ABSTRACT

Silk fibroin (SF)/cellulose acetate (CA) blend nanofibres were prepared by electrospinning with 98% formic acid as a spinning solvent, and their structure and thermal properties were investigated by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The electro-spinnability of SF solution was remarkably improved with a small quantity of CA (not more than 10%), and the blend fibres were fine and uniform with the fibre diameters ranging from 50 nm to 300 nm. While adding 10% (by weight) CA into SF, the blends showed excellent spinnability without any beads or droplets in the surface of the electrospun mats under observation in large area, and the average diameter reached 142.1 nm. However, the spinnability of SF/CA deteriorated seriously when the content of CA was more than 30% (by weight), as the electrospun production of SF/CA blends were discontinuous and tacky with beads and dendritic structures. The results from FTIR, XRD and DSC showed that adding a small quantity of CA (not more than 10%) could induce the conformation transition of SF molecules from random coil to β-sheet. Accordingly, the thermal and mechanical properties were improved. Especially, the mechanical properties of the blend mats were greatly improved with the increasing of CA content from 5-10% (by weight). The blend nanofibre mats with 10% (by weight) CA content showed excellent mechanical properties, as the tensile stress and elongation-at-break reached 88.9 cN/mm² and 12.77%, respectively. Nevertheless, the phase separation was observed between SF and CA in blend nanofibres when the content of CA was more than 30% (by weight).

INTRODUCTION

Silk fibroin (SF) is a typical natural macromolecule fibrin extracting from Bombyx mori silkworm, which endues good mechanical properties to silk fibres. Silk fibroin is a valuable candidate material for biomedical applications for its distinctive biomedical properties including good biocompatibility, blood compatibility, favourable oxygen and water permeability, biodegradability, non-cytotoxicity and minimal inflammatory reaction [1].

The degummed Bombyx mori silk, which only consists of SF, can be regenerated in various forms, such as gels, powders, membranes, composite materials and nanofibres [1]. Recently, many researchers in textile materials and biomedical fields have shown interest in
electrospun SF nanofibres, with typical applications including supports for enzyme immobilization [2], biosensors [3], long-term releasing carrier for drugs [4], culture medium for function cells [5], anticoagulant [6], man-made organ [7] and many others. However, there are some problems with SF nanofibres of being hard and brittle in dry state, lacking surface specificity and tedious degradation rate control. Electrospun SF nanofibres are inadequate to meet the diversified demands for biomaterials and other advanced applications. In order to improve the performances of the individual SF nanofibres, many blend mats have been prepared using a solution blend method, such as SF/chitin [8,9], wool keratose (WK)/SF [10], polylactic acid (PLA)/SF [11], SF/polyethylene oxide (PEO) [12,13] and SF/Tussah silk fibroin (TSF) [7].

Cellulose acetate (CA), a derivative of cellulose, can be easily obtained from natural resources and recycled in environment by biodegradation. It can be easily fabricated into films, membranes and fibres [14]. CA has been widely used in membranes for separation and medical applications, because of its good hydrolytic stability, excellent compatibility with human body environment and relatively low cost. Electrospun CA nanofibres have been reported with diameters in the range of tens of nanometers to microns and nanofibre mats with pore sizes in the range of submicron to microns [15-17].

There are no reports on SF/CA blend electrospun fibres at present. In this work, the SF was blended with CA to improve the mechanical properties of SF for it was essential for the proper use of SF nanofibres for various applications. SF/CA blend nanofibres were fabricated by typical electrospinning technique. Their structure, thermal and mechanical properties were investigated using SEM, FTIR, XRD and DSC. In addition, the relationship between CA content in SF/CA blend electrospun fibres and the spinnability and conformation of SF are also discussed.

EXPERIMENTAL

Materials

Bombyx mori cocoons were purchased from Lushan in Henan province (China). CA powder was provided by Rayonier, Inc., China (deacetylation degree: 2.5 and polymerization degree: 200). All the reagents were of analytical reagent grade and used without further purification.

