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# Rheological behaviour of native silk feedstocks

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ABSTRACT

Whilst much is known about the properties of silks, the means by which native silk feedstocks are spun still represent a gap in our knowledge. Rheology of the native silk feedstocks is germane to an understanding of the natural spinning process. Yet, an overview of the literature reveals subtle limitations and inconsistencies between studies, which has been largely attributed to sample-to-sample variation when testing these exquisitely flow-sensitive materials. This ambiguity has prevented reliable, consistent inferences from standard polymer rheology and constitutes an obstacle to further development.

To address this challenge, we present the largest study to date into the rheological properties of native silk feedstocks from *Bombyx mori* larvae. A combination of shear and oscillatory measurements were used to examine in detail the relationships between concentration, low shear viscosity, relaxation times, complex modulus and estimates of the molecular weights between entanglements. The results from this highly detailed survey will provide a sound basis for further experimental or theoretical work and lay the foundations for future bio-inspired processing of proteins.



## 1. Introduction

Silks are natural protein fibres spun by many types of arthropods in the classes Arachnida, Insecta and Myriapoda [1–11]. This may be a significant example of convergent evolution, as the ability to produce silk appears to have arisen independently at least 23 times [8]. Spiders provide the most widespread and obvious examples of silk production, with some recently evolved spider species producing at least seven different types throughout their entire lifecycle, as distinguished by chemical compositions, gland morphologies, physical properties and the ways the fibres are used by the animal [1–4]. The larvae of various lepidoptera (*i.e.* caterpillars) appear to produce only one type of silk at any time, although the quantities and compositions vary between species [5] and developmental stage [12]. Most notably, as a result of millennia of selective human intervention, the domesticated mulberry silkworm *Bombyx mori*, produces relatively large quantities of cocoon silk, which has achieved considerable importance as a textile fibre [13–16].

Although the amino-acid sequences vary, the main proteins in lepidoptera and spider silks (*i.e.* fibroins and spidroins) appear to follow a common theme. A large (up to 500 kDa) highly repetitive core section accounts for the high degree of order and partial crystallinity that can be observed in silk fibres [16–25]. The core is flanked by short non-repetitive globular terminal domains (typically 10–15 kDa), which appear to promote association with other chains in solution, through physical interactions initiated by a decrease in pH and changes in ion content [26–31].

Typically, several different proteins are present in a single type of silk fibre. In *B. mori* silk the main fibroin component consists of a 'heavy' (fibH) chain, with molecular weight around 350–400 kDa [32–35]. This is joined by a disulphide bond near its C-terminus to a 'light' (fibL) chain with molecular weight around 25–30 kDa [34–38]. This fibH-fibL dimer appears to be important for efficient secretion of fibroin and maintaining good solubility within the silk gland, as 'naked pupa' mutants lacking genes for fibL chain expression produce only small amounts of exclusively fibH silk [39–41]. The fibH-fibL dimer forms non-covalent interactions with a 'P25' chaperone (*ca.* 27–30 kDa) glycoprotein [42–45], to give a $(\text{fibH-fibL})_6\text{P25}_1$ complex of molecular weight around 2.3 MDa [46]. There is evidence that complexes of this type may be common, though not universal in lepidopteran silks [47] and may facilitate intracellular transport [46], although any subsequent role in silk spinning remains unknown.

In addition to the major fibroin components of lepidopteran silks, many minor components have been observed. Analyses of

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materials from *B. mori* silk glands revealed several components of sericin, which forms an adhesive sheath around the fibrous fibroin core, and over 500 minor species with molecular weights ranging from 15 to 100 kDa [12,34,38,48]. These appeared to include chaperones, heat-shock proteins, protease inhibitors and other metabolic enzymes, although their contributions to the spinning process also remain unknown.

Hence, given the considerable structural, compositional and functional diversities shown by silks, their means of production (*i.e.* spinning) may provide a more appropriate definition, as suggested by Porter and Vollrath [49]. It is a characteristic of all silks that they are initially produced as hydrated protein feedstocks (typically 20–30 % w/w), stored within the animal in specialised glands then, when required, extruded relatively quickly into fibres [1,2,5,8,18,49–51]. Typically, silkworms produce fibre for cocoon construction at 4–15 mm $s^{-1}$ and spiders can produce major ampullate (*i.e.* dragline) silks at 20–60 mm $s^{-1}$ [52,53], while take-up speeds of 400 mm $s^{-1}$ have been achieved during forced reeling [54]. This 'on demand' nature of silk spinning is in stark contrast to other fibres produced by animals (*i.e.* hair or wool), which grow continuously at relatively slow speeds; for example, Downes and Sharry [55] reported a growth rate of around 0.3–0.4 mm per day for sheep wool. The importance of the spinning process on silk fibre mechanical properties has been well documented and is considered to be as crucial as the feedstock itself [21,52,54,56–61].

