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ScienceDirect
Polymer 48 (2007) 3388–3392
polymer
www.elsevier.com/locate/polymer

Polymer Communication

Natural and unnatural silks

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Received 27 February 2007; received in revised form 6 April 2007; accepted 12 April 2007
Available online 19 April 2007

Dedicated to the living memory of one of the fathers of silk reconstitution and electrospinning the Late Ron Eby, who was the Robert C. Musson Professor of Polymer Science and an Ohio Eminent Scholar (Emeritus) at the University of Akron. Ron was the kindest of colleagues and most generous of friends.

Abstract

Natural silk is an important biopolymer with huge potential as it combines superb mechanical properties with environmentally sensitive production methods. Native silk dope taken straight from the gland can easily and without chemical assistance be drawn into strong fibres. Artificial silk fibres, on the other hand, rely on spinning dopes typically 'reconstituted' from natural silk fibres by strong chaotropic agents. Such fibres do not form readily, and often require chemical post-spin treatment for stabilisation. In addition these fibres tend to be brittle, and so far have been unable to match native fibres. Here we present novel rheometric data to argue that native and reconstituted silkworm silk dope differ in kind, not just in degree. While native silks behave like typical molten polymers, reconstituted silks do not. We conclude that rheology provides a powerful tool in the quest to learn from the Nature's polymer fibre technology.
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Keywords: Silk; Polymer rheology; Biomaterials

1. Introduction

Silk represents the zenith of fibre processing [1] i.e. a rapid and energetically efficient extrusion system that produces high performance and biodegradable materials with supreme properties. Therefore it is not surprising that many attempts have been made to emulate these super-fibres (e.g. Refs. [2–11]). Typically such artificial silk fibres are spun from silk that was reconstituted in a variety of ways and then extruded using techniques ranging from classical alcohol baths [2] to electrospinning [12]. Yet to date none of these reconstituted fibres were able to match the mechanical properties of their natural progenitors (Fig. 1). Since a spun fibre represents the conditions of both the spinning dope material and the processing conditions, it is only to be expected that one must be complementary to the other. We propose that our rheological characterisation methods will help us to determine whether reconstituted silk dope actually has the *potential* (or not) to form a fibre with the qualities of its natural predecessor and model.

Natural spinning relies on fine control of the energy in the spinning dope i.e. the energy introduced when the dope is pulled through the animal's spinning duct causing it to flow. Flow elongation induces the organized alignment and conformational change in the silk proteins that, assisted by changes in pH, converts the material first from a sol to a gel and then to the solid fibre [13]. We find it useful to deploy rheology as a tool to analyse – under experimental and highly controlled conditions – the deformation and flow of silk feedstocks. This allows us to analyse in great detail the conditions encountered by the silk dope during the natural spinning process as well as to compare the behaviour of native and reconstituted silks.

Despite their independent evolution, the rheologies of native spider and silkworm dopes are very similar and, quite unexpectedly, both behave like polymers, molten at room

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0032-3861/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2007.04.019

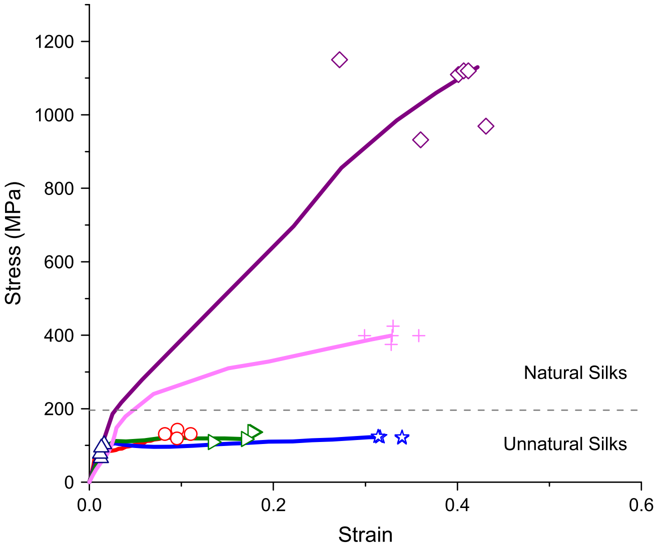

Fig. 1. Natural and reconstituted silks have substantially different material properties. A representative sample drawn from the current published literature of the mechanical properties of silk fibres created by a variety of different reconstitution/re-spinning techniques (whose stress has been reported in Pa). Lines represent exemplar stress strain curves, whilst points represent sample variation from that experiment. Natural silk, spider (*Nephila edulis*) major ampullate (purple, diamonds, from [4]) and silkworm (*Bombyx mori*) cocoon (pink, cross, from [7]) have breaking stresses much higher than silks prepared from reconstituted spider-silk spun in water (green, right facing triangles, from [3]) and reconstituted silkworm silk spun in water (red, circles, from [7]) or spun in solvent with different spinning conditions (navy, triangles, from [5] and blue, stars, from [5]).