Preparation of Regenerated SF Films

Bombyx mori cocoons were degummed three times with 0.5% (by weight), Na₂CO₃ solution at 100°C for 30 min and rinsed thoroughly in warm distilled water. After drying at room temperature, the degummed fibres were dissolved in a ternary solvent system of CaCl₂/H₂O/C₂H₅OH solution (molar ratio of 1/8/2, respectively) at 75°C for 2 h. After filtering the solution, the dialysis was continued for 3 days against running ion-exchange water to remove CaCl₂ using a cellulose semi-permeable membrane (molecular weight cut-off 12-14 kD), then dialyzed again in de-ionized water that was renewed every 2 h at room temperature for 1 day. Finally aqueous SF solution obtained was cast onto polystyrene plates and dried at room temperature. The regenerated SF films were stored in a refrigerator prior to their dissolution process.

Preparation of Spinning Solution

For the electrospinning, SF films and CA powder were dissolved in formic acid (98%) at room temperature for 4 h. The concentration of the blend solution was 12% (by weight). The solutions for electrospinning were homogeneous without any precipitates and air bubbles.

Electrospinning Process

In the electrospinning process, each spinning solution was placed in a 10 mL syringe with a stainless steel syringe needle (inner diameter of 0.7 mm) as an electrode which connected to direct current (DC) high-voltage generator (BGG, Beijing High Voltage Technology Institute, China). The syringe was loaded into a syringe pump (JZB-1800, Changsha Jianyuan Corporation of Medical Instrument, China) to control the flow rate accurately with spinning rate. A piece of aluminium foil glued onto the cardboard with double-side tapes was placed at the distance of 100 mm directly below the needle which acted as grounded collector. The polymer jets generated from the needle by the high voltage field formed the ultrafine fibres
and fibrous mats at the grounded collector. The electrospinning experiments were performed at room temperature (23°C) and 60% humidity. The applied voltage and feed rate of the spinning solution were fixed at 15 kV and 0.3 mL/L [18]. All electrospinning experiments were performed under the same processing condition.

**SEM Observation**

The morphologies of the electrospun SF/CA blend fibres were observed with a Jeol scanning electron microscope (JSM-6360LVSEM, Japan) at an accelerating voltage of 15 kV. Prior to scanning under the SEM, the samples were sputter coated for 90 s with gold. One hundred of fibre segments in SEM image were randomly used to determine the average diameter and the diameter distribution of the fibres.

**FTIR Spectroscopy**

FTIR Spectra were carried out on compressed films containing potassium bromide (KBr) pellets and products using a NEXUS670 FTIR spectrophotometer (Thermo Nicolet Co., USA). All spectra were recorded at absorption mode of 2 cm⁻¹ interval and in the range of 4000-400 cm⁻¹ wavenumbers.

**X-Ray Diffractiometry**

The powder of electrospun nanofibres was obtained with the aid of a Hardy's thin cross-section device. The XRD patterns were performed on D/max-2550PC (Rigaku Co., Japan) at 40 kV tube voltage and 40 mA tube current. CuKα radiation was used with diffraction angle, 2θ, from 5° to 60°. The scanning rate was 2°/min with powdered electrospun SF/CA fibres.

**Thermal Analysis**

DSC Analysis was performed using a Diamond DSC differential scanning calorimeter (Perkin & Elmer, USA). The samples were heated at 10°C/min from 50°C to 350°C. The nitrogen gas flow rate was 50 mL/min.

**Mechanical Properties Measurement**

For mechanical strength test, the mats were cut into strips with a dimension of 1×4 cm, and its thickness was measured by a micrometer, and then mounted on an Instron MicroTester 5848, Canton MA, USA, to test the mechanical properties at a stretching speed of 10 mm/min. Each sample was measured 5 times. All experiments were performed at 20°C and relative humidity (RH) of 65%. Average stress and strain were calculated by eqns (1) and (2), respectively.

\[
\text{Stress (cN.mm}^{-2}\) = \frac{\text{Absolute strength (cN)}}{\text{Sample width (mm)} \times \text{Thickness (mm)}}
\]

\[
\text{Strain (}) = \frac{\text{Absolute elongation (mm)}}{\text{Sample length (mm)}} \times 100
\]

**RESULTS AND DISCUSSION**

**Morphological Characteristics**

Figure 1 shows the morphological structures of the electrospun SF/CA blend nanofibres. The pure SF nanofibres were continuous and smooth in the surface except a few beads and droplets, and adhesions could be hardly identified as shown in Figure 1a, indication of good spinnability of the SF spinning solution. However, the spinnability of pure CA was poor under the condition using formic acid as a solvent in electrospinning.

