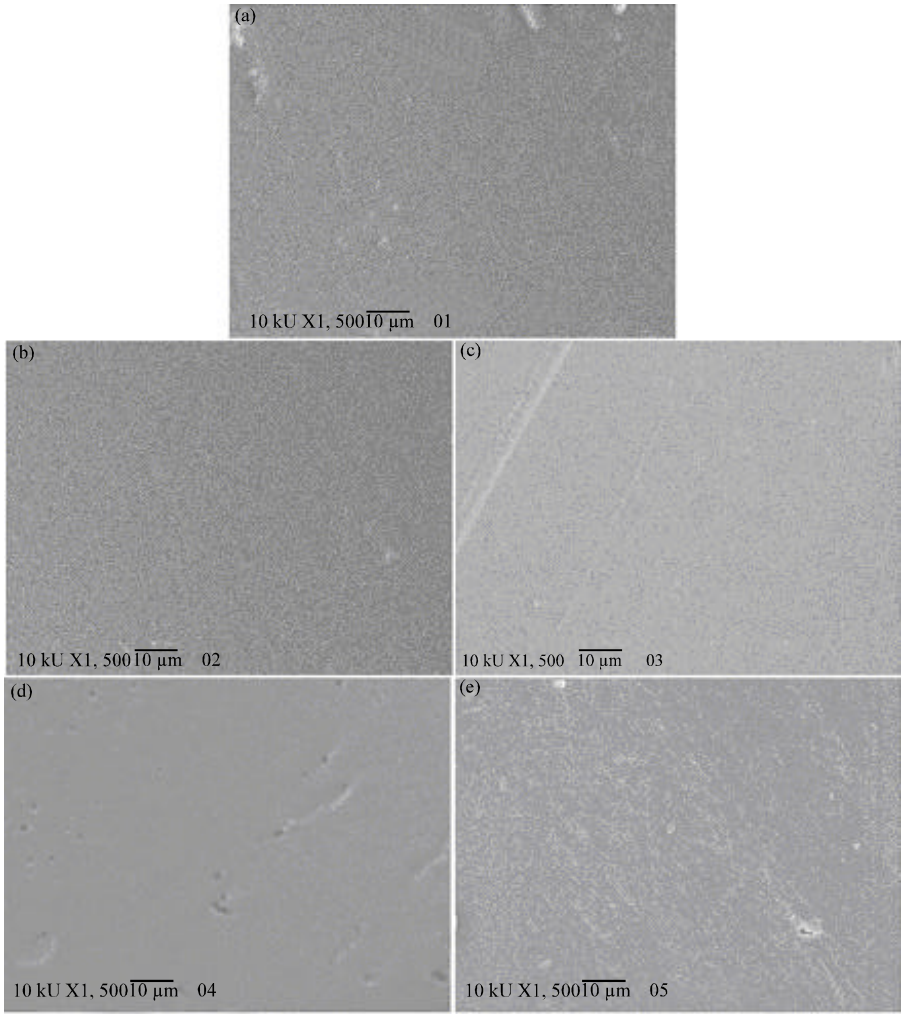

Fig. 3: SEM images of the film surfaces with SF/glucose ratios of (a) 8/0, (b) 8/1, (c) 8/2, (d) 8/4 and (e) 8/8 (w/w) (bar = 10 µm)

SEM images as shown in Fig. 2 and 3 did not occur. The phase separation between SF and poly(ethylene oxide) was also observed (Jin *et al*., 2004). Then the SF film flexibility did not improve in significantly values.

Film Transparency

The SF and the blended films were highly transparent and slight yellowish. The % transmittance at λ_{max} 660 nm ($\%T_{660}$) was used for studying the film transparency. The $\%T_{660}$ values of SF and its blended films are shown in Fig. 5. It was found that the transparency of SF film and blended films in all blended ratios did not differ by significant amounts. The results suggested that the glucose blending did not effect to the transparency of SF films.