temperature [14]. This important observation suggests that it should indeed be possible, in principle, to artificially spin high performance silks by biomimetically adapting traditional polymer spinning theory and techniques [15].

Spinning reconstituted silk is affected by a major problem caused by the harsh environments necessary to break down the silk fibres in order to prepare native silk for re-spinning. Specifically, both protein size (molecular weight) [10,16,17], and protein conformation [10,18] tend to be altered significantly by the elevated temperatures and highly chaotropic agents of the traditional processing treatments required to make the feedstock. Comparing the dope rheology of reconstituted with that of native silk, over a wide range of concentrations and varied shear loading conditions, allows us to study, indirectly but appropriately, the effect of the harsh preparation conditions on molecular integrity. Our results show that the differences between the two materials are significant. This, in turn, suggests that biomimetic (naturalistic) spinning conditions may not work for reconstituted silk, as we will discuss.

2. Experimental

2.1. Materials

2.1.1. Native silk dope preparation

L4/L5 *Bombyx mori* silkworm larvae were purchased and subsequently raised on a diet of fresh white mulberry leaves (*Morus alba*) in the lab (25 ± 2 °C 60% RH). When about to spin a cocoon they were placed into a fridge (4 °C) for 30 min, then removed, massed (1.95 g, SD 0.49), and dissected using chilled (4 °C) distilled water. Silk glands were removed (under 1 min), had their epithelium peeled and the contents were washed in 3 changes of water to remove the sericin coating (as assessed by visual inspection) for 35.29 min (SD 10.33). Spider major ampullate dope samples (*Nephila edulis*) were prepared as before [14]. Samples were then diluted with distilled water to achieve a range of concentrations and left overnight in a fridge to homogenise.

2.1.2. Reconstituted silk dope preparation

We followed the most common procedure [7]. *B. mori* cocoons were boiled twice in 0.5% w/v Na_2CO_3 solution for 30 min, washed in distilled water and soaked in warm distilled water for 30 min to remove sericin. Once dry, silk was fully dissolved in 9 M $LiBr_{aq}$ (25 °C) for 30 min to make an approximate 10% solution. Liquid silk was poured into dialysis tubing (MWCO 12.4K Sigma, UK) and dialysed against stirred distilled water changed every 3 h during the day for 3 days. For pilot studies the dialysis was performed at 25 °C and for the experiment at 4 °C, however, this made no significant difference to the results and so the data were pooled. Once dialysed the reconstituted silk solution was either diluted with distilled water or dialysed against 5, 10 or 15% polyethylene glycol (MW 15,000–20,000 Sigma, UK) at 4 °C overnight to obtain a range of concentrations.

2.2. Dry weight analysis

Remaining sample not used in the experiment was massed then dried to a constant mass (48 h 60 °C, 48 h desiccation under vacuum).

2.3. Rheological analysis

All rheological tests were run on a Bohlin Gemini HR Nano 200 (torque range 10 nNm to 200 mNm, controlled stress/rate viscometry, 3 nNm to 200 mNm, controlled stress/strain oscillation, Malvern Instruments, UK) maintained at 25 °C (Bohlin KTB 30, Malvern Instruments, UK) with environmental cuff attached to prevent the sample drying out. Native silk samples above 2% dry weight (DW), including spider major ampullate dope, used a cone and plate 1° incline, 10 mm diameter measuring geometry (CP 1/10) and for all other native and reconstituted silk samples a cone and plate 4° incline 40 mm diameter measuring geometry (CP 4/40) was used. Prior to all tests each group of concentrations had their linear viscoelastic region thoroughly characterised and subsequent oscillation tests were performed within that region. Plateau moduli were obtained from an oscillatory sweep between 623 and 0.623 rad/s (target strain 0.002 for CP 1/10 and 0.1 for CP 4/40) using the tan δ MIN method (for review see [19]). Zero shear viscosity measurements were obtained by taking a linear fit of the upper viscosity plateau during a steady shear response experiment between 0.0075 and 50 1/s for samples under a CP 1/10 (range due to higher sample viscosity and sample ejection [14,20]) and 0.1–4000 1/s under a CP 4/40 (range due to significantly lower sample viscosity and signal to noise ratio at lower shear rates).

