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Structure and characteristics of milled silk particles

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ABSTRACT

This study examined the structure, thermal property, and ion adsorption of silk particles. The particles were prepared by attritor–bead mill combination, using alkaline (pH 10) charge repulsion and surfactant steric repulsion methods. Both methods produced particles with a dominant β-sheet structure, similar to the silk fibre. There was no significant difference in the decomposition temperatures for either the silk fibre or the micro/nano silk particles. An important finding from this study is clear evidence of reduction of amorphous content during the final stage of powdering using the bead mill. As a result, despite reduction in β-sheet crystallites with the progressive milling, the relative β-sheet content actually increased during this process. However, intermolecular forces between the β-sheets reduced significantly and hence the XRD results showed significant reduction in crystallinity in nano silk particles but crystal forming segments remained with β-sheet conformations after milling. The structural change influenced the ion-adsorption property where particle-size reduction resulted in a significant increase in both the rate and volume of $HCrO_4^-$ adsorption.

1. Introduction

Silk fibre is a protein produced to form cocoon by the larva of caterpillar. A cocoon protects the silkworm from microbes, dehydration, and predators during metamorphosis [1]. In a cocoon, twin silk filaments are held together by silk gum called sericin [2,3]. The fibroin fibre itself is a bundle of several fibrils and microfibrils [4]. Fibroin has a β-sheet secondary structure with stabilized inter-chain and inter-sheet hydrogen bonds [5]. Silk fibroin is a block copolymers in which crystalline sections are built up by amino acids with short side chains such as glycine and alanine and spread into amorphous sections consisted of amino acids with bulkier side chains [5]. High wet strength, ability to withstand enzymatic breakdown, and good transfer of oxygen and drugs make silk protein a suitable biomaterial [6]. Silk particle is a useful form of silk which has been used for many applications such as drug delivery [7–11], reinforcing scaffolds [12,13], enzyme immobilization [14], cosmetics [15], and coatings [16]. Silk particles possess high potential in the biomedical field of application due to their ability to bind various ionic species and their controllable release profile [17].

Silk can be dissolved in solvents which have the ability to break down the strong intermolecular bonds [18]. Liquid silk can then be converted to various morphologies such as films, gels, fibres and particles [19]. However, during regeneration, the original crystal structure is lost. Structural stabilization can be achieved by stimulating the formation of the β-sheet structure by various means such as methanol treatment, which can improve mechanical properties and decrease the water solubility [18]. Different methods have been reported to fabricate silk particles from silk solution and improve their stability [20,21].

An alternative method to fabricate silk particles while preserving the primary structure of silk fibre is ball milling. Although mostly used for inorganic materials, ball milling processes have also been used for breaking solid organic materials. For example, ball milling was applied for reducing the particle size of drugs to increase their bioavailability [22]. In our previous study, a combination of different milling systems were also used to produce a broad size-range of silk particles from micron to nano levels [23,24]. For the production of silk nanoparticles, charge stabilization [23] or steric repulsion [24] was essential to avoid their aggregation. Charge stabilization was achieved by milling at pH 10 where electrostatic repulsion forces prevent particle aggregation [23]. The pH was restricted to 10 to be safe for silk processing as silk is sensitive to alkali hydrolysis and the hydrolysis is enhanced by increasing pH and temperature [25,26].

In addition to pH assisted milling to produce nano particles, similar silk particle can be obtained by milling at neutral pH using Tween 80, a surfactant suitable for biomedical applications [24]. Unlike alkali, Tween 80 does not hydrolyse silk. Despite progress in milling of silk to produce fine particles and understanding of the particle morphology, there is lack of understanding of the change in silk structure during milling. Understanding changes in secondary structure during milling is important as it largely determines the stability of silk against dissolution, and various forms of destabilisation process such as thermal, mechanical and enzymatic degradation. In this study, we try to correlate the secondary structure and milling process which will be helpful to engineer

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silk particles with appropriate structure for achieving desired properties for a specific application.

2. Experimental

2.1. Milling

The process flow chart for the production and drying of samples is shown in Fig. 1. Details of the powdering processes were reported in our previous works [23,24]. Briefly, degummed Eri fibres were chopped with a cutter mill (Pulverisette 19 from Fritsch) until the snippets could pass through a 1 mm grid. Wet milling of snippets was carried out in an attritor (1S from Union Process). 200 g of snippets was mixed with 1500 mL of distilled water. The slurry was treated in the attritor mill with yttrium-doped zirconium oxide balls of 5 mm in diameter in deionised water for 7 h. The slurry obtained from the attritor milling was further processed with a laboratory spray dryer (B-290 from Buchi) to produce dry powder (Powder-1). 2 g of the spray-dried powder was further milled in a bead mill (DYNO® Mill Research Lab) using100 mL deionised water. In bead milling, pH 10 buffer or Tween 80 at 30% concentration on the weight of powder (owp) was used to avoid particle aggregation. The grinding medium was cerium-doped zirconium oxide (0.5–0.6 mm in diameter) having a volume of 60 mL. The milling speed was 2000 rpm. Processing time was 10 h for pH 10, and 7 h for Tween 80 assisted milling. After milling, alkali was removed by dialysis followed by washing and centrifuging the dialysed powder with deionised water. Subsequently, washed particles were freeze dried (Powder 2). Similar procedure was followed for removing Tween 80 and drying to obtain dry powder (Powder 3).

2.2. Nano-spray dying and freeze drying of particles bead milled in pH 10

Bead milled slurry with pH 10 without removing alkali was freeze dried (Labconco FreeZone), and nano-spray dried (B-90 from Buchi) to get dry powder. The inlet temperature for nano-spray drier was 120 °C with a flow rate of 20 mL/h.

