JOURNAL OF MATERIALS SCIENCE 40 (2005) 5355–5358

Studies on spinning and rheological behaviors of regenerated silk fibroin/*N*-methylmorpholine-*N*-oxide·H_2O solutions

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In this paper, *N*-Methylmorpholine-*N*-Oxide (NMMO) was used as solvent for regenerated silk fibroin (RSF). Concentrated solutions with RSF content from 10 to 25 wt% were obtained by dissolving RSF film into NMMO monohydrate. The spinning and rheological properties of RSF/NMMO·H_2O solutions were studied by a simple piston type single filament spinning apparatus and HAAKE RS150L rheometer, respectively. The results are discussed to understand the influences of shear rate, temperature and RSF concentration on the rheological behaviors of RSF/NMMO·H_2O and to choose appropriate process condition for spinning. Spinning was performed using a dry-wet spinning process. The regenerated silk fibre was prepared successfully and the tenacity can reach to 3.07cN/dtex.

1. Introduction

Silk is a well-known natural fibre produced by the silkworm, *Bombyx mori*, which has been used traditionally in form of threads. It is composed of two kinds of protein: a fibrous one (named silk fibroin) that forms the thread core, and a glue-like one (named sericin) that surrounds the fibroin fibers to stick them together. Although the use of silk as threads is very popular, recently, interest has been increasing in the use of solubilized silk fibroin (SF) in biotechnological materials, and biomedical applications. It should be noted that silk must be regenerated into a desirable form to meet a specific biomedical application. To date, the SF is known to be soluble in several special solvents. The most common solvent is aqueous 9.0–9.5 M LiBr [1]. Moreover, mixtures of aqueous calcium chloride and ethanol $CaCl_2$-EtOH-H_2O [2, 3], calcium nitrate in methanol $Ca(NO_3)_2$-MeOH [4] or aqueous lithium bromide and ethanol LiBr-EtOH-H_2O [5, 6] were also used as well as aqueous lithium thiocyanate LiSCN [7] and aqueous sodium thiocyanate NaSCN [8]. Those solutions have been used to cast films for structure studies. In addition, aqueous SF solution can also be obtained from formic acid or H_3PO_4 solution [9]. The dissolution of SF in LiCl/N,*N*-dimethylacetamide has been investigated thoroughly [10]. Those skills in the art have been attempted to find suitable solvents for preparing silk fibroin solutions, which may be subsequently spun into fibers. A number of these solvents have been described in a series of patents assigned to the E.I. DuPont de Nemours & Co. These patents disclose further methods for producing solutions of fibroin and other fiber-forming polypeptide in hexafluoroisopropanol in combination with lithium salt solutions and the like [11–13]. Although it has been possible to produce silk fibroin fibers from such spinning solutions as described above, these solvents tend to be harsh and may be very difficult to recycle.

A number of scientific reports and patents have focused over the last two decades on the development of new organic solvents for cellulose, with a final goal of preparing regenerated cellulose films and fibers through a simple nonpolluting process. *N*-methyl morpholine *N*-oxide (NMMO) appears to be the most potential direct solvent for cellulose and it is environment friendly and easy to be recycled. In 1999, Freddi [14] first found that NMMO monohydrate is also a good direct solvent for SF and its relatively low melting point allows people to conduct dissolution and spinning experiments in a reasonably safe temperature range (90–110°C) to avoid excessive thermal degradation of SF. However, he did not obtain the silk solution suitable for fibre spinning. On the other hand, the concentration of RSF in NMMO monohydrate can easily reach up to 25 wt% by dissolving the regenerated silk fibroin (RSF) film into NMMO monohydrate. The aim of this study is to investigate the rheological behaviors of RSF/NMMO·H_2O solution

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0022-2461
DOI: 10.1007/s10853-005-4301-9

and choose appropriate process conditions for fibre spinning.

2. Experimental procedures

2.1. Samples

Fresh *Bombyx mori* cocoons were cut to remove the pupa, degummed twice with anhydrous sodium carbonate 0.5% (o.w.f.) solution at 100°C for 1 h, then rinsed thoroughly in warm deionized water and dried at room temperature.

