Production and properties of electrosprayed sericin nanopowder

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Abstract
Sericin is a proteinous substrate that envelops fibroin (silk) fiber, and its recovery provides significant economical and social benefits. Sericin is an antibacterial agent that resists oxidation and absorbs moisture and UV light. In powder form, sericin has a wide range of applications in food, cosmetics and drug delivery. Asides from other techniques of producing powder, such as precipitation and spray drying, electrospraying can yield solid nanoparticles, particularly in the submicron range. Here, we report the production of sericin nanopowder by electrospraying. Sericin sponge was recovered from Bombbyx mori cocoons through a high-temperature, high-pressure process, followed by centrifugation and freeze drying of the sericin solution. The electrospraying solution was prepared by dissolving the sericin sponge in dimethyl sulfoxide. We demonstrate that electrospraying is capable of producing sericin nanopowder with an average particle size of 25 nm, which is by far smaller than the particles produced by other techniques. The electrosprayed sericin nanopowder consists of small crystallites and exhibits a high moisture absorbance.

Keywords: sericin, electrospraying, nanopowder, particle size, dimethyl sulfoxide

1. Introduction
Sericin, a protein, envelops two silk (fibroin) filaments and keeps them together (figure 1). It also acts as a sticky layer that enables the formation of a cocoon. To prepare silk filaments for textile applications, most of the sericin is removed during a process called degumming and treated as effluent. It is estimated that the global yearly production of sericin amounts to about 50 000 metric tons [1]. Sericin is a polypeptide polymer consisting of 18 amino acids. The fractions of hydroxyl, polar and nonpolar amino acids in sericin are 45.8, 42.3 and 12.2%, respectively [2, 3]. Depending on the extraction method, the molecular weight of sericin lies in the range of 24–250 kDa [2, 4]. The microstructure of sericin consists of random and β parts. The β structure forms as a result of hydrogen bonding between the polar amino acids; it plays an important role in the stability of solid sericin and particularly prevents the dissolution of sericin in water [5]. Sericin is a biocompatible and biodegradable protein exhibiting antibacterial and UV-absorbing properties, and therefore, its recovery and application can have significant economic and social benefits. Moreover, it has a high moisture absorbency, antioxidant and, hence, antitumor activity, as well as wound-healing capacity. The properties of sericin can be further enhanced by modifications such as cross-linking, copolymerization and blending with other polymers. In powder form, sericin has a wide range of applications in food, cosmetics, drug delivery, medical and pharmaceutical industries [2].

The extraction of sericin from raw silk can be carried out through any of the high-temperature, high-pressure methods using water, aqueous alkaline or acidic media. Sericin solution can be purified through various techniques such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis.

Protein powders such as albumin, collagen, gelatin, keratin and sericin can be produced through a number of techniques including desolvation, coacervation and emulsification [6]. Electrospraying is a relatively new nanopowder production technique. In this technique, after the first phase of breaking up and emission of the Taylor cone next to the nozzle, the charged polymer solution droplets break up further...
under the influence of the so-called coulombic explosions. These explosions occur during the flight of the droplets in the electrostatic field between the nozzle and the collector. As the solvent accompanying the droplet evaporates and the droplet becomes smaller, the charge density on the smaller droplet increases and further coulombic explosions occur [7–9]. It is considered that electrospraying can produce smaller particles than those by other techniques [7]. Depending on the conditions, a variety of electrospraying modes such as microdripping, spindle, multispindle, ramified meniscus, cone, precession, oscillating, multijet, ramified and dripping jet can occur as described by Jaworek and Krupa [8]. Recent reports concerning the production of sericin powder can be summarized as follows.