2.3. Preparation of silk film

Water soluble silk films can be used as standard of amorphous silk while characterising structure of silk powder. Eri silk film was prepared using a method explained elsewhere with some modification [27]. Briefly, Eri fibre was dissolved in a 10 M lithium thiocyanate solution with a material to liquor ratio of 1 (g): 10 (mL) at room temperature. After removing undissolved parts by centrifuging at 6500 rpm for 20 min, the supernatant was dialyzed for 4 days at 4 °C against deionised water by a dialysis tube with molecular weight cut off 12,000 Da (from Sigma Aldrich). 10 mL 1% (w/v) silk solution was cast on a 10 cm diameter polyethylene disc at room temperature.

2.4. Characterisations

Mastersizer 2000 (Malvern, UK) was used for determining particle sizes and specific surface areas using deionised water as a dispersion media. The reflective index of 1.542 was used for Eri silk for the calculation of particle size distribution [28]. Measurements were repeated three times. The shapes of the snippets and particles were observed under a scanning electron microscope (SEM, Zeiss Supra 55VP) using 3–10 kV and working distance of 5–10 mm. Gold coating was applied to the samples prior to the observation. Fourier transform infrared (FTIR) spectra were obtained using a Bruker® VERTEX 70 spectrometer under the resolution of 4 cm^{-1} and 128 scans per sample. Differential scanning calorimetric (DSC) analysis was done using thermal analysis instrument DSCQ200. Scanning rate of 10 °C min^{-1} between (−30 and 400 °C), and nitrogen gas flow rate of 50 mL/min were used. Thermogravimetric analysis (TGA) was performed under the flow of nitrogen gas at a scanning speed of 10 °C min^{-1} using a Netzch STA409PC DSC/TGA instrument. XRD experiments were carried out with PANalytical X'Pert PRO instrument. CuKα radiation with a wavelength of 1.54 °A was used. The scanning speed was 0.1° with time per step of 1 s, and measurement angle 2θ from 5 to 40° under 40 kV and 50 mA.

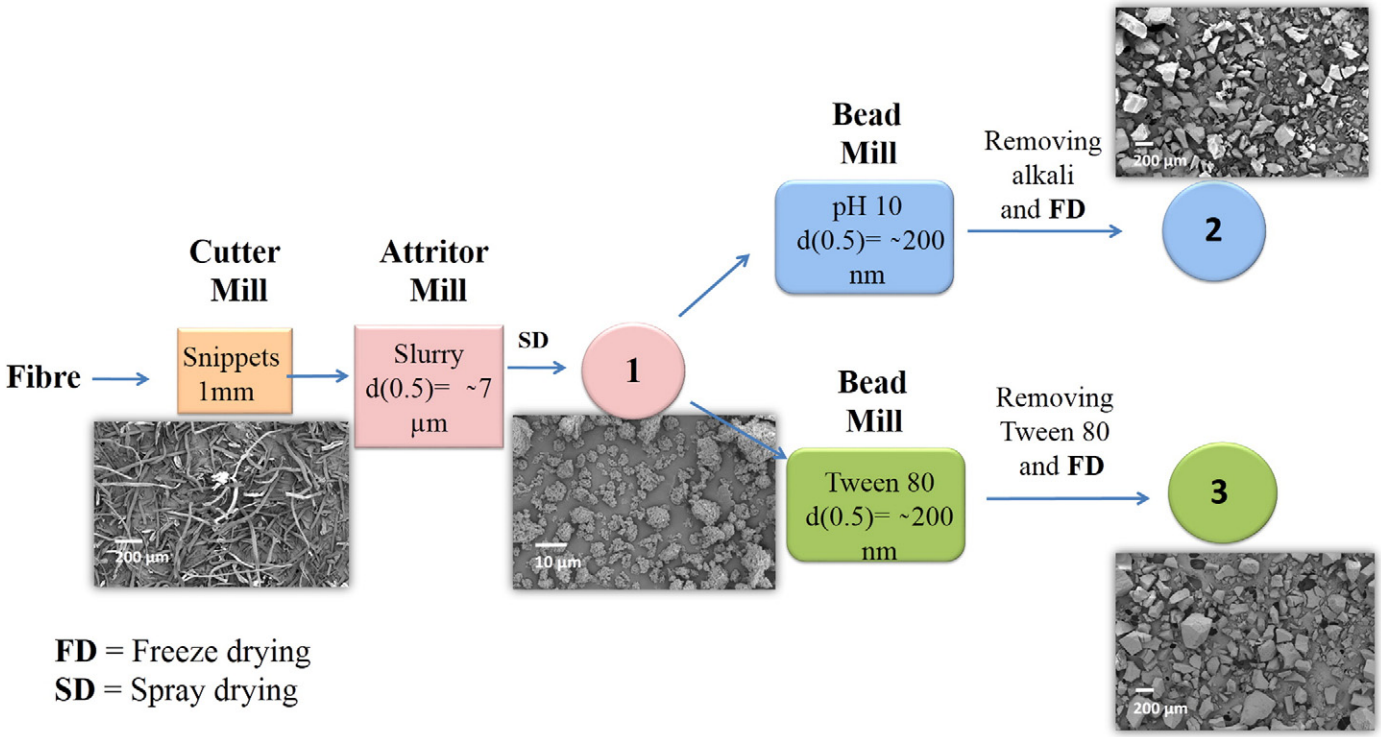

Fig. 1. Production process of dry silk particles.