

Wet-spinning of Regenerated Silk Fiber from Aqueous Silk Fibroin Solutions: Influence of Calcium Ion Addition in Spinning Dope on the Performance of Regenerated Silk Fiber*

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Abstract Ca(II) ions are added in the spinning dope to adjust the solidification rate of regenerated silk fibroin (RSF) solution during the wet-spinning process since Ca(II) ions are proved to be favorable to maintain the stable silk fibroin network in our previous work. The results show that when Ca(II)/RSF ratios are 1/50 and 1/20, the resulted RSF fibers exhibit good performance with the breaking energy more than 70 kJ/kg. However, higher Ca(II)/RSF ratio (for example, 1/10) hinders the solidification of spinning dope and results in poor RSF fibers. These observations together with earlier papers from this laboratory confirm that to produce tough silk fibers the spinning conditions must allow sufficient time for the adjustment of silk fibroin molecular chains.

Keywords: Silk fiber; Natural polymer; Wet-spinning; Protein; Calcium.

INTRODUCTION

Animal silks have been noted by biologist, chemist and material scientist for their excellent comprehensive mechanical properties and their highly evolved way for the spinning. Based on the abundant fundamental research work on the structure, property and function of silk proteins^[1], the researchers have developed several artificial spinning methods to produce regenerated silk fibroin (RSF) fibers, hoping to gain comparable mechanical properties with the natural counterparts^[2–6]. In our previous work, we successfully produced a kind of RSF fiber that have better mechanical properties than the natural silkworm cocoon silk through an environment-friendly wet-spinning method^[7]. We extruded concentrated RSF aqueous solution into the hot ammonium sulphate coagulation bath using a custom-made simplified industrial wet-spinning line with a continuous mechanical post-draw device. It has been reported that wet-spinning parameters affect the final performance of RSF fibers significantly^[6, 8–10], so we have discussed several parameters, such as the concentration of spinning dope and coagulation bath, the spinning rate and the post-draw treatment in detail based on our specific spinning device^[11]. On the other hand, we have revealed that the metallic ions played an important role in the natural spinning process^[12, 13]. Among the eight metallic elements (Na, K, Mg, Ca, Cu, Zn, Fe, and Mn) investigated, we found that only Ca(II) ions helped to maintain a stable silk fibroin network. Therefore, in this article, we report our attempt to introduce Ca(II) ions into RSF dope to study the influence of Ca(II) ion addition on the performance of RSF fibers by wet-spinning.

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EXPERIMENTAL

Preparation of RSF Spinning Dope

The preparation of RSF solution for wet-spinning is described in detail elsewhere^[7, 11]. The RSF aqueous solution was concentrated by reverse dialysis from 5% (W/W) to about 17% (W/W). Then, CaCl₂ solution was added very carefully to this concentrated RSF solution and stirred gently to make a homogeneous RSF-Ca(II) spinning dope for wet-spinning. The RSF concentration in such spinning dopes was set to 15%, and the mass ratio of Ca(II)/RSF was 1/50, 1/20 and 1/10, respectively.

Wet-spinning of RSF Fibers

RSF fibers were produced by extruding RSF spinning dope into 35% (W/W) ammonium sulphate coagulation (60 °C) bath through a 200 µm spinneret in our custom-made wet-spinning device similar to that reported previously^[7, 11]. The take-up rate of the first roller (spinning rate) was 60 r/min. The draw-down ratio was defined as the rate ratio of the second roller to the previous one. Four rollers were used in this spinning process, and the rotation rate of other three rollers was 120, 360, and 540 r/min, equivalent to the draw-down ratio of 2, 6, and 9, respectively. All resulted fibers were kept in coagulation bath for another 6 h and then immersed in deionized water to wash out ammonium sulphate. In this article, we use R0, R1, R2, R3 to represent the Ca(II)/RSF ratio of 0, 1/50, 1/20, 1/10, and 6X, 9X to represent the draw-down ratio of 6 and 9. For example, R1-9X means the RSF fiber sample was produced by Ca(II)/RSF = 1/50 and the draw-down ratio of 9.

