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Electrospinning and rheology of regenerated *Bombyx mori* silk fibroin aqueous solutions: The effects of pH and concentration

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ABSTRACT

In this study, we prepared regenerated silk fibroin (RSF) aqueous solution to approach the environmental condition in the gland of silkworm, *Bombyx mori*. Then electrospinning technique was used to prepare the silk fibers. The results showed that pH and concentration had a remarkable influence on the properties of RSF aqueous solutions. With the increase in concentration and the decrease in pH, the rheological behavior of RSF aqueous solutions exhibited a transition from Newtonion fluid to non-Newtonion fluid. At the same time, lowering the pH could induce gel formation and decrease the electrospinnable concentration of RSF aqueous solutions. With the decrease in pH and concentration, the morphology of the electrospun silk fibers changed from belt-like shape to uniform cylinder. The conformation of the electrospun silk fibers was characterized by RS, WAXD and DSC. It was found that electrospun fibers were predominantly random coil/silk I conformation.

1. Introduction

Electrospinning is an attractive method of producing fibers with diameters ranging from micrometers down to tens of nanometers [1,2]. The electrospun silk fibers, with high specific surface area, high porosity and good biocompatibility, have extensive applications in the field of biomaterials, such as wound dressing [3], scaffolds for tissue engineering [4] and drug delivery [5,6]. *Bombyx mori* silk has previously been electrospun to produce fibers from hexafluoro-2-propanol (HFIP), hexafluoroacetone-hydrate (HFA-hydrate) or 98% formic acid solutions [7–12]. Jin et al. firstly reported that *B. mori* silk could be electrospun as a blend with PEO from aqueous solutions [13], and then they obtained silk/PEO fibers with core/shell structure [14]. Recently, silk fibroin aqueous solutions from degummed *B. mori* silk or *B. mori* gland have been used to prepare silk fibers and the fibers with submicron diameter were successfully obtained [15–17].

Studies on both silkworm and spider have indicated that several physiological parameters, such as water content, pH, shear rate and elongational flow, are thought to be very important in the natural spinning process [18–24]. Magoshi et al. found that when the silk dope flows from the posterior division to the anterior division of a silk gland of silkworm, it has a decrease in water content from 88 to 70 wt% and a decrease in pH from 6.9 to 4.8. The conformation of the silk dope is also thought to be transformed from the predominant random coil into a liquid crystalline β-sheets. Spinning dope then undergoes elongation at a rate of 500 mm/min through small spinnerets to form water insoluble fiber in air [25].

The electrospinning process is somewhat analogous to the spinning process of silkworm. After undergoing a shear flow in the tube and capillary, the jet of the solution is also elongated in air, although the elongation effect of electrospinning process is much stronger than that of the spinning process of silkworm [26]. Our previous results showed that *B. mori* silk could be electrospun from aqueous solutions with high concentration at neutral pH [27]. In this paper, we further studied the effects of pH and concentration on the electrospinning and the rheology of regenerated silk fibroin (RSF) aqueous solutions. RSF aqueous solutions with high concentration were prepared and adjusted to various pHs which approach to those in the different divisions of the gland of silkworm. Then electrospinning technique was applied to prepare silk fiber. Finally, the morphology and the conformation of the electrospun silk fibers were investigated.

2. Experimental

2.1. Preparation of RSF aqueous solution

Cocoons of *B. mori* were degummed twice with 0.5 wt% Na_2CO_3 solution at 100 °C for 30 min and washed with deionized water to

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remove the sericin. After drying at room temperature, the degummed fibers were dissolved in a 9.0 M LiBr aqueous solution at 40 °C for 2 h yielding a 10% (w/v) solution. This solution was dialyzed in deionized water for 3 days with a cellulose semi-permeable membrane (molecular weight cutoff 14,000). To prepare condensed RSF aqueous solution, the water in the solution was slowly removed by forced airflow at 10 °C, the concentration of the solution was controlled by the velocity of the airflow and monitored by weighing the remaining solid after drying. After a certain time, a 20 wt% RSF aqueous solution was prepared.

2.2. Preparation of spinning solutions

In order to approach the environmental condition in the gland of silkworm, RSF aqueous solutions at pH 6.9, 6.0, 5.6, 5.2 and 4.8, which are similar to the pH in the different divisions of the gland of *B. mori* [25], were firstly prepared by adding 0.1 M citric acid–sodium hydroxide (NaOH)–hydrochloric acid (HCl) buffer reagent into 20 wt% concentrated RSF aqueous solution in the volume ratio of 1:2. Then the solutions were condensed by forced airflow at an airflow rate of 5 ± 1 m/s to different concentrations at 10 °C and 50 ± 5% relative humidity. The solutions for electrospinning were homogeneous without any precipitates and air bubbles.

