

## Dissolution and Regeneration of *Bombyx mori* Silk Fibroin Using Ionic Liquids

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Natural silk fibers have outstanding mechanical properties that rival the most advanced synthetic polymers.<sup>1</sup> Compared to the limited number of molecular solvents available today, ionic liquids have taken the spotlight as green, designer solvents, with many combinations that offer an unprecedented versatility and tunability. In this work, we test the suitability of ionic liquids for dissolving and regenerating silkworm (*Bombyx mori*) silk and examine the structural properties of silk films cast from ionic liquid solutions.

A single strand of natural cocoon silk fiber contains two silk fibroin cores surrounded by a protective, glue-like sericin coating. The actual fibroin cores consist of two macromolecules, with molecular weights of 391 and 26 kDa for the heavy and light chains, respectively.<sup>2</sup> The crystalline regions of the heavy chain occur primarily in the repeated GAGAGS amino acid motifs. These regions assume a hydrogen bonded, antiparallel,  $\beta$ -sheet structure in the natural cocoon fiber.<sup>3,4</sup> The hydrogen bonding and the hydrophobic nature of these crystalline regions make dissolution of silk a formidable task.<sup>5</sup>

Multiple methods have been utilized to prepare silk fibroin solutions. These generally involve stripping the sericin in a Na<sub>2</sub>CO<sub>3</sub> wash, rinsing and drying the silk, and dissolving the silk in a high-concentration, aqueous lithium salt solution or a CaCl<sub>2</sub>/ethanol/water solution.<sup>6</sup> These solutions are subsequently dialyzed to remove the salt and have a shelf life of about 1 week before the onset of gelation. Stable fibroin solutions can be prepared by lyophilizing the dialyzed solutions and dissolving the resulting silk powder in another solvent such as 1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroacetone.<sup>7</sup>

Ionic liquids have been used to successfully dissolve other biological macromolecules such as cellulose.<sup>8,9</sup> Swatloski et al. found that the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIM Cl) can be used to prepare solutions of up to 25% (w/w) cellulose.<sup>8</sup> They also reported that cellulose was soluble in BMIM Br and BMIM SCN but with less than half the degree of solubility of BMIM Cl. The success of BMIM Cl has been attributed to the ability of the chloride anion to disrupt the hydrogen bonding present between the cellulose chains.

Swatloski et al. also found that the solution preparation method greatly affected the solubility.<sup>8</sup> In particular, they used microwave heating on a BMIM Cl/cellulose solution to dissolve 25% (w/w) cellulose, as compared to a 10% (w/w) cellulose solution heated in an oil bath at 100 °C. However, they did not report the peak temperature that was reached during the microwave dissolution or analyze for cellulose degradation.

The ability of BMIM Cl to disrupt hydrogen bonding makes it an attractive solvent for silk fibroin. In addition to BMIM Cl, the

suitability of several other ionic liquids was tested for solubilizing silk. BMIM<sup>+</sup> Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, and BF<sub>4</sub><sup>-</sup>; 1-butyl-2,3-dimethylimidazolium (DMBIM<sup>+</sup>) Cl<sup>-</sup>; and 1-ethyl-3-methylimidazolium (EMIM<sup>+</sup>) Cl<sup>-</sup>, BF<sub>4</sub><sup>-</sup>, and AlCl<sub>4</sub><sup>-</sup> ionic liquids were evaluated to determine their ability to dissolve silk.

The cocoon silk utilized in these experiments originated from silkworms raised on a diet of Silkworm Chow (Mulberry Farms, Fallbrook, CA). The pupae were extracted from the cocoons intact two to 7 days after spinning by cutting open the cocoons. This method avoids possible contamination and thermal degradation from the industrial process of baking the pupae in the cocoons.

For most of the experiments, the sericin was extracted from the silk prior to solubilizing in the ionic liquids. However, for the BMIM Br, BMIM I, and BMIM BF<sub>4</sub> experiments, the sericin remained on the fibers, which were dried under vacuum. In the BMIM Cl and DMBIM Cl experiments, the sericin was stripped in a 0.05 M Na<sub>2</sub>CO<sub>3</sub> and 0.05 M Na<sub>2</sub>EDTA solution with 9% (w/w) cocoons at 65 °C for a day. The silk was rinsed thoroughly and lyophilized prior to solubility testing. In the EMIM Cl experiments, the sericin was stripped in a 0.2 M Na<sub>2</sub>CO<sub>3</sub> solution by boiling for 2 h. These cocoons were rinsed thoroughly and dried at 100 °C in a vacuum oven overnight prior to solubility testing.

