

# Fibroin Membrane Preparation and Stabilization by Polyethylene Glycol Diglycidyl Ether

Piyarut Moonsri,<sup>1</sup> Ruangsri Watanesk,<sup>1</sup> Surasak Watanesk,<sup>1</sup> Hataichanoke Niamsup,<sup>1</sup> Richard L. Deming<sup>2</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup>Department of Chemistry and Biochemistry, California State University, Fullerton, California 92833

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**ABSTRACT:** Membranes prepared by drying aqueous *Bombyx mori* silk fibroin (SF) solution and modified silk fibroin (MSF) solutions, prepared by adding the low molecular weight crosslinking agent, polyethylene glycol diglycidyl ether (PEGDE) MW 526, 0–10% w/w, were investigated by Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy, and UV–vis spectroscopy. Weight gain in aqueous solutions and their mechanical properties (tensile strength, elongation, and Young's modulus) were then characterized. SEM measurements revealed greater porosity in MSF membranes. IR spectra showed transformation from the largely  $\alpha$ -helical/random coil structures in SF membranes to predominantly  $\beta$ -sheet in MSF membranes. Results from UV–vis spectro-

scopy showed that the MSF membranes were largely insoluble within the pH range of 4–10. Water absorbability of the MSF membranes improved with increasing the amounts of cross-linker, up to 4%. The MSF membranes showed greater pliability and tenacity, but lower tensile strength, with increasing PEGDE concentrations. In the wet condition, PEGDE levels up to 4% can improve both tensile strength and tenacity of the MSF membrane, but higher levels (up to 10%) did not significantly change these properties. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 1402–1406, 2008

**Key words:** silk fibroin; modified silk fibroin; PEGDE crosslinking agent

## INTRODUCTION

Silk, derived from the silkworm *Bombyx mori*, is composed of two proteins: fibroin (about 70%) forming the central core of the fiber and imparting strength, and sericin (30%), the outer glue-like protein coating that helps protect the fiber against water and other environmental factors (microorganisms, insects, etc.).<sup>1</sup> Sericin can be removed by chemical treatment, such as boiling in 5% aqueous sodium carbonate. The resulting silk fibroin (SF) can be dissolved by treatment with reagents such as  $\text{CaCl}_2\text{-EtOH-H}_2\text{O}$ ,  $\text{Ca(NO}_3)_2\text{-MeOH-H}_2\text{O}$  or aqueous  $\text{LiBr}$ ,<sup>2</sup> and purified by dialysis against deionized water. It can be incorporated into a wide range of materials that have high biocompatibility, thermal stability, microbial resistance, and oxygen permeability. These materials have been utilized for wound protection,<sup>3</sup> as substrates for cell cultures,<sup>4</sup> in drug-release

agents,<sup>5</sup> and as substrates for enzyme immobilization in biological sensors.<sup>6</sup>

The primary structure of SF consists of the repeating amino-acid sequence Gly-Ala-Gly-Ala-Gly-X, where X is Ala (64%), Ser (22%), Tyr (10%), and other amino acids (4%).<sup>7,8</sup> Two crystalline polymorphs are observed for SF: Silk I, containing  $\alpha$ -helical and random coil structures, and Silk II, containing the antiparallel  $\beta$ -sheet structure.<sup>9</sup> In aqueous solution, the SF molecules exhibit the random coil conformation with an average hydrodynamic radius of 139 nm and a molecular weight of  $\sim 25$  kDa.<sup>10</sup> Silk fibroin membranes can be prepared by casting SF solutions onto different surfaces, such as polyethylene, polytetrafluoroethylene, polystyrene, or glass. Silk I membranes are flexible, but the fibroin can dissolve when in contact with water or solvents. A stable SF membrane which contains high proportions of the Silk II can be prepared by (a) casting at room temperature, followed by soaking in  $\geq 75\%$  methanol in water;<sup>11</sup> (b) casting at temperatures above  $60^\circ\text{C}$ ;<sup>12</sup> or (c) dissolving silk fibroin in 98% formic acid prior to casting and drying.<sup>13</sup> While these treatments are useful in stabilizing the SF membrane against water, the  $\beta$ -sheet-rich membranes are generally rigid and brittle in the dry state, causing difficulty in practical applications. Chemical modification by crosslinking or chemical blending results in improvements in

Correspondence to: R. Watanesk (scirwtns@chiangmai.ac.th).