Scanning Electron Microscopy (SEM)

The cross-section of RSF fibers was observed using a Tescan 5136MM SEM at 20 kV after sputtering with gold. The cross-section of the fibers was obtained by fracturing them perpendicular to the fiber axis under liquid nitrogen. The diameters of the RSF fibers were calculated from the sectioned fibers using the software provided with the SEM.

Mechanical Testing

An Instron 5565 mechanical testing instrument (at 25 ± 0.5 °C and 50 ± 5% R.H.; gauge length: 30 mm; cross-head speed: 15 mm/min) with a load cell of 2.5 N was used for testing. During the testing, we ensured that the silk samples did not glide through the clamps. At least 10 individual fibers were used in each mechanical test.

Raman Spectroscopy

Raman dichroism spectra of RSF fibers were collected with a Renishaw inVia Reflex Raman spectrometer. Single RSF fiber was aligned perpendicular or parallel to the direction of polarization of the laser beam. At least 3 individual fibers were used in each Raman measurement.

EXPERIMENTAL RESULTS

Influence of Ca(II) Content on the Morphology and Mechanical Properties of RSF Fibers

During the previous wet-spinning testing, we found our RSF fibers always exhibited dumbbell-like shapes. In the meantime, they often had typical skin-core structures and considerable large voids inside, as shown in Fig. 1(a). This means when RSF dope extruded into the coagulation bath, a solid skin formed rapidly, which prevented the diffusion of coagulant into inner core and thus hindered the further coagulation of the as-spun fiber. Thus, the collapse of hard skin led to the dumbbell-like shape of the resulted RSF fibers. However, after the addition of a small amount of Ca(II) ions into the spinning dope, it was found that the morphologies of RSF fibers were improved (Figs. 1b and 1c). The cross-section of R1-6X fiber was nearly round with less skin-core structure and voids inside compared to that of R0-6X fiber. For R2-6X fiber, it had a rather homogeneously dense structure (there was no skin-core structure and voids found), though the cross-section was not as round as R1-6X fiber. However, the R3-6X fiber showed the worst morphology with porous tabular cross-section. In addition, it was very difficult to get the RSF fibers with 9X draw-down ratio without Ca(II) ions in the spinning dope, but it became easy after Ca(II) ion addition. As expected, those 9X RSF fibers showed the similar morphologies as the 6X fibers (figures not shown).

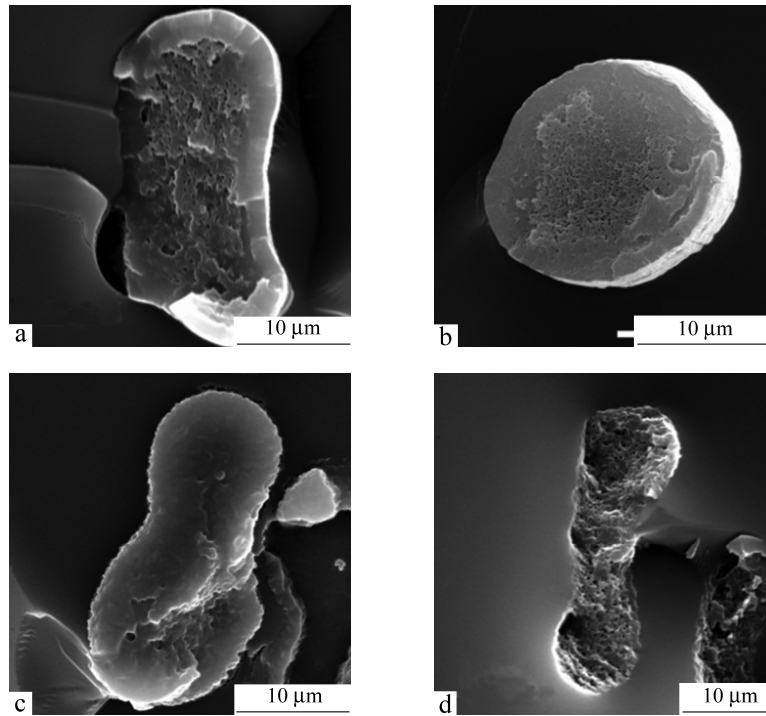


Fig. 1 SEM images of cross-sections of RSF fibers: (a) R0-6X, (b) R1-6X, (c) R2-6X and (d) R3-6X