2.3. Electrospinning process

In the electrospinning process, a high potential was applied to a wire connected with a syringe filled with 5 mL of RSF solution, while a collection plate covered with an aluminum foil was grounded. The internal diameter of the syringe tip was 0.6 mm and the flow rate of the solution was 2.0 mL/h. The electric field was 4 kV/cm and the electrospinning process was carried out at ambient temperature.

2.4. Characterization

The rheological measurements were performed on a HAAKE RS150L Rheometer (Therm Haake Co., Germany) with a 35 mm cone plate (Ti, 35/1°). The shear rate was linearly increased from 0.01 to 1000 s^{-1} and the temperature was controlled at 25 ± 0.1 °C.

The morphology of the electrospun silk fibers was observed with a JSM-5600LV (JEOL Co., Japan) scanning electron microscope (SEM). Hundred fibers were randomly used to determine the average diameter and the diameter distribution of the fibers.

Raman spectra were obtained using a LabRam-1B microscopy Raman spectrometer (Dilor, France) coupled with an Olympus BX 40 optical microscope (100× objective). The electrospun mats were fixed on the appropriate viewing frames. A He–Ne laser at 632.8 nm with about 6 mW of power was focused on the individual fiber. Spectral data were accumulated for a 200 s period at a fixed grating position and recorded from 900 to 1800 cm^{-1}.

The wide-angle X-ray diffractograms were obtained on a D/Max-BR diffractometer (RigaKu, Japan) with Cu Kα radiation in the 2θ range of 5–50° at 40 kV and 300 mA.

The differential scanning calorimetry (DSC) analyses were carried out using an MDSC 2910 differential scanning calorimeter (TA Instruments Co., USA). The sealed samples were heated at 3 °C/min from 25 to 330 °C. The nitrogen gas flow rate was 40 mL/min.

3. Results and discussions

3.1. Gel formation and rheological behavior of RSF aqueous solutions with different pHs

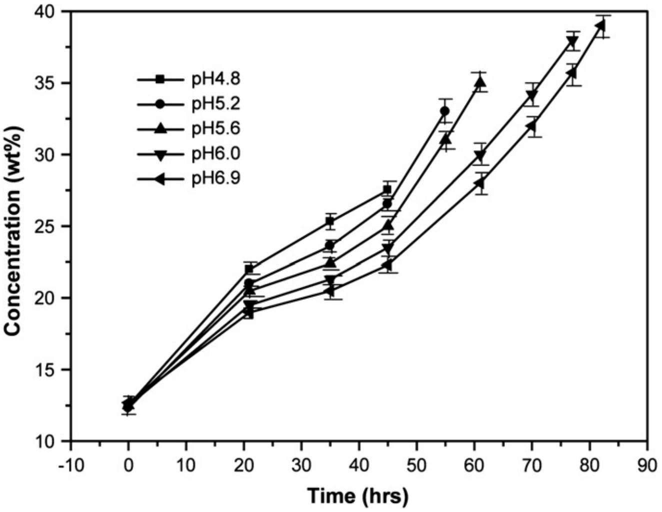

Fig. 1. The relationship between the concentration of RSF aqueous solutions at various pHs and concentration time. (-◄-) pH 6.9, (-▼-) pH 6.0, (-▲-) pH 5.6, (-●-) pH 5.2 and (-■-) pH 4.8. Values are average ± standard derivation of three samples.

pH and water content are thought to be two important factors in affecting conformations [28–30]. Fig. 1 shows the relationship between the concentration of RSF aqueous solutions at various pHs and concentration time. It can be seen that the concentrations of the solutions increased with increasing time and the solution with a low pH had a high concentration at the same concentration time. With the reduction in pH, the solutions were easy to evaporate water and become gels. We defined the concentration just before gelation is the critical concentration, which decreased from 35 to 27 wt% when the pH of the solutions reduced from 5.6 to 4.8. This result indicated that lowering the pH induced gel formation and the solutions near neutral pH were difficult to gelate even for the highly concentrated solutions at 10 °C. It also suggested that the optimal pH value for gel formation was fairly closed to the isoelectric point of H-chain fibroin (pI = 4–5) and the near neutral pH in the lumen of the posterior of the silkworm may provide a safe environment for storing the protein. Similar results were also found by Terry et al. [22]. We suggested that lowering the pH suppressed repulsive forces between the negative charges in two regions of the fibroin molecule. Thus the hydrophobic blocks approached one another to produce hydrophobic interactions and induced the gel formation [21].