Oh et al. [10] produced sericin particles through desolvation of the sericin solution in lithium chloride/dimethyl sulfoxide. The average size of sericin particles was 1 mm. Capar et al. [11] precipitated sericin from its solution using ethanol and prepared a powder by freeze-drying the precipitated sericin. The size of the sericin particles was not specified. Lee et al. [12] employed a similar approach and used methyl alcohol instead of ethanol. The size of the sericin particles obtained in this work was about 2 μm. Genç et al. [13] produced sericin powder with a particle size in the range of 1–20 μm through spray drying. They claimed that the produced sericin powder is suitable for drug delivery to the lungs. Gulrajani et al. [4] employed spray drying and produced sericin powder with a particle size of about 2 μm. Kurioka et al. [14] produced sericin particles (10–100 μm size) from citric acid-degraded sericin by freeze-drying the dialyzed solution. Literature review also reveals two works on the blends of sericin with other polymers as follows. Cho et al. [15] produced blended sericin-polyethylene glycol nanoparticles (200–400 nm) through self-assembly. In this work, sericin was made to react with polyethylene glycol. Song et al. [16] blended sericin and methyl acrylate through graft copolymerization, yielding 100–150 nm particles suitable for drug delivery applications.

As our literature review showed no reports on the electrospun sericin nanopowder, this study aimed at producing sericin nanopowder through electrospraying and studying the effect of electrospraying parameters on the average size of sericin particles. Moreover, some properties of the electrospayed sericin particles were investigated.

2. Experimental details

2.1. Electrospraying sericin nanopowder

To recover sericin from cocoons, the cocoons were cut into small pieces and rinsed thoroughly. This was followed by boiling in pressurized distilled water at 120 °C for 1 h (the ratio of water volume to the weight of cocoon sample was 30). This method is known as the high-temperature, high-pressure method and has the highest efficiency amongst all the methods used to recover sericin from cocoons [14]. In the next stage, the solution obtained from boiling was purified by paper filtering to remove fibroin and other suspended impurities. This solution was further purified using a glass filter under vacuum. The final stage of purification consisted of dialysis of the vacuum-filtered solution against distilled water for 48 h using a tubular cellulose membrane (D9527-100FT Sigma-Aldrich, USA), which allowed ions and molecular chains lighter than 12 000 Da to pass from the inside of the tube into the distilled water bath. The bath containing distilled water was stirred using a magnet and changed every 6 h. The yellow solution inside the membrane was taken out and centrifuged (Heraeus, Germany) for 30 min at 7500 rpm. The thin top level of the centrifuged solution was removed, and the remaining thicker solution was dried in a vacuum freeze dryer (Alfa 2–4 LD, Germany) yielding solid, sponge-like sericin (figure 1). Because heating above 50 °C can cross-link proteins, the sericin solution was not heated directly and it was dried by freeze drying. The sample was cooled to −20 °C, then the chamber pressure was lowered to 153 × 10⁻⁶ kgf cm⁻² within 1 h, and these conditions were kept for 10 h.

To prepare the electrospraying solution, warm water (45 °C), dimethyl sulfoxide (Merck), and a mixture of lithium chloride and dimethyl sulfoxide (Merck) were employed to dissolve the sericin sponge with a molecular weight of over 12 000 Da; however, only the dimethyl sulfoxide solution was suitable for electrospraying. Sericin solution in warm water is unstable and precipitation occurs as the temperature decreases. The presence of lithium chloride (even in small amounts) in the lithium chloride–dimethyl sulfoxide solution leads to the agglomeration of the particles at the collector owing to the high stability of lithium chloride (decomposes at 1382 °C). Formic acid dissolves sericin but also degrades it.

Electrospraying was carried out at a voltage of 15, 20 or 25 kV, needle-collector distance of 15, 20 or 25 cm, concentration of 0.1, 0.5 or 0.9% (sericin in dimethyl sulfoxide, w/w) and feed rate of 0.022 or 0.044 ml h⁻¹. These parameters were chosen in a series of preliminary experiments. The setup used for electrospraying keratin solution consisted of a dosing pump, syringe with a needle, aluminum foil collector and high-voltage supply. The syringe (2 ml) connected to a needle of gauge 23 (internal diameter
0.6 mm) was filled with the solution of keratin and connected to the dosing pump (TERUMO, STC-527 Japan). The syringe needle (horizontal) and aluminum foil collector (vertical) were connected to the positive and negative electrodes of a high-voltage supply (Emersun, 220 V, ac input–up to 35 kV dc output), respectively.