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both stability and mechanical properties of the modified silk fibroin (MSF) membrane, by crosslinking reaction or chemical blending demonstrated improvement in both the stabilization and mechanical properties of the modified silk fibroin (MSF) membranes. Casting the membrane with a crosslinking agent, such as high molecular weight polyethylene glycol diglycidyl ether (PEGDE) at 40°C, produces a flexible and stable membrane.<sup>14</sup> Other chemicals to produce more flexible SF membranes include poly(vinyl) alcohol,<sup>15</sup> cellulose,<sup>16</sup> polyethyleneglycol,<sup>17</sup> and Nylon 66.<sup>18</sup>

In this work, we utilize low molecular weight PEGDE to prepare flexible and stable silk fibroin membranes. They are characterized by examining surface morphologies by scanning electron microscopy (SEM), conformational structures by Fourier transform infrared spectroscopy (FTIR), solubility in aqueous media by ultraviolet visible (UV-vis) spectroscopy, water absorption by weight gain, and mechanical properties by measuring tensile strength, elongation at break, and Young's modulus.

## EXPERIMENTAL

### Materials and methods

The waste silk from silk-processing at Jun Mai Thai Co., Ltd, Phetchabun, Thailand, was used in this study. All of chemicals were analytical reagent grade. The sericin was removed by degumming three times with 5 g/L Na<sub>2</sub>CO<sub>3</sub> solution at 98–100°C for 30 min each, rinsing with water and drying at 60°C for 6 h (deionized water was used throughout the study). Subsequently, 10 g of the degummed silk fiber were dissolved in 100 mL of the ternary solvent, CaCl<sub>2</sub>-ethanol-water (mole ratio = 1 : 2 : 8)<sup>2</sup> at 110 ± 5°C for 2 h. The solution was then filtered, dialyzed continuously in dialysis tubes (molecular cutoff = 10,000) against water for 3 days. The resulting aqueous silk fibroin (SF) solution was obtained and the composition was ~ 3% w/v. The modified silk fibroin (MSF) solution was prepared by mixing the SF solution and PEGDE (Aldrich Chemical Co., USA) in various weight ratios of 0, 2, 4, 6, 8, and 10% w/w PEGDE/SF. Then 20 mL of the mixed solutions were poured separately into 6 cm × 9 cm polystyrene dishes and dried at 60°C for 9 h. The dried membranes were soaked in water for 1 h (to wash the membrane and remove excess PEGDE) and then freeze-dried (Flexi-Dry™ MP, FTS Systems, USA) for 3 h to obtain the final form of the fibroin membranes, ~ 200 μm each in thickness.

### Measurements

SEM was performed with a JEOL microscope (JSM-6335F, JEOL, Japan) operating at 15 keV. The freeze-

dried membrane was coated with gold in the vacuum chamber to provide a conductive surface. IR spectra were obtained (model Tensor 27, Bruker, Germany) at room temperature. Water solubility was determined by shaking 250 mg of the membrane (SF or MSF) in 10 mL of water at room temperature. The pH of the solution was adjusted using small amounts of 1M HCl (for pH 4), 0.1M sodium phosphate buffer (for pH 7), or 1M KOH (pH 10). The membrane was dried in the oven at 60°C until its weight remained constant.

The water solubility was determined using a UV-vis spectrometer (model UV-1700, Shimadzu, Japan). The SF solutions show a maximum absorption at 276 nm. The percent mass loss was determined by measuring the absorbance of 10.0 mL of water in contact with 250 mg of the membrane at 276 nm, after shaking for 60 min and standing for 24 h. Using known amounts (mg) of freeze-dried silk fibroin powder, the absorptivity, *a*, in the Beer's Law equation  $A = abC$  (where  $b = 1$  cm,  $C$  is in mg mL<sup>-1</sup>) was determined to be 1.18 cm<sup>-1</sup> mg<sup>-1</sup> L at 276 nm.