As shown in Figure 1f, the fibres were discontinuous and intensively tacky with many beads in their surfaces. The poor spinnability of CA solution might be caused by the poor solubility of CA in formic acid and poor conductivity of its solution.

Adding CA into SF solution could affect the morphology of electrospun SF/CA blend nanofibres significantly. While adding 5% (by weight) CA into SF solution, a significant reduction in size and the number of beads is observed on the surface of the SF/CA blend nanofibres (Figure 1b), compared with that of pure SF nanofibres. The SF/CA blend nanofibres, shown in Figure 1c, excellent spinnability without any beads or droplets on the surface of the electrospun mats when the content of CA increased to 10% (by weight).

It is known that conformation transition of SF molecules from random coil to β-sheet could be induced by hydrophilic polymers, such as chitosan and sodium alginate [19], because of the formation of
intermolecular hydrogen-bonds. Therefore, intermolecular hydrogen-bonds might be formed between carboxyl and amido groups of SF molecules and hydroxyl groups of CA molecules in the blend spinning solution, leading to a reduction in the content of random coil conformation in SF molecules, which was advantageous to draw the spinning jet in the high voltage electric field, and further improved the spinnability. However, the spinnability of SF/CA solution deteriorated seriously when the content of CA was beyond 30% (by weight). As shown in Figures 1d and 1e, the SF/CA nanofibres are discontinuous and adhesive with dendritic structures and beads. This might be due to the excessive content of

Figure 1. SEM Micrographs of SF/CA blend nanofibres with different contents of CA: (a) 0%, (b) 5%, (c) 10%, (d) 30%, (e) 40% and (f) 100% (all by weights).
CA, which could reduce the spinnability of SF/CA blends seriously.

Diameter Distributions of SF/CA Blend Nanofibres
As it is shown in Figure 2 and Table 1, the electrospun SF/CA blend nanofibres with CA content of 0%, 5% and 10% (all by weights) were fine and uniform with the fibre diameters ranging from 50 nm to 300 nm, and their diameter distributions were relatively concentrated. The diameters were finer and more uniform at the CA content of 5% (by weight) in SF/CA blends with average diameter of 95.6 nm and coefficient of variance (CV) value of 26.55%, but there were few beads and droplets (as shown in Figure 1b). While adding 10% (by weight) CA into SF, the blend solution showed excellent spinnability without any beads or droplets in the surface of the electrospun mats under observation in large area, and the average diameter reached 142.1 nm.

FTIR Spectra of Electrospun SF/CA Blend Nanofibres
FTIR spectrum is a powerful and common tool for the protein conformation analysis, and therefore it is widely used in the study of silk protein [20,21]. The FTIR spectra of SF/CA blend nanofibres are shown in Figures 3 and 4. Spectrum a in Figures 3 and 4 show the FTIR spectrum of pure SF nanofibres. The C=O stretching vibration attributed to amide I region, a peak was observed at 1651 cm\(^{-1}\) due to random coil conformation. The N-H group deformation vibration and C-N stretching vibration attributed to amide II

<table>
<thead>
<tr>
<th>CA content (%)</th>
<th>Average diameter (nm)</th>
<th>Range (nm)</th>
<th>CV value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>127.5</td>
<td>222.7</td>
<td>29.94</td>
</tr>
<tr>
<td>5</td>
<td>95.6</td>
<td>142.6</td>
<td>26.55</td>
</tr>
<tr>
<td>10</td>
<td>142.1</td>
<td>226.1</td>
<td>32.21</td>
</tr>
</tbody>
</table>
region double peaks appeared around 1540 cm\(^{-1}\) and 1518 cm\(^{-1}\) due to random coil and β-sheet conformation, respectively [21]. The characteristic absorption band at 1235 cm\(^{-1}\) represents the amide III band which is attributed to N-H group bending and C-N stretching vibrations. All these suggest that the pure SF nanofibres mainly contained random coil and α-helix conformation as well as the existing β-sheet conformation [7]. The amide III could not be used in SF molecular conformation analysis in SF/CA blend nanofibre mats, because it overlapped with the acetyl ester band of CA at 1236 cm\(^{-1}\) (as shown in Figure 3, spectrum f) [22].