Moisture Uptakes

The moisture uptakes of SF films were measured instead of water uptake (immersion in water) because of the partial dissolution of SF. The moisture (%) uptakes were calculated from Eq. 1 and are

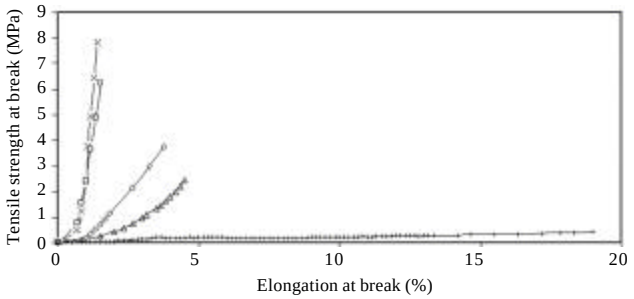

Fig. 4: Stress-strain curves of the blended films with SF/glucose ratios of (×) 8/0, (□) 8/1, (Δ) 8/4 and (+) 8/8 (w/w)

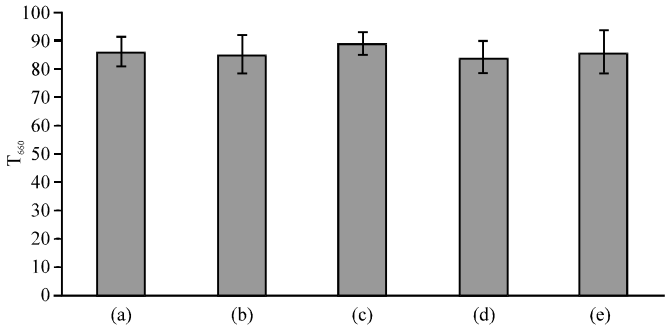

Fig. 5: Transmittances (%) at 660 nm (T_{660}) of the blended films with SF/glucose ratios of (a) 8/0, (b) 8/1, (c) 8/2, (d) 8/4 and (e) 8/8 (w/w)

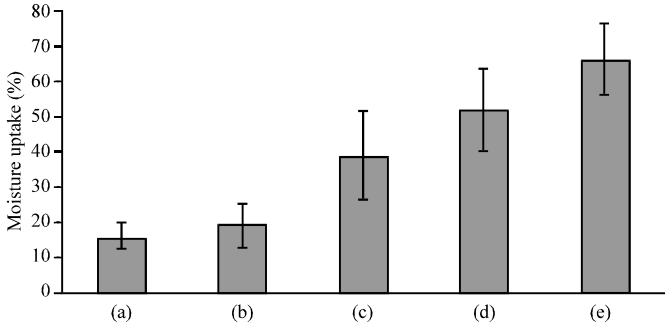

Fig. 6: Moisture uptakes (%) of the blended films with SF/glucose ratios of (a) 8/0, (b) 8/1, (c) 8/2, (d) 8/4 and (e) 8/8 (w/w)

shown in Fig. 6. The moisture uptakes (%) of the SF films increased in significant values as the glucose blended ratio increased. This may be explained that the glucose fraction had higher moisture adsorption than the SF film matrices. Then glucose blending can enhance wettability of the SF films.

From present results, it is indicates that the flexibility of SF films can be improved by blending with glucose. Thus, the flexible SF films are the novel biomaterial films which appropriate for handing in widely applications.

CONCLUSION

The flexibility of SF films can be enhanced by blending with glucose. The FTIR results showed that the intermolecular interactions between SF and glucose of the blended films had occurred. The morphology of blended films observed from the SEM images was uniform and homogeneous throughout the film matrices. The elongation at break of blended films increased and tensile strength at break decreased as the glucose blend ratio increased. The moisture (%) uptakes of blended films increased but the film transparency did not change with the glucose blended ratio. The glucose shows potential for use as a good biocompatible plasticizer to improve flexibility of the SF films. These SF blended films might be of interested for biomedical, pharmaceutical and packaging applications.

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