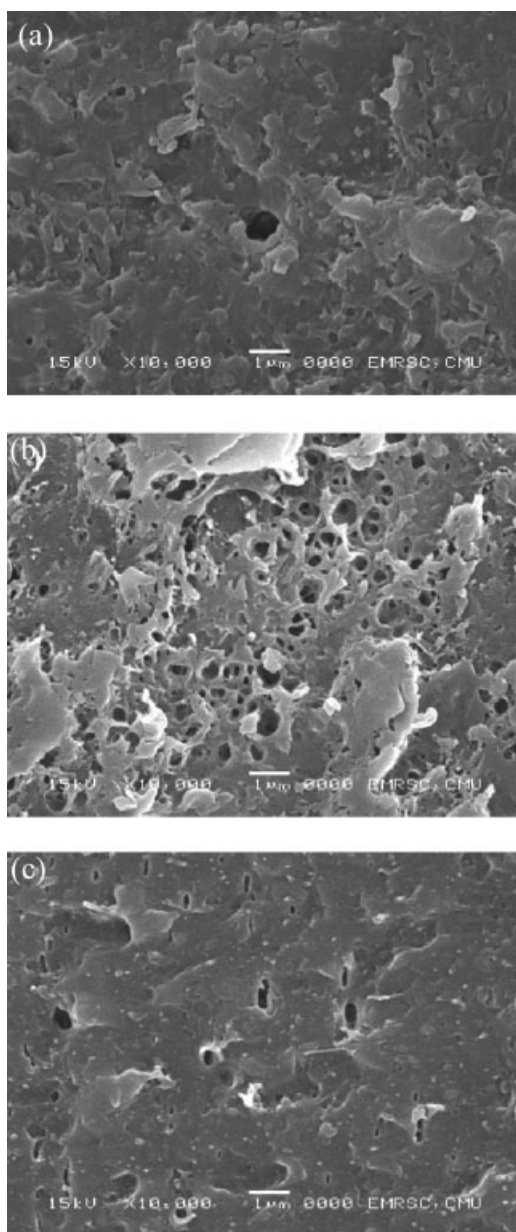
The water absorbability of the final form of the membrane was determined by measuring the mass gain after soaking in deionized water at room temperature for 24 h. The change was converted to % gain based on the original mass. Since the SF membrane (predominantly α-helical/random coil structure) is quite soluble, it was not possible to measure a weight gain. However, the more stable β-sheet form of the SF was prepared by pretreatment with 75% methanol for 15 min<sup>11</sup> for comparison.

Tensile strength, elongation at break, and Young's modulus were determined using a universal tester (model LRX, LLOYD instruments, UK) at 25°C and ambient relative humidity of 55 ± 5%, with head speed of 20 mm min<sup>-1</sup>. Membrane thickness was measured by using the thickness equipment (model ID-C112BS, Mitutoya, Japan), to a precision of 1 μm. The specimen, with accurately measured thickness (close to 200 μm), was cut into 10-mm wide strips and mounted to have 40 mm of exposed sample between the clamp points. The measurement was repeated three or more times and averaged.

## RESULTS AND DISCUSSION

### Morphology

For SEM measurements, membranes were cut into sections, exposing a fresh surface along the cut which is representative of the bulk of the material. As shown in Figure 1(a), the SF membrane exhibited a more dense structure than the MSF membranes with 4% PEGDE, Figure 1(b) and 10% PEGDE, Figure 1(c), respectively. The pore sizes are generally less than 1 μm in all samples. Addition of PEGDE



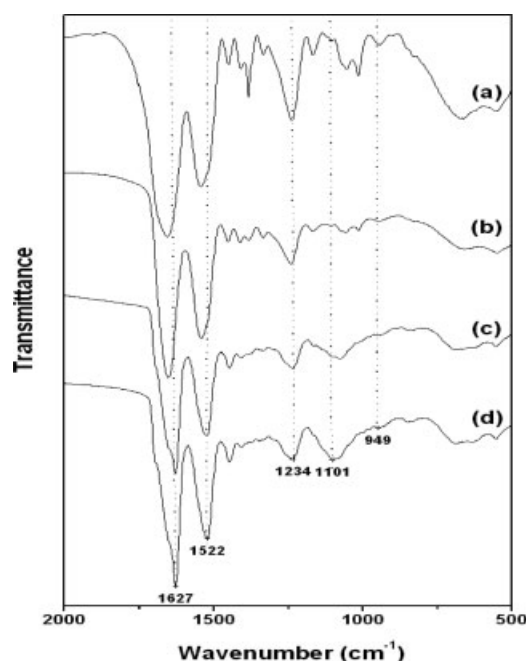
**Figure 1** SEM image of cross section of (a) the SF membrane; (b) the MSF membrane with 4% PEGDE; and (c) the MSF membrane with 10% PEGDE.