Fig. 2 shows the rheological behavior of RSF aqueous solutions with different concentrations at pH 6.9, 6.0, 5.6, 5.2 and 4.8, respectively. At each certain pH, the solutions with higher concentrations showed higher viscosities, as might be expected for common polymeric solution. When the concentrations of the solutions were as low as 20 wt%, the viscosities were almost independent of shear rate and the solutions were Newtonion fluid (Fig. 2a–d). With the increase in concentration and the decrease in pH, shear thinning behavior could be obviously observed at very low shear rates, while shear thickening behavior also was observed at high shear rates ($\gtrsim 500\ s^{-1}$, Fig. 2a–d). The findings indicated that the solutions exhibited a transition from Newtonion fluid to non-Newtonion fluid. Actually, for the solutions with higher concentration, e.g., 33 wt% at a pH of 5.2, the shear flow response of the solutions became unstable and some white tough granules were separated from the sample. Therefore, we think shear thickening behavior was a stress induced phase separation and crystallization, which had been observed previously in the solutions removed from the gland of silkworm [22]. However, for the solutions at a pH of 4.8 (Fig. 2e), no obvious shear thickening behavior was observed since only the solutions with low concentrations (≤ 27 wt %) could be obtained at this pH value.

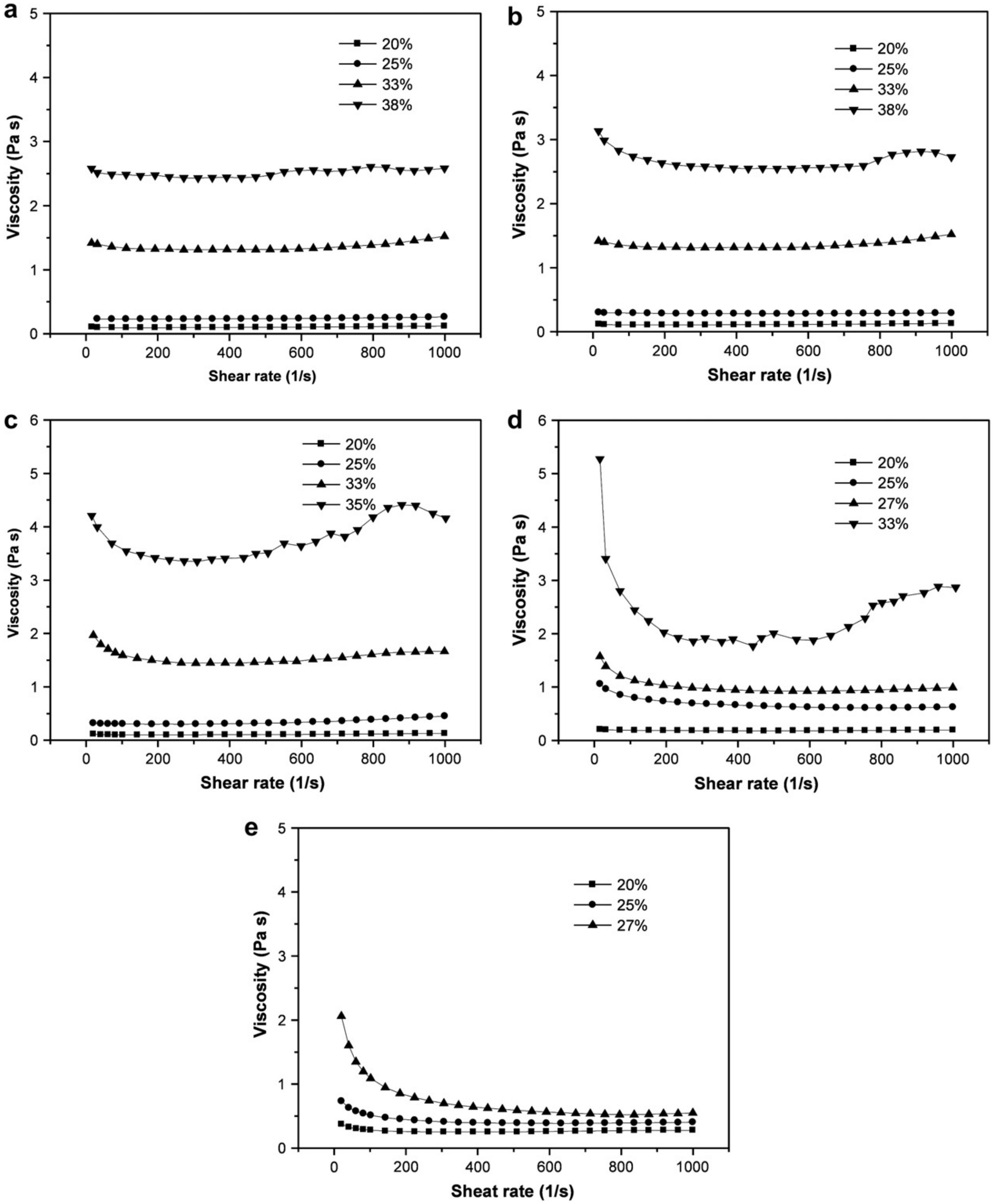

Fig. 2. The rheological behaviors of RSF aqueous solutions with different concentrations at various pHs. (a) pH 6.9, (b) pH 6.0, (c) pH 5.6, (d) pH 5.2 and (e) pH 4.8.

3.2. Effects of pH and concentration on the morphology and the diameter of the electrospun silk fibers

In this work, various silk fibers were electrospun from RSF aqueous solutions with different concentrations and pHs. The fiber diameter and the diameter distribution results are summarized in Table 1. It shows that with the reduction in pH, the electrospinnable concentration of RSF aqueous solutions decreased. Moreover, the average