The viscosity and surface tension of the sericin solution were measured with a shearing viscometer (Brookfield, USA) and a surface tension measurement instrument (Data Physics, DCAT11, Germany), respectively.

2.2. Characterization of sericin nanopowder

The average particle size of the electrosprayed sericin nanopowder was measured by applying measurement software (Manual microstructure distance measurement, Nahamin Pardazan Asia Co.) to the micrographs obtained by scanning electron microscopy (SEM, Philips XL30, Holland). An x-ray diffractometer (XRD, Philips Xpert MPD) was employed to study the microstructure of electrosprayed sericin nanoparticles. The crystallinity index of the samples was calculated from XRD patterns using the Origin software (Data Analysis & Graphic Software, OriginLab Corporation). Fourier transform infrared spectroscopy (FTIR, Hartmann & Braun MB Series BOMEM) was employed to investigate changes in the basic structural bonds of sericin as a result of dissolving it in dimethyl sulfoxide.

Moisture absorption was measured by first drying the samples in a closed oven at 45 °C for 24 h and then measuring the sample weight. The sample was exposed to air with 65% relative humidity for 24 h at 25 °C; its moisture absorption was measured relative to the dried weight and expressed in %.

3. Results and discussion

3.1. Sericin particle size

Typical SEM images of electrosprayed sericin nanoparticles are shown in figure 2 with the corresponding electrospraying conditions mentioned in the caption. As can be seen, electrospraying produced spheroidal nanoparticles from the sericin solution.

Figure 3 shows the variation of the average size of electrosprayed sericin nanoparticles with sericin
concentration in dimethyl sulfoxide. Each datapoint was averaged over 50 randomly chosen particles. As can be seen, increasing the sericin concentration leads to a larger average particle size. It is worth mentioning that electrospraying failed at sericin concentrations in dimethyl sulfoxide exceeding 2%. This failure is related to the fact that breaking down droplets through coulombic explosions is hindered at high concentrations, because viscosity increases with concentration and, hence, overcoming the adhesion forces between the sericin macromolecules becomes more difficult. Figures 4 and 5 show the concentration dependences of the viscosity and surface tension of sericin solution in dimethyl sulfoxide, respectively. As can be seen, increasing the concentration above 0.1% leads to a sharp drop in the surface tension. Combining these results with the data of figure 3, we can relate a higher surface tension to a smaller size of electrosprayed particles; this can be explained on the basis of the Hartmann relationship \[ d = \alpha \left( \frac{Q^2 \epsilon_0 \rho_1}{\sigma_1 \gamma_1} \right)^{1/6}, \]

where \( d \) is the particle size, \( \alpha \) is a constant depending on the solution dielectric constant, \( Q \) is the feed rate, \( \epsilon_0 \) is the vacuum dielectric constant, \( \rho_1 \) is the density, \( \sigma_1 \) is the surface tension and \( \gamma_1 \) is electrical conductivity.

Figure 6 shows the variation of the average size of sericin nanoparticles with electrospraying voltage, revealing that higher voltages lead to the formation of smaller sericin nanoparticles. This is expected as higher voltages induce a higher amount of charge on the droplets, and as a result, the breaking up of the droplets occurs with more forceful coulombic explosions. It is obvious that the initiation of coulombic explosions has a voltage threshold, which is above 10 kV for sericin. However, when the nozzle is too close to the collector, a higher voltage can result in a greater acceleration and, hence, a shorter flight time of the particles, leaving less time for solvent evaporation and thus larger particle size. This situation was observed for sericin concentrations of 0.5 and 0.9%, feed rate of 0.022 ml h\(^{-1}\), and nozzle–collector distance of 15 cm, where increasing the voltage from 15 to 20 kV led to a larger particle size. It is worth mentioning that with a sericin concentration of 0.1% and nozzle–collector distance of 15 cm, electrospinning proved impossible as there was insufficient time for the solvent to evaporate. Increasing the voltage further from 20 to 25 kV for a sericin concentration of 0.1%, feed rate of 0.022 ml h\(^{-1}\), and nozzle–collector distance of 25 cm resulted in an unstable jet and large particles.