2.2. Preparation of RSF film and RSF/NMMO·H_2O solution

Degummed silk fibres from *Bombyx mori* cocoons were dissolved in 9.3 M LiBr aqueous solution under constant stirring for several hours at room temperature. The salt was removed by hollow fibers dialysis. After being filtered, the aqueous solution of RSF was cast onto flat polyethylene sheets and allowed to stand at room temperature for drying overnight. A transparent, cellophane-like RSF films were prepared.

NMMO aqueous solution with 50 wt% water content (purchased from BASF) was distilled at 70–100°C under vacuum to prepare NMMO·H_2O solution with 13.3 wt% water content.

Dissolution of RSF was performed in a dissolving apparatus, in which the RSF films and the melted solvent were added. With continuing vigorous mixing for 4–5 h, a thick, clear, and light yellowish solution was obtained.

2.3. Spinning of RSF/NMMO·H_2O solution

Spinning was performed on a simple piston type single filament spinning equipment using a dry-wet spinning process. Methanol was used as coagulation bath.

2.4. Measurements

Rheological measurements were performed on a HAAKE RS150L rheometer (manufactured by Hakke Company, Germany) using a C35/1°C cone and plate system. The range of shear rate was from 0.01 to 250 s^{-1} and the measurements were carried out at the range of temperature from 70 to 100°C. The rheological curves were obtained for the SF/NMMO·H_2O solution under different temperatures.

The tenacity of resultant fibre was measured on a XQ-1 tensile strength tester (manufactured by Donghua University, China) at the condition of 20°C and R.H. 65%.

3. Results and discussions

3.1. Influence of shear rate

Typical flow behaviors for the apparent viscosity of a molten polymer as a function of shear rate can be described in power-law equation 1:

$$\eta_a = K\dot{\gamma}^{n-1} \quad (1)$$

where constant K is called the consistency index, constant n is known as the power-law index and $\dot{\gamma}$ is

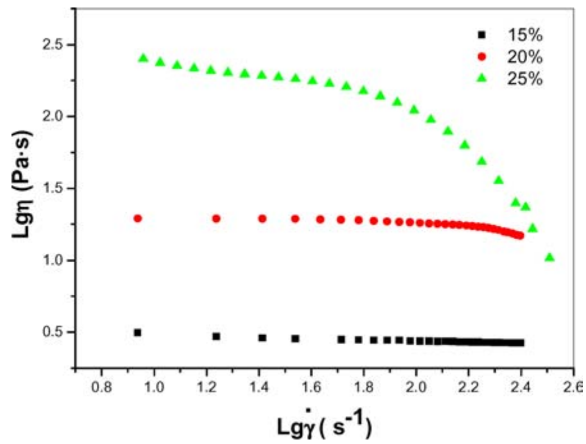

Figure 1 Flow curves of RSF/ NMMO·H_2O solutions with different concentration at 85°C.

shear rate. The flow curves of RSF/NMMO·H_2O solutions with different concentrations at 85°C are shown in Fig. 1. It can be seen that the apparent viscosity of RSF/NMMO·H_2O solution with a concentration of 15 wt% is very low. It has a slightly decrease at low shear rates and levels off into a single line at high shear rates. One of the possible reasons is that the fibroin macromolecule chains are intrinsically flexible [15]. Moreover, individual protein molecules exist as random coils in dilute solution and the concentration is too low to make the molecules to entangle. Therefore, the RSF/NMMO·H_2O solution with a concentration of 15 wt% is dilute and not very sensitive to shear. Fig. 1 also reveals that zero-shear viscosity η_o increases apparently while RSF concentration becomes higher. In addition, shear thinning is observed in other two cases. This is because that individual globular molecules are allowed to form entanglement with the increase of RSF concentration. In other words, when the RSF concentration exceeds a critical value, protein molecular chains begin to be entangled with each other. At low shear rates, the shear stress is not high enough to completely destroy the entanglement and the solution has a relatively constant viscosity due to the constant entanglement density. This region is known as the lower Newtonian region. At the higher range of shear rates, however, the viscosity decreases sharply because of the disentanglement of molecular chains.