When adding 5-30% (by weight) CA into SF solution, the absorption peak at 1651 cm\(^{-1}\) shifts to 1656 cm\(^{-1}\) (as shown in Figure 4), which could be attributed to α-helix conformation. This change suggests that the conformation of SF molecules in SF/CA blend nanofibre mats changes when adding CA into SF solution. In amide II region, the intensity of the peak at 1518 cm\(^{-1}\) is enhanced slightly, which indicates that adding a small quantity of CA (not more than 10%) in SF could facilitate the conformational transition of the SF molecules in electrospun nanofibres from random coil to β-sheet conformation, because the intermolecular hydrogen bonds were formed between the carboxyl and amido groups of SF molecules and hydroxyl groups of CA molecules, leading to the formation of crystalline structures in SF molecules. However, the intensity of the peak at 1518 cm\(^{-1}\) is reduced when the content of CA in SF/CA blend nanofibres is beyond 30% (by weight), indicating a reduction of β-sheet conformation. These changes might have been due to the hydrogen bonds formed in the superfluous CA molecules.

**X-Ray Diffraction Analysis**

X-Ray diffraction was carried out to study the crystalline structure of the SF/CA blend nanofibres. Figure 5 shows the X-ray diffraction patterns of the electrospun SF/CA nanofibres. A broad peak around 2θ = 20.7°, shown in Figure 5a, suggests that the pure SF nanofibres mainly contained random coil and α-helix conformations [19,23]. As shown in curve f in Figure 5, the X-ray diffraction curve of pure CA electrospun fibres shows two broad peaks at around

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**Figure 4.** The varieties of the main characteristic peaks of SF/CA blend nanofibres with different contents of CA: (a) 0%, (b) 5%, (c) 10% and (d) 30% (all by weights).

**Figure 5.** X-Ray diffraction patterns of the SF/CA blend nanofibres with different contents of CA: (a) 0%, (b) 5%, (c) 10%, (d) 30%, (e) 40% and (f) 100% (all by weights).
$\theta = 10.2^\circ$ and $21.7^\circ$, meaning that their structure is also amorphous [24].

Compared with the pure SF nanofibres, no obvious changes in the position of the main diffraction peaks in the blend electrospun nanofibre mats are observed, which indicates that the crystalline form of SF in the blend nanofibre mats does not change with increases in CA content of SF/CA blends. However, a shoulder peak at $2\theta = 9.1^\circ$ appears when adding 5% (by weight) CA into SF, which may be attributed to $\beta$-sheet conformation. When the CA content in SF/CA blends increased to 10% (by weight), the intensity of the peak at $2\theta = 9.1^\circ$ is enhanced, which means that an addition of a small quantity of CA (not more than 10%) could induce the conformation transition of SF molecules from random coil to $\beta$-sheet. This transformation is due to the intermolecular hydrogen-bond taking place between the carboxyl and amido groups of SF and the hydroxyl groups of CA molecules, leading to crystallization of molecular morphology. Nevertheless, because of less polar groups of CA the ability to induce conformational transition is lower than that of chitosan and sodium alginate.

Two broad peaks of CA are observed at $2\theta = 10.2^\circ$ and $21.7^\circ$ clearly in the blend electrospun fibres with CA content of above 30% (by weight) (curves d and e in Figure 5). This indicates that the phase separation occurred between SF and CA, is the reason why the spinnability of SF/CA blends diminished seriously when the content of CA was above 30% (by weight).