3. Results and discussion

Both natural and reconstituted silk dopes were subjected to the same rheological characterisation, consisting of an oscillatory sweep, which was used to determine the response of the material to energy input over different timescales. The oscillatory sweep was then followed by a viscometry test, in order to provide information on the degree of intermolecular associations present in the dope [14,21].

Oscillation tests on natural silk confirmed previous measurements (Fig. 2a), with higher concentration dope displaying the characteristic crossover point between G' and G'' [14,20] and more dilute samples appearing to have gelled [22], as determined by a frequency independent G' value. The comparison of natural silks with samples of reconstituted silk having the same dry weight of material (see Fig. 2a legend) revealed a clear difference in both the absolute value of the modulus and the shape of the profiles. It appears that reconstituted silk does not posses a frequency dependent crossover point and behaves entirely like a liquid over the full concentration range tested.

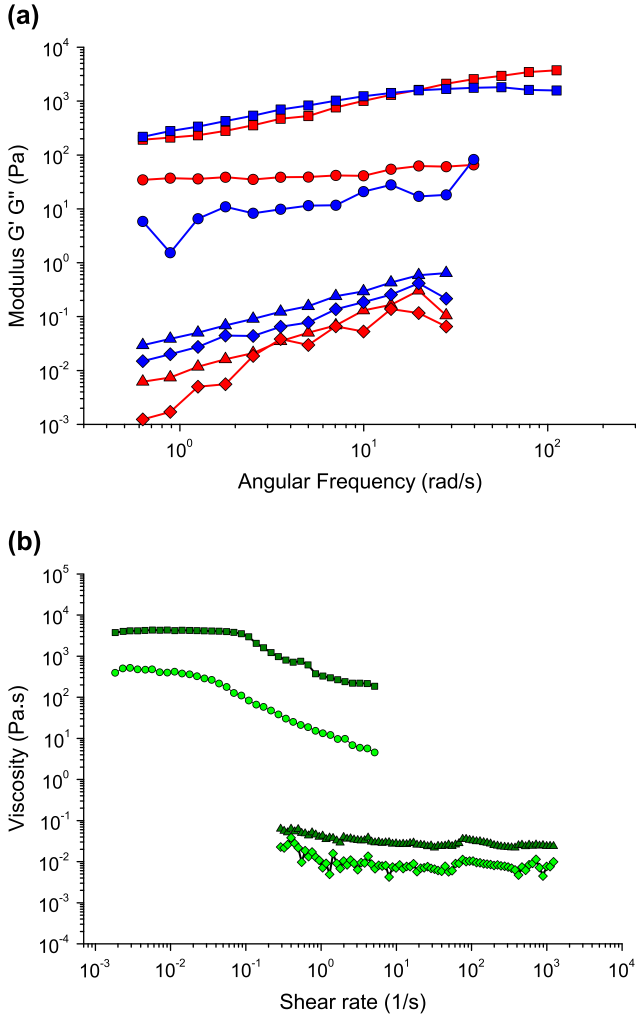

Fig. 2. Representative oscillatory (a) and viscosity (b) measurements of natural and reconstituted silkworm dope. Dry weight concentrations of natural dope are 18.6% (squares) and 4.6% (circles) and of reconstituted dope they are 18.5% (triangles) and 4.5% (diamonds). For each concentration the same sample is represented in both graphs for consistency. Oscillation graph depicts elastic modulus G' (red) and viscous modulus G'' (blue).

Viscosity measurements over a range of shear rates further emphasise the great differences between these two materials (Fig. 2b). Over the range of all concentrations tested, natural silk dope consistently exhibited a zero shear viscosity and power law shear-thinning typical of a molten polymer, with higher concentrations representing higher overall viscosities. When compared on the same scale, reconstituted silks behaved like Newtonian fluids with significantly lower viscosities, in agreement with previous findings [23,24]. The concentrations of reconstituted silk dope that we tested had only slightly different rheologies, which suggest that for this material there is a direct, positive link between amount of material and viscosity. Taken together these data indicate that, despite the origin of the reconstituted molecules from native silk, the reconstitution process did substantially alter the integrity of the silk molecules. This alteration must have been sufficiently invasive to render the reconstituted molecules unable to respond to shear in the same way as their native predecessors.