3.2. Influence of temperature

It is well known that temperature has an important effect on the rheological properties of polymer solutions. Fig. 2 shows the flow curves of RSF/NMMO·H_2O solutions with different concentrations at different temperatures. As illustrated in Fig. 2b and c the zero-shear viscosity η_o and the apparent viscosity η_a decrease obviously with increasing temperature. The onset point of shear thinning shifts to higher shear rate as the temperature increases. One possible explanation is that protein chain segments have enough space to move in the case of high temperature. Thus, it becomes easy for the protein chains to flow due to the weak van der Waals

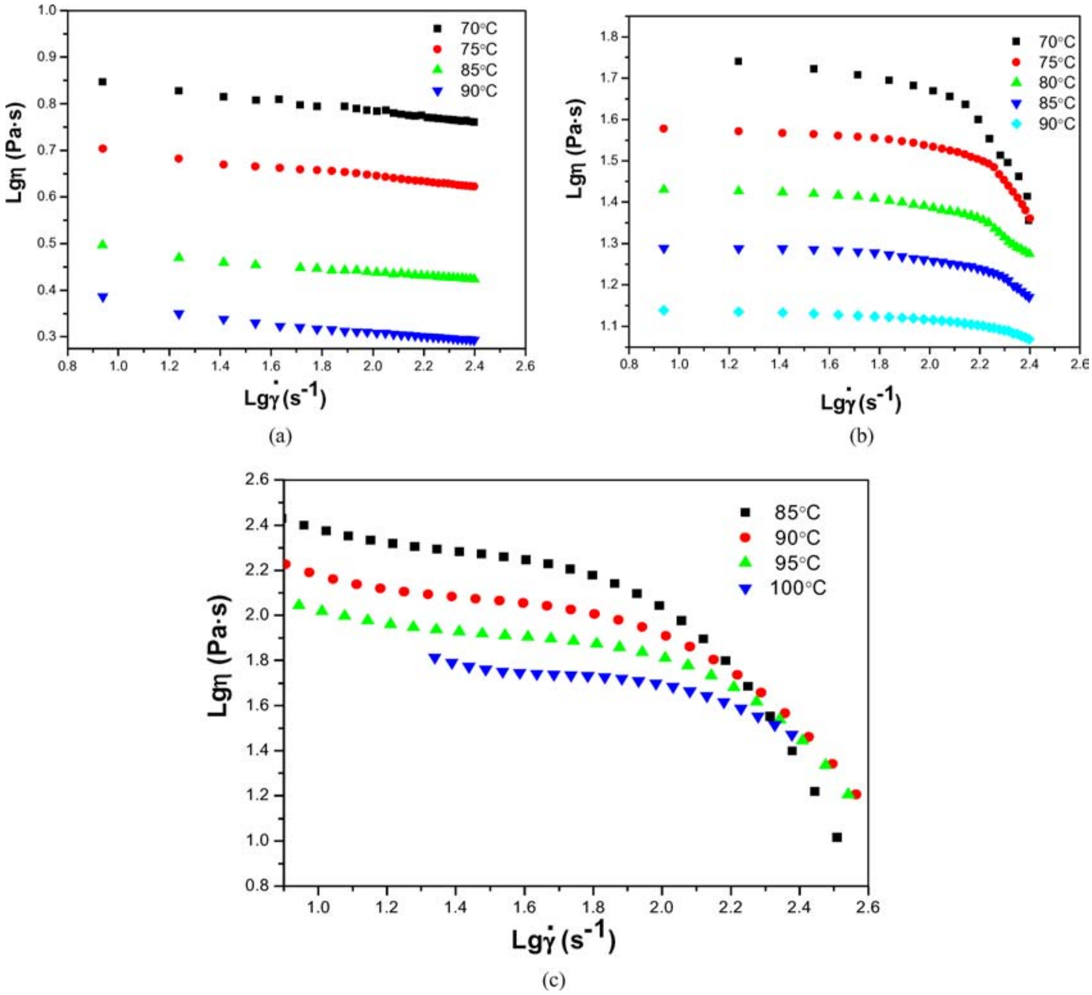

Figure 2 Flow curves of RSF/ NMMO·H_2O solutions at different temperatures. The concentration of RSF is (a) 15 wt% (b) 20 wt% (c) 25 wt%.