diameter and the diameter distribution of the electrospun silk fibers became smaller. This indicated that lowering of pH could induce fiber formation at low concentration. Due to the decrease in the concentration of the spinnable solutions, the average diameter and the diameter distribution of resultant electrospun silk fibers became small and narrow. When the pH of the solutions was 6.9, the viscosities of the solutions with concentrations lower than 33 wt% were too low to be electrospun. When the pH of the solutions was 4.8, the solutions with concentration higher than 25 wt% could not be electrospun either due to the gelation of the solutions.

Table 1
Effects of pH and concentration on the diameter and the diameter distribution of the electrospun silk fibers

pH	Concentration (wt%)								
	22	25	27	30	31	33	35	38	39
4.8	N/F	265 nm (s.d.96)	N/F	N/F	N/F	N/F	N/F	N/F	N/F
5.2	N/F	N/F	453 nm (s.d.129)	__	__	1064 nm (s.d.452)	N/F	N/F	N/F
5.6	N/F	N/F	N/F	617 nm (s.d.194)	__	840 nm (s.d.331)	958 nm (s.d.443)	N/F	N/F
6.0	N/F	N/F	N/F	N/F	707 nm (s.d.202)	718 nm (s.d.212)	__	803 nm (s.d.257)	N/F
6.9	N/F	N/F	N/F	N/F	N/F	893 nm (s.d.299)	823 nm (s.d.234)	__	887 nm (s.d.265)

Value is average diameter and value in parentheses is standard deviation; N/F: no fiber was obtained.

Fig. 3 shows the morphology and the diameter distribution of the electrospun silk fibers from RSF aqueous solutions with different pHs. With the reduction in pH and in concentration, the morphology of the electrospun silk fibers changed from belt-like shape to uniform cylinder. The uniform cylindrical fibers with a smaller average diameter of 265 nm could be obtained by using the solution at a pH of 4.8. Fig. 4 shows the morphology of the electrospun silk fibers prepared from the solutions with a concentration of 33 wt% at different pHs. With the reduction in pH, the morphology of the electrospun silk fibers changed from belt-like shape to uniform cylinder then to viscous belt-like shape. As the solution at a pH of 5.2 was approaching gel state and had a high