Clearly, reconstituted silk is different from native silk. But how different? And does this difference prevent reconstituted silk from serving as dope for biomimetic extruders? In order to compare reconstituted silks to their native counterparts for 'spinnability', we propose, initially, two rheological indicators: one such indicator is *zero shear viscosity*, the strength of intermolecular association, internal friction, between silk molecules in the dope. This would indicate how the applied energy flows through the material. The second indicator would be the *plateau modulus*, which reveals how much energy the silk molecules are able to absorb, required for complete transition from stored gel to final solid fibre [13].

At the same concentration, native dope has a zero shear viscosity nearly 3 orders of magnitude higher than standard reconstituted silk (Fig. 3a). Variation within and between samples of the same concentration may be attributed to sample handling [14]. The trend of a zero shear viscosity that increases with concentration is seen for all samples of natural dope. However, the reconstituted silks appear to plateau below 10% dry weight, implying that a critical amount of reconstituted silk molecules must be present before they can have an effect on viscosity. Our observations suggest that reconstitution appears to seriously degrade the associations between silk molecules, thus altering the way in which shear energy is distributed through the material.

A similar trend can be seen in the plateau modulus of the natural silk dope, with the ability to store energy increasing with concentration of silk molecules (Fig. 3b, over 4 orders of magnitude in the range tested). However, this is not the case for reconstituted silk dope, whose energy storage barely increases 1 order of magnitude over the entire range.

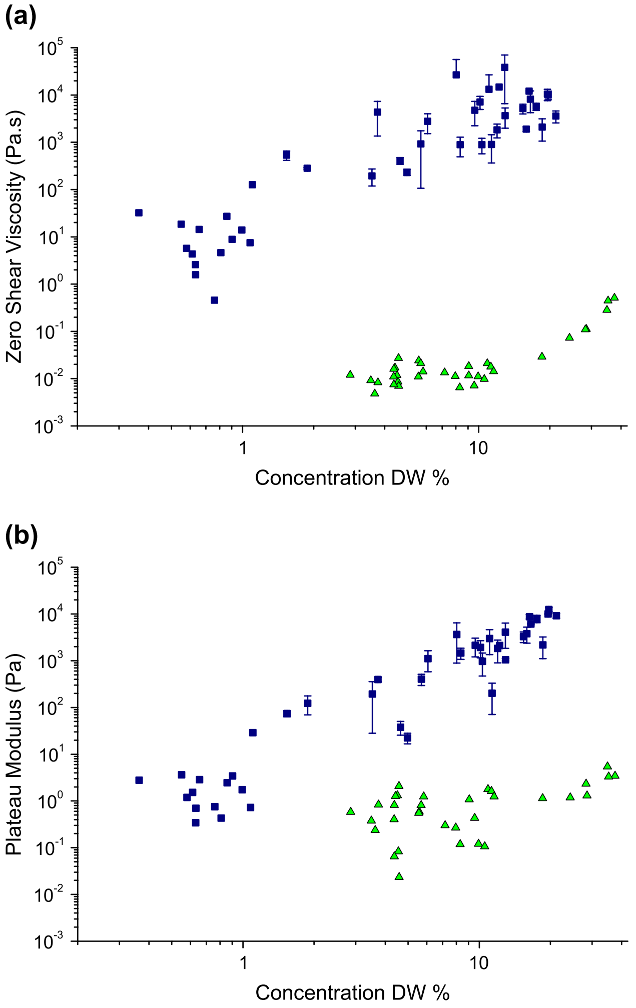

Fig. 3. Concentration of natural (blue squares) and reconstituted (green triangles) silkworm dope plotted against zero shear viscosity (a) and plateau modulus (b). Error bars for native silkworm silk samples >1.5% dry weight, represent standard error based on 3 repeats from the same silkworm gland contents, lower concentration native and reconstituted silkworm dope points represent individual tests due to the increased amount of sample required for accurate characterisation.