Figure 2 shows the typical stress-strain curves of RSF fibers with different Ca(II) ion contents and draw-down ratios, and Table 1 summarizes their mechanical properties. It can be found that although the higher draw-down ratio did not improve the morphology of RSF fibers, it increased their mechanical properties. The influence of Ca(II) ion addition on the improvement of mechanical properties was obvious for 6X fibers (R1-6X and R2-6X) and more significant for 9X fibers (R1-9X and R2-9X). The R1-9X fiber exhibited the largest break strain of 57% while the R2-9X fiber showed the highest break stress of 314 MPa. The breaking energies of both R1-9X and R2-9X fibers were larger than 70 kJ/kg, which was considerable higher than that of natural *B. mori* cocoon silk^[5]. However, both R3-6X and R3-9X fibers showed very poor mechanical properties.

Table 1. Comparison of the mechanical properties of RSF fibers with different Ca(II)/RSF ratios and draw-down ratios

Sample	Breaking stress (MPa)	Breaking strain (%)	Breaking energy (kJ/kg)
R0-6X	156 ± 7	44 ± 4	40.5 ± 4.6
R1-6X	201 ± 8	47 ± 5	62.5 ± 6.4
R2-6X	214 ± 11	48 ± 5	61.8 ± 6.5
R3-6X	158 ± 6	18 ± 6	17.6 ± 2.8
R1-9X	206 ± 11	57 ± 6	72.0 ± 7.0
R2-9X	314 ± 19	37 ± 4	78.0 ± 7.4
R3-9X	200 ± 6	27 ± 5	33.8 ± 3.5
Natural cocoon silk	400 ± 20	20 ± 1	42.3 ± 3.3

Influence of Ca(II) Content on the Molecular Alignment of RSF Fibers

Raman dichroism has widely been used to determine the molecular orientation in polymer fibers including silk fibers^[7]. Figure 3 shows the Raman dichroism spectra of RSF fibers and natural cocoon silk. By calculating the intensity ratio of the amide I band in perpendicular and parallel directions, we can investigate the changes in molecular alignment of RSF fibers with the addition of Ca(II) ions. The ratio of the natural cocoon silk was 3.4, which exhibited the high level of molecular orientation along the fiber axis. The ratios of R1-9X and R2-9X fibers were 2.4, which reached about 70% of the ratio of natural cocoon silk. Although these RSF fibers with

Ca(II) ion addition still cannot have the same level of the molecular alignment as the natural silks, they were much better than the one without Ca(II) ions (for instance, the ratio of R0-6X fiber was 1.8). Moreover, such a value was very low for R3-9X RSF fiber, which was only 1.4.