forces among many chains. The temperature dependence of zero-shear viscosity follows the Arrhenius equation to a good approximation:

$$\eta_0 = Ae^{E_\eta/\mathrm{RT}} \tag{2}$$

In this equation, A is a constant characteristic of the polymer, E_η is the activation energy for the process, R is the universal gas constant, and T is the temperature in degrees Kelvin. Table I lists the values of energy of activation E_η for different RSF solutions, which were calculated by equation 2. It can be seen that the energy of activation E_η increases with increasing concentration of the RSF solution, which indicates that the viscosity of the concentrated dope is more sensitive to variation of temperature than those of dilute solutions.

3.3. Influence of RSF concentration

The concentration of polymer in the solution has important effects on choosing process conditions for spinning and product properties as well as the temperature. If the polymer concentration is too low, it is impossible for fibre formation due to lack of chain entanglements in solutions. If the polymer concentration is too high, however, the solution is difficult to process or need higher temperature to process, and it may be led to a strong degradation. Therefore, the RSF content in the spinning dope is an important factor in preparing regenerated silk fibres.

As shown in Fig. 2, RSF concentration, on the other hand, is also an important factor that can control the rheological properties of the solutions. The zero-shear viscosity can be obtained from the flow curves and are listed in Table II. It can be seen that the zero-shear viscosity η_o of the solution with 15 wt% is 3.135 Pas when the temperature is set to 85°C, while that of the solution with 25 wt% is increased to 211 Pas. As a result, the dilute RSF solution with 15 wt% cannot be used to spin fibres due to the very low viscosity. The solutions with concentration of 20 wt% were investigated to spin in the range of temperature from 70 to 90°C, whereas the solution with 25 wt% was tested in the higher range of temperature i.e., 85 to 100°C, since the solution with 25 wt% will be frozen below 85°C. It can also be found from Fig. 2 that the viscosity is stable in the range of low shear rates and it is worthy to point out that the solution with 20 wt% at 70°C and the solution with 25 wt% at 100°C have analogous rheological behaviors, such as zero-shear viscosity and

TABLE I Energy of activation E_η for different RSF solutions

RSF concentration (wt%)	15	20	25
E_η(KJ/mol)	23.36	30.91	45.97

TABLE II Zero-shear viscosity of solutions with different concentrations

	η_o/Pa·s		
Temperature/°C	15 wt%	20 wt%	25 wt%
70	7.025	59.94	/
75	5.057	37.82	/
80	/	26.92	/
85	3.135	19.46	211.1
90	2.435	13.97	167.3
95	/	/	108.9
100	/	/	70.04

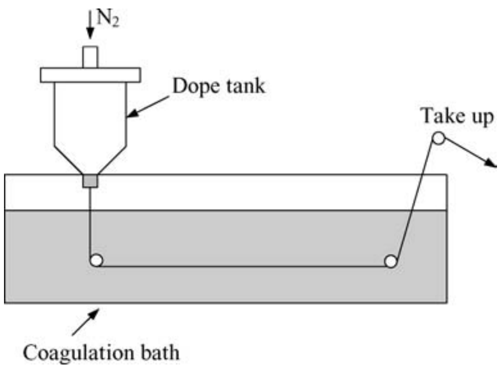

Figure 3 Scheme of single filament spinning apparatus.

critical shear rate. That indicates the solution with 20 wt% can be spun at relatively low temperature and the solution with 25 wt% has to be spun at relatively high temperature. It is well known that processing temperature higher than 110°C may partially lead to active thermal degradation of NMMO in which the liberated atomic oxygen may also lead to degradation of SF [16–18]. It is concluded that the appropriate concentration of RSF in NMMO·H_2O for spinning ranges from 20 to 25 wt% and the processing temperature is from 85 to 100°C.

