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A study on the flow stability of regenerated silk fibroin aqueous solution

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Abstract

The flow stability of silk fibroin (SF) aqueous solutions with different concentrations under different temperatures was investigated. It was found that the flow stability decreased quickly with the increase of solution concentration and temperature. X-ray diffraction, Fourier transform infrared (FTIR) and Raman spectroscopy analysis showed that silk fibroin in aqueous solution was mainly in random coil and α-helix conformation. However, it turned into α-helix and β-sheet conformation after gelation, and both silk I and silk II crystalline structures appeared accordingly. The investigation implies that the original dilute regenerated SF aqueous solution should be stored under low temperature and concentrated just before spinning.

Keywords: Silk fibroin; Gel; Conformation; Aqueous solution; Flow stability

1. Introduction

Spiders and silkworms produce silks with outstanding mechanical properties despite being spun at ambient temperatures and pressures using water as the solvent [1–3], so researchers from different fields are interested in mimicking the native silk fiber and hope to produce high-performance artificially spun silk products in the near future [4,5]. Because there is still not enough spider silk protein that can be supplied to do spinning and the composition of silkworm fibroin is very similar to spider protein, silk fibroin of silkworm is selected as a model system in our work. The spinning process of silkworms is very complicated. Silk fibroin (SF) is produced in a single type cell lining a pair of long tube-like glands since the fifth larval instar of silkworm [6]. The gland consists of three relatively distinct regions: the posterior section where SF is synthesized by epithelial cells, the middle section where SF is stored and the sericin is synthesized, and the anterior section which tapers toward the spinneret. Both of the glands connect together before spinneret to produce a single duct. Sericin surrounds SF as a layer without mixing in the gland. The concentration of SF aqueous solution in the posterior gland is 12–15 wt%, while the concentration of SF and sericin aqueous solution is approximately 30 wt% (about 7 wt% sericin coating) in the middle gland [7]. At the end of the fifth larval instar, SF and sericin aqueous solution is extruded through the spinneret into the air and the proteins are converted from a concentrated viscous solution into a strong filament. During the process, it takes several days since SF aqueous solution is produced and moves along the gland before spinning, and the silkworm can keep the proteins in solution and spinnable.

In mimicking the spinning process for silk fiber, we are hoping to obtain spinnable SF aqueous solution with fluidity. That means we have to make a dope with high SF concentration (around 30 wt% as in silkworm) and processable, it should be a fluid. However, in making such kind of regenerated SF aqueous solution, it is found that the solution is unstable, thus the problem of the flow stability of SF aqueous solution arises. It would turn into gel and could not flow very quickly if the storing time and temperature are not controlled. How the time and temperature affect the gelation of regenerated SF aqueous solution and what changes in the gelation? In order to answer these questions and to obtain optimal storing conditions for SF aqueous solution, the influences of temperature and SF concentration on the flow stability of the solution was investigated, and the structure

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of concentrated regenerated SF aqueous solution before and after gelation was also studied.

2. Experimental

2.1. Preparation of regenerated SF aqueous solution

Cocoons of *B. mori* silkworms were boiled twice in 0.5% (w/w) $NaHCO_3$ solution for 30 min each, then rinsed thoroughly with distilled water and allowed to air dry at room temperature. The degummed silk was dissolved in 9.3 M LiBr aqueous solution at room temperature to a concentration of 10 wt%. It was diluted with five times of water and then dialysed against distilled water for three days. Then the solution was filtered, and the final SF aqueous solutions with desired concentrations were prepared under 4 °C.

2.2. Measurements

Wide-angle X-ray diffraction (WAXD) patterns were measured by a reflection method and recorded on a Rigaku D/Max-BR diffractometer, using Cu Kα radiation ($\lambda = 1.54$ Å) in the 2θ range of 5–40° at 40 kV.