The dissolution experiments were conducted under an inert atmosphere of N<sub>2</sub> (BMIM and DMBIM) or He (EMIM) due to the hygroscopic nature of the ionic liquids. To determine the solubility of the silk in each of the ionic liquids, silk was added slowly to the ionic liquid melt while providing agitation. The resultant ionic liquid/silk solutions were clear with an amber color and quite viscous above 10% (w/w).

The temperatures of the ionic liquid solutions were maintained with a temperature-controlled oil bath at 100 °C. Attempts to dissolve the silk fibroin in BMIM Cl using a microwave were unsuccessful due thermal decomposition of the silk. The solubility results listed in Table 1 are samples dissolved using an oil bath as the heat source.

The solubility of silk fibroin in ionic liquids depends on the identity of both the cation and anion, with the anion having a much larger effect. The more the cation and anion are able to participate in hydrogen bonding, the greater the solubility of the silk fibroin.<sup>10</sup> Presumably, the ionic liquid disrupts the hydrogen bonding present in the  $\beta$ -sheets. The BMIM Br, BMIM I, and BMIM BF<sub>4</sub> were tested with both silk and sericin. Sericin is soluble in BMIM Br and I but not in BMIM BF<sub>4</sub>.

The solubility of the silk fibroin in BMIM Cl was also examined with wide-angle X-ray scattering (WAXS) by examining the crystal structure. In Figure 1a, the data from a 12.24% (w/w) silk solution sealed between Kapton windows show a broad amorphous halo centered near a  $2\theta$  of 20°, indicating that amorphous silk, but no

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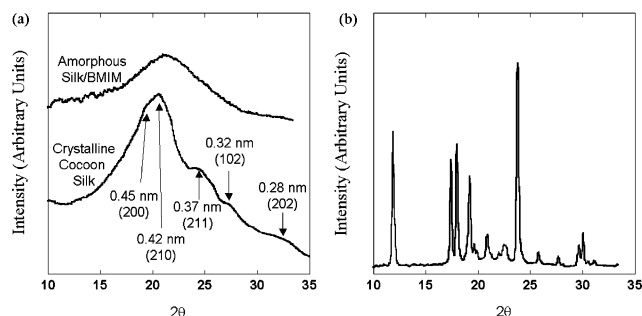
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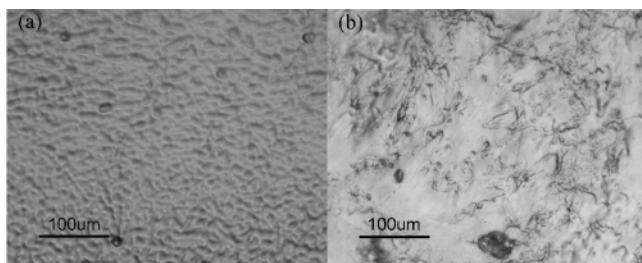
**Table 1.** Saturated Solubility by Weight for Silk in Ionic Liquids

cation	anion				
	Cl <sup>-</sup>	Br <sup>-</sup>	I <sup>-</sup>	BF <sub>4</sub> <sup>-</sup>	AlCl <sub>4</sub> <sup>-</sup>
BMIM <sup>+</sup>	13.2%	0.7% <sup>a</sup>	0.2% <sup>a</sup>	0.0% <sup>a</sup>	c
DMBIM <sup>+</sup>	8.3%	c	c	c	c
EMIM <sup>+</sup>	23.3%	c	c	0.0%	0.0% <sup>b</sup>

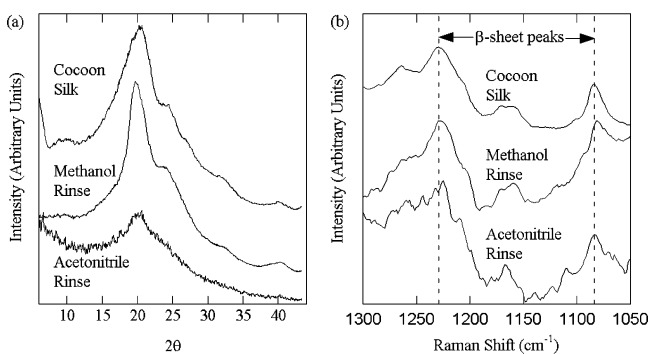
<sup>a</sup> Both sericin and fibroin added to the solvent; only the sericin is soluble. With the exception of BF<sub>4</sub><sup>-</sup>, these solutions are not saturated. <sup>b</sup> Solvent is an EMIM Cl/EMIM AlCl<sub>4</sub> mixture with a 1.0:0.7 molar ratio. <sup>c</sup> System not tested.