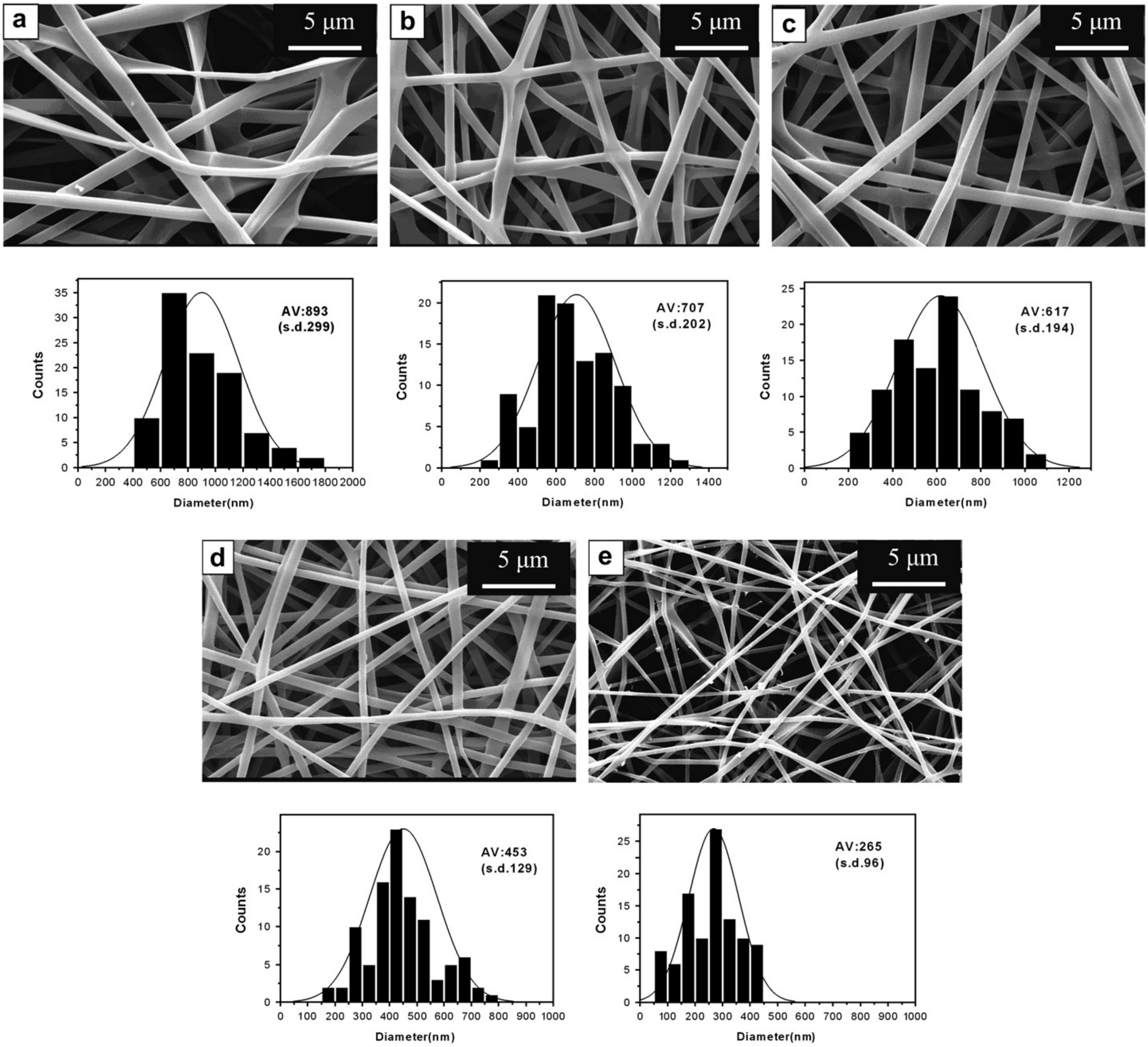

Fig. 3. The morphology and the diameter distribution of the electrospun silk fibers from RSF aqueous solutions. (a) pH 6.9, concentration 33%; (b) pH 6.0, concentration 31%; (c) pH 5.6, concentration 30%; (d) pH 5.2, concentration 27% and (e) pH 4.8, concentration 25%.