3.4. Regenerated silk fibre spun from RSF/NMMO·H_2O solution

According to the rheological properties of RSF/NMMO·H_2O solution, the RSF solution with a concentration of 20 wt% was selected as spin dope and a dry-wet spinning process was used to spin regenerated silk fibre. Fig. 3 shows scheme of single filament spinning equipment to which the spinning solution was transferred. After deaeration, compressed nitrogen gas was applied to force the solution passing through a 0.3 mm diameter stainless steel spinneret into a bath containing methanol at room temperature, whereby the fibroin was precipitated in the form of continuous filaments. The spinning temperature is 90°C and the air gap length is around two centimeters. These filaments were wound on a bobbin at 3.2 m/min and allowed to stand in methanol overnight. Then, while still wet with methanol, these filaments were drawn to 3.6 times before air drying. Mechanical testing of the dry samples showed that it has a average tenacity of 3.07 cN/dtex, which is a little bit lower than the tenacity of the native silk fibre (3.58 cN/dtex).

4. Conclusion

The rheological results showed that characteristic of the RSF/NMMO·H_2O solutions is shear-thinning, i.e., the apparent viscosity decreases as the shear rate increases. With increasing RSF concentration, the apparent viscosity tends to increase and the non-Newtonian behavior becomes obvious. The investigation on rheological behaviors indicates that the appropriate concentration of RSF in NMMO·H_2O for spinning is from 20 to 25 wt% and the processing temperature is from 85 to 100°C.

According to these investments, RSF/NMMO·H_2O solution with relatively high RSF concentration are viscous and suitable for fibre spinning. The regenerated silk fibre was prepared successfully and the tenacity can reach to 3.07cN/dtex.

Acknowledgment

The authors thank for the financial support by Hi-Tech Research and Development Program of China (863), No. 2002AA336060.

References

1. X. CHEN, Z. SHAO, N. S. MARINKOVIC, L. M. MILLER, P. ZHOU and M. R. CHANCE, *Biophys. Chem.* **89** (2001) 25.
2. M. LI, S. LU, Z. WU, H. YAN, J. MO and L. WANG, *J. Appl. Polym. Sci.* **79** (2001) 2185.
3. M. LI, Z. WU, C. ZHANG, S. LU, H. YAN, D. HUANG and H. YE, *ibid.* **79** (2001) 2192.
4. A. B. MATHUR, A. TONELLI and T. RATHKE, S. HUDSON, *Biopolymers* **42** (1997) 61.
5. K. MATSUMOTO, H. UEJIMA, *J. Polym. Sci. Polym. Chem.* **35** (1997) 1949.
6. K. MATSUMOTO, H. UEJIMA, Y. SANO and H. SUMINO, *ibid.* **35** (1997) 1955.
7. N. AGARWAL, D. A. HOGLAND and R. J. FARRIS, *J. Appl. Polym. Sci.* **63** (1997) 401.
8. Y. SUN, Z. SHAO, M. MA, P. HU, Y. LIU and T. YU, *ibid.* **65** (1997) 959.
9. H. ISHIZAKA, Y. WATANABE, K. ISHIDA and O. FUKUMOTO, *J. Seric. Sci. Jpn.* **58** (1989) 87.
10. K. I. FURUHATA, A. OKADA, Y. CHEN, Y. XU and M. SAKAMOTO, *ibid.* **63** (1994) 315.
11. R. L. LOCK, US Patent5171505 (1992).
12. *Idem*, US Patent 5252285 (1993).
13. *Idem*, US Patent 5252277 (1993).
14. G. FREDDI, G. PESSINA and M. TSUKADA, *Int. J. Bio. Macro.* **24** (1999) 251.
15. C. VINEY, A. E. HUBER, D. L. DUNAWAY, K. KERKAM and S. T. CASE, in "Silk Polymers: Materials Science and Biotechnology" (American Chemical Society, Washington DC, 1994) p.120.
16. T. ROSENAN, A. POTTHAST, P. KOSMA, H. FIRGO and H. T. SIXTA, in Proceedings of the 4th International Symposium "Alternative Cellulose-Manufacturing, Forming, Properties," Rudolstadt, Germany, September 6-9, 2000, p. 1.
17. T. RAAENAU, A. POTTHAST, P. KOSMA, C. CHEN and J. S. GRATZL, *J. Org. Chem.* **64** (1999) 2166.
18. F. A. BUIJTENHUIJS, M. ABBAS and A. J. WITTEVEEN, *Das Papier* **40** (1986) 615.

Received 10 July 2004
and accepted 14 February 2005