While much has been written concerning the remarkable properties and uses of silk fibres [16,19,21,49–53,62], we suggest that the natural spinning route represents the most inspirational feature of silks. During silk spinning, the animal is able to convert a high molecular weight polymer from an aqueous (solution or gel) phase into a water-insoluble fibre, rapidly and at ambient temperatures [3,18,19,48,52,53,55,63,64]. This transition to a solid silk fibre appears to be initiated by flow-induced alignment of the protein chains [54,65,66], probably after the system has been activated by changes in pH and ionic content within the silk duct [26–31,66–69]. The only significant energy input for this process would appear to be the work required to convey the protein feedstock along the silk duct and draw it into a fibre.

By comparison, melt-spun fibres of thermoplastic polymers (*e.g.* polyolefins or polyesters) are typically extruded around 200–300 °C above ambient temperature, while wet- or dry-spun fibres (*e.g.* viscose rayon, acrylics or cellulose acetate) involve large amounts of harmful and potentially dangerous solvents or other processing chemicals [70,71]. Each of these processing routes incurs a significant energy penalty (*e.g.* for process heating or solvent recovery, where applicable) and may also produce pollution in the form of spent or unrecoverable process chemicals. This disparity in energy efficiency between natural silks and synthetic polymers has been recently highlighted by Holland *et al.* [64], who estimated that the energy required to initiate the phase-change in silk would be several orders of magnitude smaller than that required to melt-spin high density polyethylene.

Rheology provides a convenient and informative method for characterising the behaviour of polymer-based and colloidal systems [72–76]. Meaningful measurements can be made on a wide range of flowable media (including solutions, suspensions, melts, gels, pastes and granular systems) under realistic conditions, using modest quantities of materials (*i.e.* consistent with the volumes of silk feedstock contained in the ducts of *B. mori*, other silkworms and reasonably large spiders). Moreover, in polymer-based systems, a suitable analysis of oscillatory measurements can yield information on chain entanglements, relaxation rates and molecular weight. In view of the role generally ascribed to interchain interactions, flow-induced orientation and phase transformations, information of this type is considered to be germane for a complete understanding of natural silk spinning. Consequently, a large body of work has already been published concerning rheological properties of native silk feedstock from *B. mori* [65,66,77–85], other silkworms [82,83], spiders [84,86,87], as well as reconstituted silk feedstocks prepared using various chaotropic solvents [85,88–94].

This has revealed some important generic rheological characteristics across all native silk feedstocks tested to date. Specimens undergo shear-thinning (*i.e.* the viscosity decreases as shear rate increases) [65,78–87] accompanied by flow-induced orientation [65,66,81]. They also exhibit significant viscoelasticity, with the elastic or storage modulus ($G'$) exceeding the viscous or loss modulus ($G''$) at high frequency and a cross-over to viscous behaviour ($G'<G''$) at low frequency [65,78,80–85]. These observations are typical of a concentrated polymer solution above the overlap threshold, where the rheology is dominated by interchain entanglements [72–76]. Moreover, comparisons between specimens from *B. mori* and wild types suggest that silkworms can produce superficially similar fibres from protein solutions with somewhat different rheological characteristics [82,83].

Nevertheless, a careful examination of the details reveals several limitations and inconsistencies amongst this work. For example, for native silk feedstocks from *B. mori*, the reported values of low shear-rate (*i.e.* $\dot{\gamma}$ <0.1 $s^{-1}$) viscosity range from $10^3$ to $10^8$ Pa s [65,66,77–85]. Also, the cross-over from elastic to viscous behaviour (at $G' = G''$) has been reported at angular frequencies ranging from 1 to 10 rad $s^{-1}$ [65,78,80–85]. By contrast, the data presented by Ochi *et al.* [77] was more characteristic of a gel, with $G'>G''$ over the entire frequency range. It is possible that these apparent inconsistencies may reflect inherent variations in a natural biological system, or they may have resulted from poor handling of a highly sensitive material. This ambiguity has prevented reliable, consistent inferences based on standard polymer rheological analysis and constitutes a considerable obstacle to further development of the field. We propose that this may be addressed by adopting a more statistical approach to the analysis of native silk rheology and studying the distributions of properties.

The purpose of the present work was to provide a thorough analysis of the rheological characteristics exhibited by native silk protein feedstock from a statistically meaningful population of the silkworm *B. mori* at 25 °C. To that effect, combinations of shear and oscillatory measurements were used to extract information on molecular relaxation behaviour and chain entanglements. This will provide the background for subsequent work on silk protein solutions, including measurements of the activation energy of flow, investigating shear- and temperature-induced gelation, comparisons between native feedstocks from other animals and the characterisation of redissolved silk protein systems.

## 2. Experimental

*B. mori* silkworms in the 5th instar were housed at ambient humidity until ready for use. Once the silkworms had stopped feeding, they were housed at around 10 °C, in order to delay pupation (normally by no more than 10 days). In addition to visual examination, the weights of some silkworms were monitored in order to assess their condition during storage.

Native silk feedstocks were extracted from silkworms that had just started to construct the cocoon. The silk glands were removed following methods similar to those described previously [65,82,85]. Using a dissection microscope, glands were carefully separated from the rest of the tissue, then transferred to cold (*ca.* 5 °C) distilled water in order to peel off the epithelial membrane using tweezers. This was achieved as quickly as possible (typically within 5 min) to minimise dilution of the silk gland contents. The coiled