Fourier transform infrared (FTIR) spectra were obtained using a NEXUS-670 (Nicolet Corp.) spectrometer in the spectral region of 4000–350 cm^{-1}.

Raman spectra were obtained using a Lab RAM-1B microscopy Raman microscope. The 632.8 nm red line of a He–Ne laser was focused on the sample.

In order to quantitatively describe the flow stability about this system, here we define it as follows [8].

Regenerated SF solutions with different concentrations are sealed in glass syringes (with diameter of 15 cm) respectively under different temperature. After a certain time, the gelation phenomenon takes place, it can be judged by the appearance of insoluble SF clots on one hand. And on the other hand, lean the syringe at a gradient of 45°, if SF aqueous solution keeps immobile for 30 s, the gelation process would be thought to have completed and SF aqueous solution loses its flow ability. Accordingly, the flow stability of SF aqueous solution would be characterized by the time needed for completing gelation process, which is called gelation time later for short. The longer the time is, the better the flow stability SF aqueous solution has.

3. Results and discussion

3.1. Effect of SF concentration and temperature on the flow stability of SF solution

As we all know, the protein aqueous solution is very sensitive to solution concentration, temperature, pH value and ions, etc. [9]. The pH value of regenerated SF aqueous solution used in this work was around 7 and no any ions was added, only the influence of SF concentration and temperature on the flow stability of SF aqueous solution was investigated. Keep SF aqueous solutions with different concentrations in each glass syringe under the same temperature, the effect of SF concentration was firstly investigated. The result was shown in Table 1 and Fig. 1, the temperature was controlled at 45 ± 0.5 °C.

Table 1
Gelation time of SF aqueous solutions with different concentrations under 45 °C

Sample code	Temperature (°C)	SF concentration (wt%)	Gelation time (h)
1	45	15	144
2	45	25	56
3	45	30	32
4	45	35	16
5	45	40	6
6	45	45	4
7	45	50	0.25

It is found that it takes 144 h (about 6 days) to complete the gelation process under 45 °C when the concentration of SF aqueous solution is 15 wt%. However, the gelation time decreases quickly with the increase of the concentration of SF aqueous solution. It takes only a quarter of an hour in the case of 50 wt% SF concentration as shown in Table 1. This means that the flow stability of SF aqueous solution decreases with the increase of SF concentration. Therefore, it is impossible to store the SF aqueous solution with high concentration for a long time.

Fig. 1 shows the relationship between the concentration and the gelation time of SF aqueous solution when temperature is 45 ± 0.5 °C. It can be estimated from Fig. 1 that a key concentration is about 27 wt%, below which the gelation time decreases sharply with increasing SF concentration. However, the gelation time decreases slowly with increasing concentration when the concentration is higher than 27 wt%. It is interesting that the concentration of the SF aqueous so-

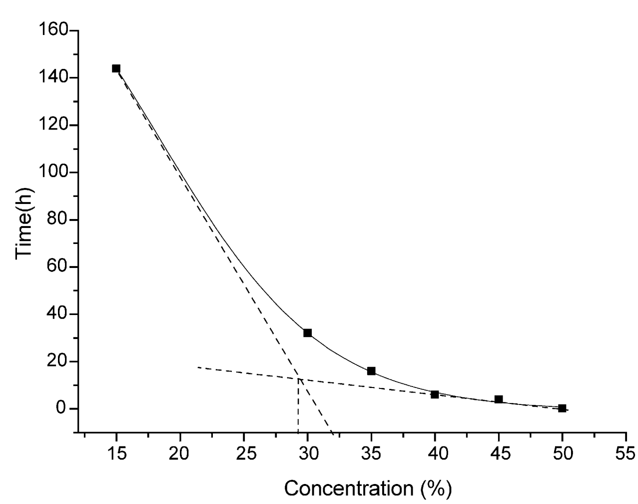

Fig. 1. Relationship between gelation time and concentration of SF aqueous solutions under 45 °C.