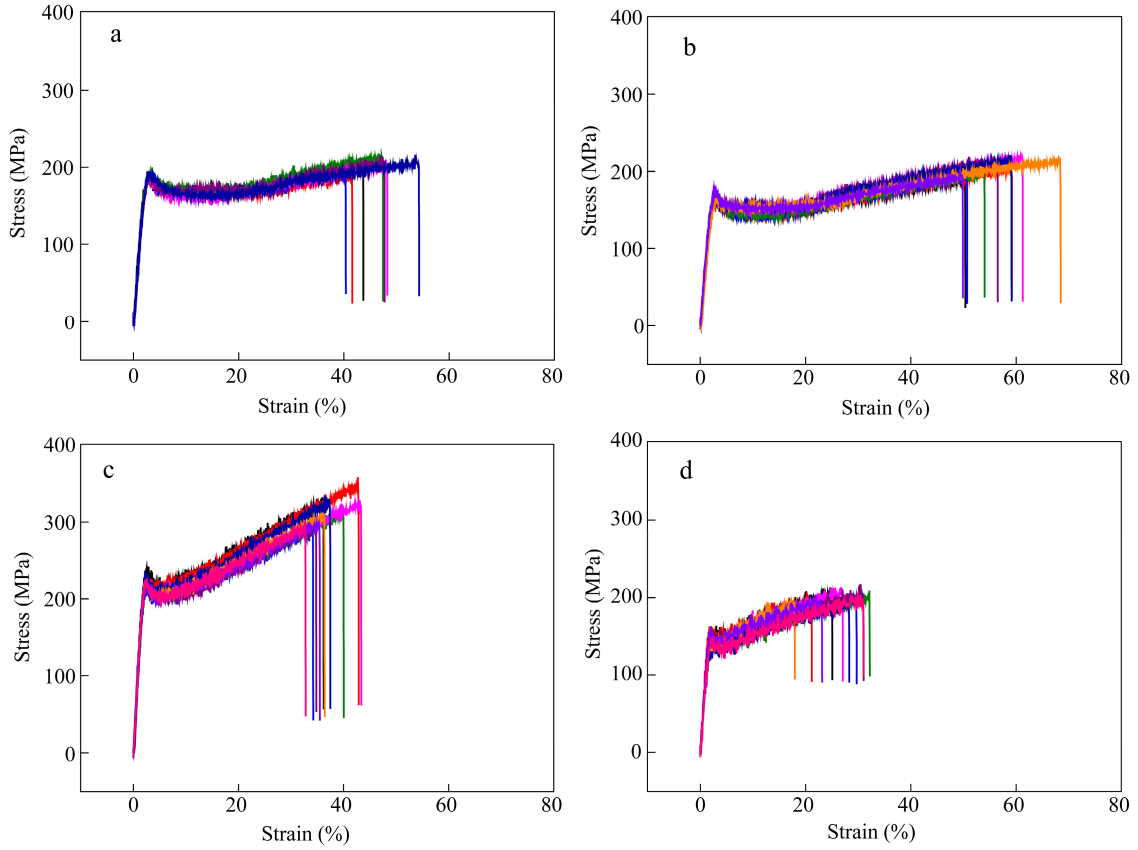


Fig. 2 Stress-strain curves of RSF fibers: (a) R1-6X, (b) R1-9X, (c) R2-9X and (d) R3-9X

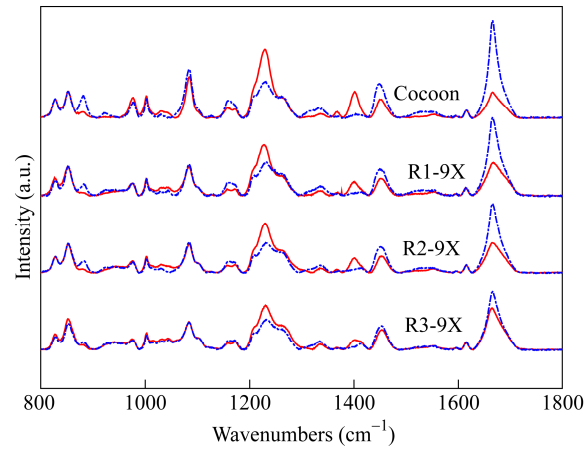


Fig. 3 Raman dichroism spectra of RSF fibers and natural *B. mori* cocoon silk. Fiber aligned perpendicular (dotted line) or parallel (solid line) to the polarization direction of the laser beam.