**Figure 1.** WAXS crystallinity analysis for (a) 12.24% (w/w) silk in BMIM Cl (labeled "Amorphous Silk/BMIM") at 30 °C after cooling from 100 °C compared with crystalline cocoon silk and (b) pure BMIM Cl at 30 °C. The BMIM Cl disrupts the  $\beta$ -sheet crystalline structure in the silk.



**Figure 2.** Reflectance optical microscopy images of films on glass slides cast from 9.51% (w/w) silk in BMIM Cl and rinsed with (a) acetonitrile and (b) methanol. The acetonitrile-rinsed film has a convoluted surface and scatters light. The methanol-rinsed film appears to be clear and is birefringent. Neither film has any residual fiber structure.



**Figure 3.** Crystallinity analysis of silk films compared to the natural cocoon fiber. Both (a) WAXS and (b) Raman data indicate a  $\beta$ -sheet structure for the films and the cocoon. The film rinsed with acetonitrile has only a small amount of crystallinity.

$\beta$ -sheet structure, exists at 30 °C. The data are compared to crystalline cocoon silk, which shows several peaks that can be indexed according to an antiparallel  $\beta$ -sheet structure.<sup>4</sup> Furthermore, none of the BMIM Cl peaks are prominent at 30 °C due to the interaction between the BMIM Cl and silk. The pure BMIM Cl data are shown in Figure 1b. These data confirm that the BMIM Cl disrupts the hydrogen bonds in the crystalline domains of the cocoon silk. In conjunction with the WAXS data, the elimination

of any cocoon silk fiber structure by optical inspection in BMIM Cl indicates that the silk has dissolved.

With the solubility of silk confirmed, a 9.51% (w/w) silk in BMIM Cl solution at 100 °C was used to cast films on both silicon wafers and glass slides. The BMIM Cl was removed by rinsing with either acetonitrile or methanol. Raman analysis indicated that both the acetonitrile and methanol rinses removed the BMIM Cl. Attempts to rinse the BMIM Cl with water resulted in dissolution of the silk film. Figure 2 shows optical microscopy images of silk films. The acetonitrile-rinsed film is white in color due to light scattering and has a visible surface topography. The methanol-rinsed film is not smooth but is transparent. This film is birefringent with 50  $\mu$ m domains, whereas the acetonitrile-rinsed film is not birefringent. Neither film shows residual fiber structure, further supporting our assertion that the silk was solubilized.

The crystal structures of films cast on silicon wafers were analyzed with both WAXS and laser Raman at 514 nm. Figure 3 compares both films with a cocoon fiber. Figure 3a shows the WAXS data for the three samples. It is clear that the methanol-rinsed film exhibits a high degree of crystallinity that is similar to the cocoon fiber. This is in agreement with previous results that have shown methanol treatment of silk induces the  $\beta$ -sheet structure.<sup>11</sup> In contrast, the acetonitrile-rinsed film only has a small degree of crystallinity. The Raman data in Figure 3b indicate that the crystal structure for both films and the cocoon fiber are indeed  $\beta$ -sheet, with peaks at 1229 and 1084  $\text{cm}^{-1}$ .<sup>12</sup>

In conclusion, BMIM Cl, DMBIM Cl, and EMIM Cl are able to disrupt the hydrogen bonding in silkworm silk fibroin to form solutions that have no residual resemblance to the original cocoon fibers. WAXS results confirm that the crystal structure of silk fibroin in BMIM Cl is eliminated after heating to 100 °C. The structure of films cast from silk in BMIM Cl solutions is highly dependent on the rinse treatment; acetonitrile yields a convoluted surface structure with little crystallinity, and methanol yields a transparent film with a high degree of crystallinity. Our results confirm that ionic liquids are viable solvent systems for dissolving and regenerating silk fibroin.

**Acknowledgment.** Funding for this research was provided by the Air Force Office of Scientific Research. We gratefully acknowledge the National Research Council for providing post-doctoral fellowships at the Air Force Research Laboratory (D.M.P. and L.F.D.).

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JA046079F