has been shown to result in crosslinking with the polar amino acid groups in the SF molecules, including Asp, Tyr, and Arg.<sup>14</sup> In the present study, at 4% PEGDE, the resulting crosslinked structure is more stable than the SF membrane, but appears to be sufficiently open to allow excess PEGDE to be washed out, leaving relatively large pores, Figure 1(b). At 10% PEGDE, more extensive cross-linking is anticipated, but there may also be regions where excess PEGDE is trapped within the matrix so that excess PEGDE cannot be washed out. It has been shown that for polyethyleneglycol (PEG) there are PEG-rich

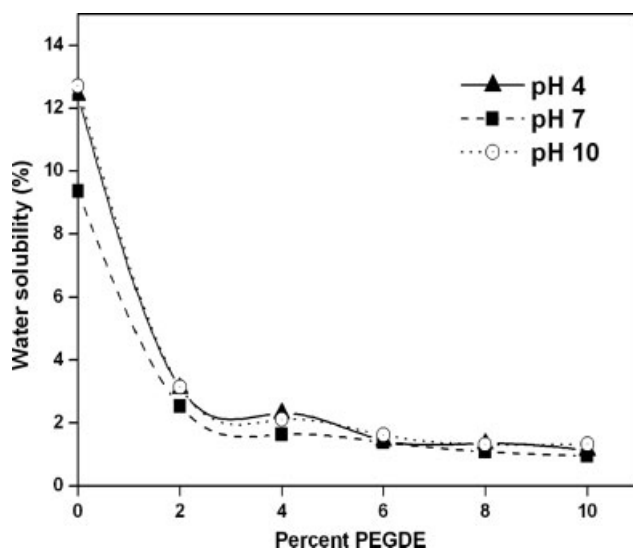
domains within the SF membrane due to poor miscibility.<sup>17</sup> Consequently, the MSF membrane structure in the present study appears less porous at 10% PEGDE, Figure 1(c).

### Structure characterization

The secondary structure of proteins can be characterized using FTIR spectroscopy by examining the absorptions in the range 2000–500  $\text{cm}^{-1}$ . The spectrum of dried SF powder, obtained from fresh SF solution, frozen and freeze dried, illustrated in Figure 2 (a) exhibits that it contains largely  $\alpha$ -helical and random coil conformations,<sup>10</sup> as indicated by the strong absorptions at 1652  $\text{cm}^{-1}$  (amide I, C=O stretching), 1542  $\text{cm}^{-1}$  (amide II, N–H bending vibration), and 1242  $\text{cm}^{-1}$  (amide III, C–N stretching). The SF membrane shows absorptions at these same frequencies, suggesting that the formation of the membrane after casting and drying at 60°C for 9 h does not significantly change the ratio of conformations. In contrast, the spectra of the MSF membranes (4% and 10% PEGDE) show shifts in the absorption bands to 1627  $\text{cm}^{-1}$  (amide I), 1522  $\text{cm}^{-1}$  (amide II), and 1234  $\text{cm}^{-1}$  (amide III), assigned to the  $\beta$ -sheet conformation.<sup>10</sup> In addition, bands at 1101 and 949  $\text{cm}^{-1}$  increase in intensity with increasing PEGDE concentration. These frequencies have been assigned to the asymmetrical stretching of the C–O–C bond of PEG.<sup>14</sup>