Figure 7 shows the variation of the average size of electrosprayed sericin nanoparticles with the nozzle–collector distance. As can be seen, increasing the distance from 20 to 25 cm leads to a decrease in the particle size. This is related to a longer flight trajectory, giving more time for the coulombic explosions to occur. Like voltage, the nozzle–collector distance must lie in a suitable range to allow electrospraying of nanoparticles.

Figure 8 shows the variation of the average size of electrosprayed sericin nanoparticles with the feed rate. In all the cases, increasing the feed rate leads to larger particles. This can be explained by the fact that higher feed rates provide higher amounts of sericin solution emerging from the nozzle and, hence, bigger initial droplets. It is worth mentioning that with the exception of voltage at concentrations of 0.1 and 0.5%, the other three parameters proved to be statistically significant at a 5% significance level.
Figure 6. Average size of electrosprayed sericin nanoparticles versus electrospraying voltage. The feed rate is 0.044 ml h\(^{-1}\) and the nozzle–collector distance is 20 cm (A) or 25 cm (B).

Figure 7. Average size of electrosprayed sericin nanoparticles versus nozzle–collector distance. The feed rate is 0.044 ml h\(^{-1}\) and the voltage is 20 kV (A) or 15 kV (B).

3.2. FTIR analysis

Figure 9 shows the FTIR spectra of sericin sponge and electrosprayed sericin nanoparticles. In the FTIR analysis of proteins, the absorption bands at 1650, 1530 and 1230 cm\(^{-1}\) are related to the C=O, –NH and C–N bonds in the amide 1, 2 and 3 groups, respectively [18]. The FTIR spectra of the sponge and powder are very similar, indicating that the electrosprayed sericin nanoparticles have maintained the basic structure of sericin in the sponge.

3.3. XRD analysis

Sericin is known to have a characteristic XRD peak at 2\(\theta\) = 20° [12]. This peak is present both for the sericin sponge and electrosprayed nanopowder in figure 10, but in slightly different forms, indicating a minor difference in the microstructure of the electrosprayed sericin nanoparticles and the sponge. Applying the Origin software to the XRD patterns yielded crystallinity indices of 35.94% and 27.13% for the sericin sponge and electrosprayed nanoparticles, whereas the crystallite sizes were 6.13 and 1.14 Å, respectively. These results suggest that the sericin produced in this work has a low crystallinity and its ordered parts are composed of very small crystallites known as \(\beta\) sheets.

3.4. Moisture absorption

Moisture absorption measurements yielded a moisture regain of 9.8% for the sponge and 56.2% for the electrosprayed sericin nanoparticles. The much higher moisture absorption by the sericin nanoparticles is related to their higher specific surface; it could be very valuable for the application of sericin nanopowder in moisturizing and sun creams.

3.5. Comparison of the size of particles produced by different methods

Considering the average size of sericin particles produced by various methods (see Introduction), it can be concluded that electrospraying yields the smallest particles. This size has been reduced from 2000 nm [4, 12] to 24–175 nm in this work, putting the electrospraying technique far ahead of its rivals.
Figure 8. Average size of electrosprayed sericin nanoparticles versus feed rate. The voltages and nozzle–collector distances are 20 kV, 20 cm (A); 20 kV, 25 cm (B); 15 kV, 15 cm (C).

Figure 9. FTIR spectra of sericin sponge and electrosprayed sericin nanoparticles.
Figure 10. XRD patterns of sericin sponge and electrosprayed sericin nanoparticles.

4. Conclusions

The results of this study show that electrospraying can produce sericin particles with a uniform spherical shape and an average size below 80 nm. Lower concentrations, lower feed rates and longer needle–collector distances lead to a decrease in the average particle size. The particle size decreases with electrospraying voltage up to 20 kV, but increases at higher voltages. FTIR and XRD studies showed that the structure of electrosprayed sericin is similar to that of sericin sponge, but has a lower crystallinity index and contains smaller crystallites. The humidity absorbance is about six times higher for the sericin nanopowder than for the sericin sponge. Electrospraying is capable of producing sericin particles in the range of 25–175 nm, which is much smaller than those obtained using rival techniques.

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