DISCUSSION

The above results indicate that the addition of a small amount of Ca(II) ions in spinning dope, *i.e.*, when the Ca(II)/RSF ratio is 1/50 and 1/20, results in RSF fibers with relatively dense and homogeneous structures as well as good mechanical properties. The reason should lie on the fact that Ca(II) ions are favorable to maintain the stable RSF network. In our previous works, we found when we added Ca(II) ions into natural *B. mori* silkworm dope, its β -sheet content decreased but its intermediate state content increased by means of Raman spectroscopy. Same phenomenon was found when we added Ca(II) ions into 26% (W/W) RSF solution, *i.e.*, the content of intermediate state increased from 30% to 35%^[12]. Therefore, when spinning dope with Ca(II) ions was extruded into coagulation bath, the coagulation rate slowed down because of the presence of Ca(II) ions. Such an effect can be found even if when Ca(II)/RSF ratio was only 1/50. The less skin-core structure in R1-6X fiber was an evidence for the decrease in coagulation rate, which weakened the hard skin formation. The thin skin in as-spun fiber was favorable for coagulator to further diffuse into the core of as-spun fiber, resulting in fewer voids. In the meantime, the thin skin was also favorable for the even shrinkage of the as-spun fiber and thus maintaining its round-shaped cross-section (Fig. 1b). When Ca(II)/RSF ratio increased to 1/20, the coagulation rate was even slower, therefore the whole fiber solidified in the same rate, resulting in a homogeneously dense structure (Fig. 1c). For the cross-section, we think the reason for the tabular shape in R2-6X fiber may be different from the dumbbell-like one in R0-6X fiber. In the spinning process of R2-6X fiber, as the coagulation rate was quite slow, during taking out from coagulation bath and winding on the first roller, the fiber was still soft and was easy to be flattened by pressing onto the roller. Similarly, when Ca(II)/RSF ratio further raised to 1/10, the effect of Ca(II) was too strong that hindered the solidification of the spinning dope, so that the cross-section of R3-6X fiber was more tabular (Fig. 1d).

Obviously, post-draw improves the molecular alignment of RSF fibers during wet spinning process^[7]. Therefore, the slow coagulation rate from the presence of Ca(II) ions gave the opportunity for the further alignment of molecular chains (Fig. 3), so the level of molecular orientation of R1-9X and R2-9X fibers reached about 70% of that of the natural cocoon silk. If there was no Ca(II) ions addition, the level of molecular orientation of RSF fiber was much lower. For instance, R0-6X fiber only reached about half of the natural cocoon silk, and cannot be post-drawn further. It is well known that the increase in molecular orientation in RSF fiber improves its mechanical properties^[7], thus both R1-9X and R2-9X fibers exhibited good mechanical properties (Table 1). However, because of the difference in coagulation rate during the wet-spinning process, the mechanical properties of R1-9X and R2-9X fibers were slightly different. For R2-9X fiber, because of the complete coagulation for the entire fiber that formed more β -sheet structure, it had the highest breaking strength (314 MPa). While for R1-6X fiber, as the coagulation in the center of fiber was slow, so the molecular chains had more extensibility, therefore it showed the highest breaking strain (57%). Moreover, though R1-9X fiber seems more flexible and R2-9X fiber is stronger, their breaking energy is almost the same and is larger than that of the natural cocoon silk. As we can see, the level of molecular orientation of our RSF fibers just reached about 70% of that of the natural *B. mori* silkworm cocoon silk, so if we could modify the spinning conditions to further increase the molecular alignment, the mechanical properties of RSF fibers may be comparable to those of the spider dragline silk or the force-reeled silkworm silk^[14].

If the Ca(II) ion content in RSF spinning dope was too high (for instance, 1/10), the effect of the maintenance on the stable silk fibroin network was too strong, which prevented the formation of enough β -sheet structures and the alignment of molecular chains in RSF fibers. Therefore, both the mechanical properties and molecular orientation of R3-9X fiber were worse than those of R1-9X and R2-9X fibers.

CONCLUSIONS

In this article, we report our findings that suitable amount of Ca(II) ion addition in spinning dope is favorable to produce RSF fibers with good mechanical properties, which are significantly tougher than the natural silkworm cocoon silk. We conclude such an improvement is because Ca(II) ions alter the coagulation rate of spinning

dope to fiber, and subsequently give the opportunity for the well alignment of the molecular chains during the post-draw process. This work confirms that an appropriate coagulation rate is critical to produce high performance RSF fibers that was also found in other wet-spinning systems in our laboratory^[15, 16].

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