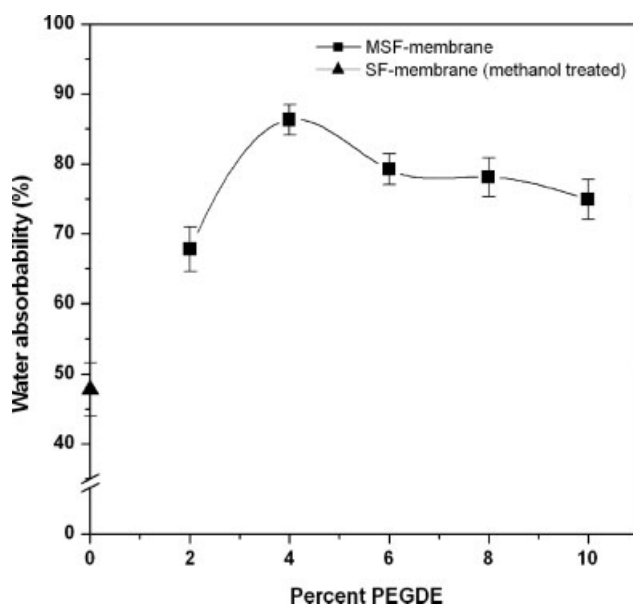
**Figure 2** IR spectra of (a) the SF powder; (b) the SF membrane; (c) the MSF membrane with 4% PEGDE; and (d) the MSF membrane with 10% PEGDE in the range 2000–500  $\text{cm}^{-1}$ .



**Figure 3** The effect of PEGDE on water solubility of the MSF membrane at pH 4–10.

### Water solubility

The water solubility of the SF membrane and MSF membrane (with varying levels of PEGDE and different pH's) is shown in Figure 3. Without PEGDE (SF membrane only), very high solubility is observed (9–12% by mass). With only 2% PEGDE (MSF membrane), the solubility drops dramatically to 3% or less, and the mass loss stabilizes at 6% PEGDE and above. At pH 4 and pH 10, there is enhanced solubility but it quickly stabilizes at 6% PEGDE and higher. These results suggest that the PEGDE greatly stabilizes the membrane against solubilization. In



**Figure 4** The effect of the percent PEGDE on the percent PEGDE on water absorbability of the MSF membranes at pH 7.0 comparing with the SF membrane.

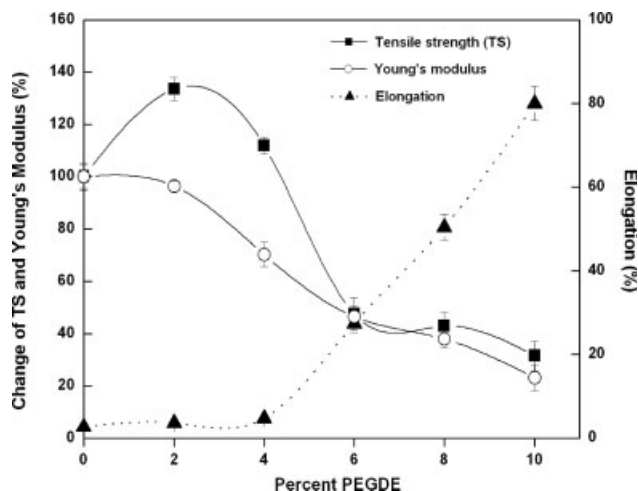
addition, the formation of PEG-rich phase separated from the SF domain may affect the water diffusion through the MSF membrane with 6% PEGDE and above.

### Water absorbability

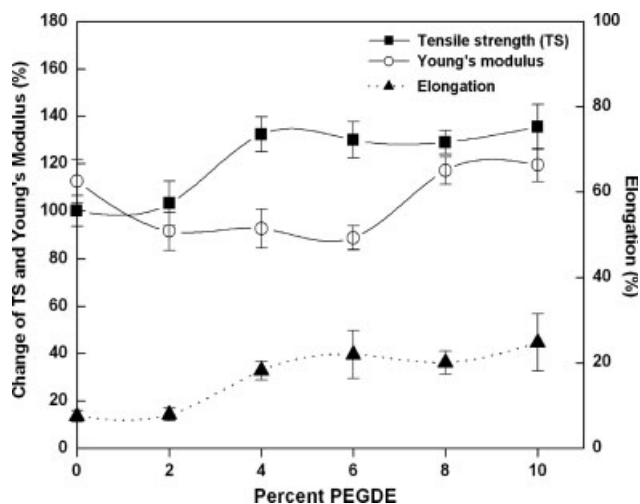
Figure 4 shows the relationship between PEGDE percentage and water content of the membranes. In comparison to the membrane without PEGDE (SF membrane only, stabilized by treatment with methanol as noted above), the maximum absorbability is achieved at 4% PEGDE (90% mass gain), with a decrease to 10% PEGDE (75% mass gain). As noted in Figure 1(b) above, the 4% PEGDE MSF membrane contains large numbers of pores that would be capable of accommodating more water molecules. At 10% PEGDE, these pores decrease in number and size, restricting access by the water molecules.