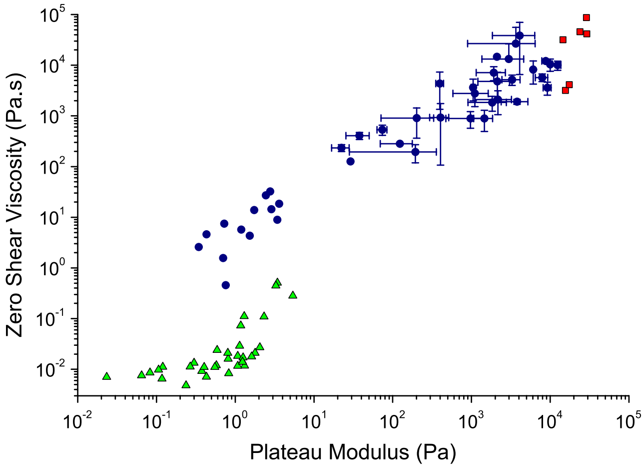

Fig. 4. Zero shear viscosity vs. plateau modulus for native (blue circles), reconstituted (green triangles) silkworm silk dope, with native spider dragline silk dope (red squares) for reference. Error bars for native silkworm silk samples >1 Pa, represent standard error based on 3 repeats from the same silkworm gland contents, lower concentration native and reconstituted silkworm dope points represent individual tests due to the increased amount of sample required for accurate characterisation. Spider silk samples represent single tests on individual glands due to the limits of sample availability.

Therefore the act of reconstitution does not only hamper the way in which energy flows through the material, as reflected in the reduced viscosity of the dope, but it also affects the ability of the silk molecules to store energy. Energy conversion, from kinetic to chemical (bonding), of course drives the conformational change of the protein during the spinning process [13].

By now plotting these two ‘spinnability’ indicators against one another for the two sets of samples, we are able to visualise inherent material properties, specifically how these two dopes control the flow of energy (Fig. 4). For native dope the zero shear viscosity is strongly coupled with the plateau modulus at all concentrations (adjusted $R^2 = 0.851$, $p < 0.0001$ on log transformed data), which is not observed in the reconstituted dope. It also appears that, due to the nature of this linear relationship, natural silk dope has a constant relaxation time and is able to self-regulate the flow-through and storage of energy throughout a range of concentrations. This, of course, is a property of great interest to, but not yet seen in, the field of polymers.

To corroborate our proposition that natural silks possess a similar set of rheological parameters, and thus confirm the suitability of our ‘spinnability’ indicators, a small sample of spider major ampullate dope (*N. edulis*) was tested under the same conditions and included in the comparison (Fig. 4). Due to the constraints upon sample size for spider major ampullate silk dope, it is currently not possible to obtain exact sample concentrations. However when compared to silkworm dope these samples not only occupy the space predicted by their expected concentration, but also appear to possess a similar ability to self-regulate their material properties.

4. Conclusions

The origin for the vast difference in the observed rheologies of native and reconstituted silks is in all probability due to the severe degradation of the silk proteins during the reconstitution process, which appears to reduce size [16,17] as well as affect conformation [18]. Such changes would directly influence the impact of shear energy and could explain the reduced viscosities and modulus values we observed. Determining the individual contributions of, amongst others, size, shape, stability and integrity of reconstituted silk molecules to their ‘spinnability’ will allow us to devise sensitive protocols aimed to minimise both physical and chemical damage to the silk proteins. This will not only increase our understanding of the requirements for silk to be spinnable, but also our comprehension of the natural spinning processes. Thus, hopefully, comparative studies of the rheological behaviour of native and reconstituted silk dopes *in vitro* will contribute to a better grasp of the *in vivo* behaviour of the many thousand silks evolved in nature. Hence these novel insights may, one day, be conducive to the design of synthetic silks that can be spun by a fully functional biomimetic extruder.

The massive gap between these two dopes may go some way to explain why it has not been possible to create a reconstituted 'silk' fibre with the mechanical properties [2−10], or structural complexity [25,26], of a natural silk, let alone process it in the same way. Clearly these two materials are already different before they are even spun, and it is questionable whether the first can even be called a silk. For biomimetic spinning i.e. the important step towards the economic and ecological production of artificial silks, it will be necessary, so we believe, to first match the rheologies of reconstituted and native dopes. This then, perhaps, will ultimately lead to landing one of natures greatest catches − high performance fibres, naturally produced.

Acknowledgements

We thank the European Commission (grant G5RD-CT-2002-00738 as well as the AFSOR of the United States of America (grant F49620-03-1-0111) for funding. We also thank Cheng Cheng, Yi Liu and Cedric Dicko for their help with reconstituted silk sample preparation and comments. Finally we are indebted to Dr. Alex Sponner (EC-MTKD-CT-2004-014533) for his generosity in helping with various molecular techniques and for sharing his valuable insights into silk molecular structure.

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