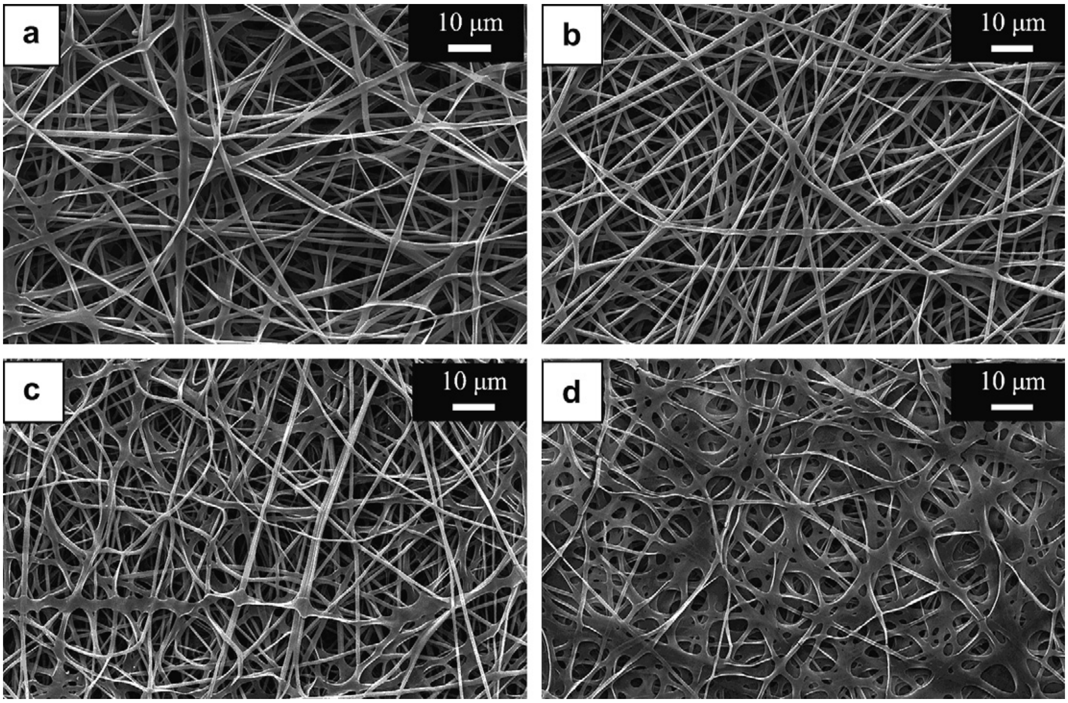

Fig. 4. The morphology of the electrospun silk fibers from RSF aqueous solution with a concentration of 33 wt% at a pH of (a) 6.9, (b) 6.0, (c) 5.6 and (d) 5.2.

viscosity, it was difficult to split into filaments. In addition, fibers were also difficult to completely dry. When the pH of the solution with a concentration of 33 wt% was 6.0, uniform cylindrical fibers with an average diameter of 718 nm were obtained.

3.3. *Characterization of the electrospun silk fibers*

In order to understand the effect of pH on the electrospun silk fibers, the conformation of the fibers from the RSF solutions with a concentration of 33 wt% at different pHs was investigated.

Fig. 5 shows Raman spectra of the electrospun silk fibers. It was found that with the reduction in pH, in the amide I region, the band at 1660 cm^{-1} (random coil or silk I) shifted to 1666 cm^{-1} (silk II), and in C–C stretch region, the intensity of the band at 1107 cm^{-1} (sensitive to silk I) decreased [31]. This indicated that lowering the pH facilitated the conformational transition of the electrospun silk fibers from random coil/silk I conformation to silk II conformation. But in the amide III region, all the electrospun silk fibers had the band ranging from 1276 to 1242 cm^{-1} (random coil or silk I), which suggested that the electrospun silk fibers were predominantly in random coil or silk I conformation [32].

Fig. 6 shows wide-angle X-ray diffractograms (WAXD) of the electrospun silk fibers. We noticed that the electrospun silk fibers showed a very weak peak at 27.8° (silk I). With the reduction in pH, the peak disappeared and other two very weak peaks appeared simultaneously at 20.6° and 24.7° (silk II) [33,34]. However, the similar shape of the four curves indicated that lowering the pH had a limited influence on the structure of the electrospun silk fibers. The main structure of the electrospun silk fibers was amorphous.

Fig. 7 shows the DSC curves of the electrospun silk fibers. It could be found that the four curves of the electrospun silk fibers displayed the weak endothermic shift at about 175 °C, which indicated the temperature of glass transition (T_g). And the four

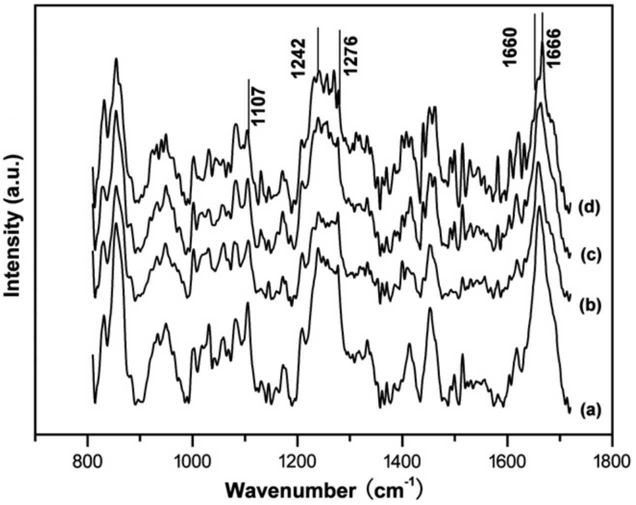

Fig. 5. Raman spectra of the electrospun silk fibers from RSF aqueous solutions with a concentration of 33 wt% at a pH of (a) 6.9, (b) 6.0, (c) 5.6 and (d) 5.2.