### Mechanical properties

The influence of the PEGDE percentage on the mechanical properties of the MSF membranes in the dry or rewetted (24 h in water at 25°C) states was studied. Figure 5 summarizes the results as relative change. Increasing PEGDE levels results in initial increases in tensile strength with a maximum about 2–3% PEGDE, followed by a rapid decrease after 4% PEGDE. This is attributed to the elongation at break and a decrease in Young's modulus (increase in compliance) of the MSF membranes as the range of 2–4% PEGDE. At more than 4% PEGDE, the MSF membrane became more compliant and tenacious but lost its tensile strength. The degree of the crystallinity of the SF chains can be reduced by the PEG chain separated in the SF matrix.<sup>17</sup> Thus, the results



**Figure 5** Influence of PEGDE on mechanical properties of the MSF membrane in the dry state.



**Figure 6** Influence of PEGDE on mechanical properties of the MSF membrane in the wet state.

of decreasing in the degree of the crystallinity of SF by introduced PEG chains revealed a decrease in the tensile strength of the MSF membrane with more than 6% PEGDE. In the wet condition as shown in Figure 6, the MSF membrane exhibits higher tensile strength and elongation at break than the SF membrane at increasing PEGDE concentrations. The results show that the MSF membrane is more tenacious than the SF membrane as the PEGDE exceeds 4%. The tenacity can be explained by the chemical bonding between the fibroin residues and PEGDE, in that the crosslinking greatly stabilizes the membrane so that the ductile behavior of the MSF membrane is governed by the ductile PEG matrix.<sup>17</sup> However, the tensile strength and elongation at break of the MSF membranes as the PEGDE exceeds 4% do not change significantly. Whereas the water absorbability of the MSF membrane evidently attains the maximum absorbability at 4% PEGDE (90% mass gain), with a decrease of about 15% as the PEGDE concentration is increased to 10%. The tenacity of the MSF membranes with more than 4% PEGDE, however, is not significantly different. In the wet state, the Young's modulus remains relatively constant with increasing amounts of PEGDE in the range of 2–4%, but as PEGDE exceeds 6%, an increase in the Young's modulus is observed. This may be explained by the low porosity of the MSF membranes and an increase in small pores that is apparent at 10% PEGDE.

### CONCLUSION

The MSF membranes were prepared using varying levels of the crosslinking agent, PEGDE, at 0–10% w/w PEGDE/SF added to the SF solutions. The resulting modified membranes show decreased water solubility and improved mechanical properties. The SEM images of the cross-sectional surface

of freeze-dried samples show dense structures for both the SF and the high (10%) PEGDE membranes, but a more porous structure for the 4% PEGDE MSF membrane. The water absorbability measurements support the more microporous nature of the 4% PEGDE MSF membrane, compared to the (methanol-stabilized) SF and 10% PEGDE MSF membranes. IR spectra support the transition from  $\alpha$ -helical/random coil to  $\beta$ -sheet structures with addition of PEGDE. Partial crosslinking appears to produce more open structures that can hold water molecules. The water solubility of the MSF membrane in the pH 4–10 range decreased significantly when the percent PEGDE was more than 2%. The MSF membrane exhibited higher water absorbability compared to the methanol-treated SF. PEGDE improved the pliability and the tenacity of the dried membrane, but tensile strength decreased with more than 4% PEGDE. In the wet condition, PEGDE levels up to 4% can improve both tensile strength and tenacity of the MSF membrane, but higher levels (up to 10%) did not significantly change these properties.

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