**Thermal Properties**

DSC Thermogram is a powerful and common tool for thermal properties analysis [25]. Figure 6 shows the DSC thermograms of SF/CA blend electrospun nanofibre mats. The thermal properties of the regenerated SF which were investigated in 2001 by Um et al., indicated that the exothermic peak at 224°C did not appear when SF was dissolved in formic acid before being fabricated into films [23]. In the present study, it is found that pure SF nanofibre mats show three characteristic peaks (Figure 6, curve a). The first endothermic peak which may be attributed to the existence of water bonds with SF molecules appears around 80°C. The next peak at 224°C, as an exothermic peak which could be attributed to the movement of random coil is further confirmation of electrospun SF nanofibres mainly containing random coil and $\alpha$-helix conformation. This might be due to the rapid volatilization speed of solvent (formic acid) during the electrospinning process, leading to low induction capacity. The third, as an endothermic peak which appeared around 280°C, could be attributed to the thermal decomposition of ordered SF molecules in the electrospun SF nanofibre mats [23].

The DSC curves of SF/CA blend nanofibres have demonstrated notable changes with increased CA content. The intensity of the exothermic peak at 224°C is obviously depressed by adding 5-10% (by weight) CA into SF, an effect shown by a small quantity of CA into SF solution which induces the conformation transition of SF molecules from random coil to $\beta$-sheet conformation. However, the intensity of the exothermic peak at 224°C increased when the CA content was 30% (by weight) (Figure 6, curve d), indicating a reduction of $\beta$-sheet conformation. These results are consistent with the results of FTIR and XRD analyses. In addition, the
decomposition temperatures of SF/CA blends were slightly higher than those of the pure SF nanofibres, a clear confirmation of improved thermal properties due to addition of CA to SF.

**Mechanical Properties**
The results of tensile strength testing of the SF/CA blend fibre mats are shown in Table 2. The pure SF nanofibre mats give poor tensile strength and elongation-at-break, which reach 35.88 cN/mm² and 8.03%, respectively. This might be caused by lower flexibility of large molecules of SF proteins [26]. The mechanical properties of the blend fibre mats are greatly improved with the increased CA content from 5-10% (by weight). The blend nanofibre mats with 10% (by weight) CA content showed excellent mechanical properties, due to more developed β-sheet conformation contained in the SF molecules, as the tensile stress and elongation-at-break values reach 88.9 cN/mm² and 12.77%, respectively. However, the mechanical properties of the blend nanofibre mats with more than 30% (by weight) CA content could not be tested, because the fibres were discontinuous with dendritic structures.

**CONCLUSION**
In this paper, SF/CA blend nanofibres were prepared by electrospinning with 98% formic acid as a spinning solvent, and their structure and thermal properties were investigated by SEM, FTIR, XRD and DSC analyses. The spinnability of SF solution was remarkably improved with a small quantity of CA (not more than 10%), and the blend fibres were fine and uniform with the fibre diameters ranging from 50 nm to 300 nm. When adding 10% (by weight) CA into SF, the blends showed excellent spinnability without any beads or droplets. However, the spinnability of SF/CA deteriorated seriously when the content of CA was more than 30% (by weight), as the electrospun production of SF/CA blends were discontinuous and tacky with beads and dendritic structures. The results from FTIR, XRD and DSC analyses showed that adding a small quantity of CA (not more than 10%) into SF could induce the conformation transition of SF molecules from random coil to β-sheet. Accordingly, the thermal and mechanical properties were improved. Nevertheless, for SF molecules the ability to undergo conformational transition from random coil to β-sheet was lower than that of chitosan and sodium alginate. Phase separation was observed between SF and CA when the content of CA was higher than 30% (by weight).

**ACKNOWLEDGEMENT**
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**ABBREVIATIONS**
SF : Silk fibroin  
CA : Cellulose acetate  
SEM : Scanning electron microscope  
FTIR : Fourier transform infrared spectroscopy  
XRD : X-Ray diffraction  
DSC : Differential scanning calorimetry  
WK : Wool keratose  
PLA : Polylactic acid  
PEO : Polyethylene oxide  
TSF : Tussah silk fibroin  
CV : Coefficient of variance

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