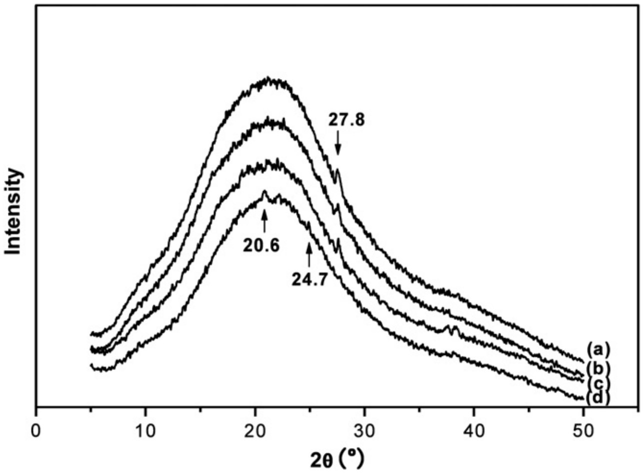

Fig. 6. WAXD of the electrospun silk fibers from RSF aqueous solutions with a concentration of 33 wt% at a pH of (a) 6.9, (b) 6.0, (c) 5.6 and (d) 5.2.

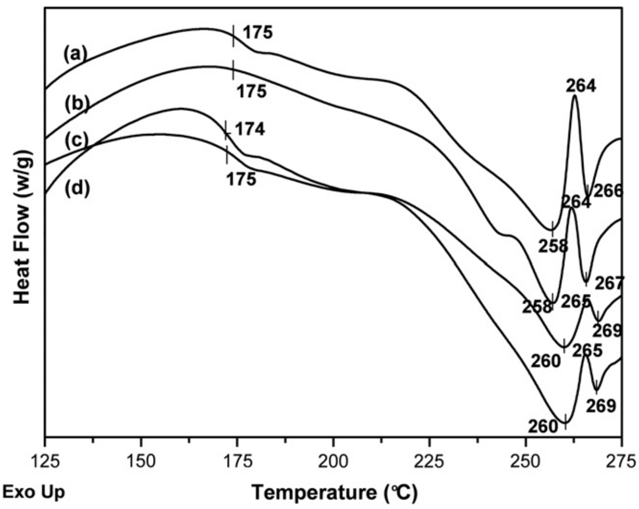

Fig. 7. DSC curves of the electrospun silk fibers from RSF aqueous solutions with a concentration of 33 wt% at a pH of (a) 6.9, (b) 6.0, (c) 5.6 and (d) 5.2.

endothermic peaks at 266–269 °C were attributed to the thermal decomposition of the electrospun silk fibers [35,36]. The four endo–exothermic peaks at around 258–265 °C may indicated conformational transitions from random coil/silk I to β-sheet [37]. This further confirmed that the electrospun silk fibers had mainly random coil and silk I conformation.

Our investigation revealed that the conformation of the electrospun silk fibers was predominantly random coil/silk I conformation. This indicated that lowering the pH in the solution had no apparent effect on the crystallization of the electrospun fibers. This could be explained that the evaporation of water and the short travel time of the jet in air (milliseconds) before deposition on the collecting plate limited the time allowed for molecular rearrangement and crystallization [38]. Thus, the crystallization of the electrospun silk fibers was still lower than that of native silk. Zarkoob et al. and Wang et al. also reported similar results [8,39]. The β-sheet-rich structure of the electrospun silk fibers may be obtained by other methods.

4. Conclusions

Our results showed that pH had a remarkable influence on the properties of RSF aqueous solutions. Lowering the pH promoted the evaporation of water from the solutions and induced gel formation. RSF aqueous solutions had the optimal pH value for gel formation, fairly close to the isoelectric point of H-chain fibroin (4–5). With the decrease in pH and the increase in concentration, the rheological behavior of RSF aqueous solutions exhibited a transition from Newtonion fluid to non-Newtonion fluid and a stress induced phase separation and crystallization could be observed at higher shear rates. In addition, lowering the pH decreased the electrospinnable concentration of RSF aqueous solutions and the average diameter of the electrospun silk fibers. With the decrease in pH and concentration, the morphology of the electrospun silk fibers changed from belt-like shape to uniform cylinder. Although lowering the pH facilitated the conformational transition of the electrospun silk fibers from random coil/silk I conformation to silk II conformation, the electrospun silk fibers were still predominantly random coil/silk I conformation, the pH of the solution had a limited influence on crystallization of the electrospun silk fibers. We believe that these results help us to further understand how the physiological parameters, in particular pH and concentration, affect the assembly of silk proteins during natural processing.

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References

[1] Li D, Xia YN. Adv Mater 2004;16(14):1151–70.
[2] Subbiah T, Bhat GS, Tock RW, Parameswaran S, Ramkumar SS. J Appl Polym Sci 2005;96:557–69.
[3] Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH. Biomaterials 2004;25:1289–97.
[4] Sombatmankhong K, Sanchavanakit N, Pavasant P, Supaphol P. Polymer 2007;48:1419–27.
[5] Suwantong O, Opanasopit P, Ruktanonchai U, Supaphol P. Polymer 2007;48:7546–57.
[6] Tungprapa S, Jangchud I, Supaphol P. Polymer 2007;48:5030–41.
[7] Zarkoob S, Reneker DH, Eby RK, Hudson SD, Ertley D, Adams WW. Polym Prepr 1998;39(2):244–5.
[8] Zarkoob S, Eby RK, Reneker DH, Hudson SD, Ertley D, Adams WW. Polymer 2004;45:3973–7.
[9] Ogho K, Zhao CH, Kobayashi M, Asakura T. Polymer 2003;44:841–6.
[10] Sukigara S, Gandhi M, Ayutsede J, Micklus M, Ko F. Polymer 2003;44:5721–7.
[11] Sukigara S, Gandhi M, Ayutsede J, Micklus M, Ko F. Polymer 2004;45:3701–8.
[12] Ayutsede J, Gandhi M, Sukigara S, Micklus M, Chen HE, Ko F. Polymer 2005;46:1625–34.
[13] Jin HJ, Fridrikh SV, Rutledge GC, Kaplan DL. Biomacromolecules 2002;3:1233–9.
[14] Wang M, Yu JH, Kaplan DL, Rutledge GC. Macromolecules 2006;39:1102–7.
[15] Wang H, Zhang YP, Shao HL, Hu XC. J Appl Polym Sci 2006;101:961–8.
[16] Chen C, Cao CB, Ma XL, Tang Y, Zhu HS. Polymer 2006;47:6322–7.
[17] Putthanarat S, Eby RK, Kataphinan W, Jones S, Naik R, Reneker DH, et al. Polymer 2006;47:5630–2.
[18] Tanaka T, Kobayashi M, Inoue S, Tsuda H, Magoshi J. J Polym Sci Part B Polym Phys 2003;41(3):274–80.
[19] Jin HJ, Kaplan DL. Nature 2003;424:1057–61.
[20] Dicko C, Vollrath F, Kenney JM. Biomacromolecules 2004;5:704–10.
[21] Foo Po Wong C, Bini E, Hensman J, Kinght DP, Lewis RV, Kaplan DL. Appl Phys A 2006;82:223–33.
[22] Terry AE, Knight DP, Porter D, Vollrath F. Biomacromolecules 2004;5:768–72.
[23] Chen X, Knight DP, Vollrath F. Biomacromolecules 2002;3:644–8.
[24] Moriya M, Ohgo K, Masubuchi Y, Asakura T. Polymer 2008;49:952–6.
[25] Magoshi J, Magoshi Y, Becker MA, Nakamura S. In: Salamone JC, editor. Polymeric materials encyclopedia. Boca Raton, FL: CRC Press; 1996. p. 667–79.
[26] Feng JJ. Phys Fluids 2002;14:3912–26.
[27] Zhu JX, Shao HL, Hu XC. Int J Biol Macromol 2007;41:469–74.
[28] Zhou P, Xie X, Knight DP, Zong XH, Deng F, Yao WH. Biochemistry 2004;43:11302–11.
[29] Dicko C, Knight D, Kenne JM, Vollrath F. Biomacromolecules 2004;5:2105–15.
[30] Dicko C, Kenne JM, Knight D, Vollrath F. Biochemistry 2004;43:14080–7.
[31] Monti P, Taddei P, Freddi G, Asakura T, Tsukada M. J Raman Spectrosc 2001;32:103–7.
[32] Monti P, Freddi G, Bertoluzza A, Kasai N, Tsukada M. J Raman Spectrosc 1998;29:297–304.
[33] Um IC, Kweon HY, Park YH, Hudson S. Int J Biol Macromol 2001;29:91–7.
[34] Ha SW, Park YH, Hudson SM. Biomacromolecules 2003;4:488–96.
[35] Magoshi J, Nakamura S. J Appl Polym Sci 1975;19:1013–5.
[36] Magoshi J, Magoshi Y, Nakamura S, Kasai N, Kakudo M. J Polym Sci Polym Phys Ed 1977;15:1675–83.
[37] Tsukada M, Freddi G, Kasai N. J Polym Sci Part B Polym Phys 1994;32:1175–82.
[38] Dersch R, Liu T, Schaper AK, Greiner A, Wendorff JH. J Polym Sci Part A Polym Chem 2003;41:545–53.
[39] Wang M, Jin HJ, Kaplan DL, Rutledge GC. Macromolecules 2004;37:6856–64.