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Thermochimica Acta 315 (1998) 61–66

thermochimica acta

Thermal analysis of major ampullate (drag line) spider silk: the effect of spinning rate on tensile modulus[1]

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Abstract

Spiders use major ampullate silk (MAS) in web building and as a 'safety line' when escaping predators. In the former case, the fibre is spun at approximately 1 cm s^{-1}; in the latter case the rate is an order of magnitude faster. The different spinning rates do not result in identical microstructures, implying that the mechanical properties should not be identical either. However, neither conventional tensile testing, nor the characterisation of storage modulus with DMA, yields reproducible data that could be used to correlate stiffness to the spinning rate. Any correlation is masked by the consequences of MAS having a cross-section that is non-circular, difficult to measure and highly variable along the fibre length. Also, DSC shows that fibres contain bound moisture even after drying in a desiccator; fibre thickness affects the ability of samples to lose this moisture and gain stiffness on heating in the DMA. Thermal expansion data obtained with the DMA are reproducible below the MAS glass transition (approximately 160°C): these results do not depend on the accurate measurement of the sample cross section, they are insensitive to the moisture content of samples and they are not affected significantly by energy loss arising from friction between individual fibres in the specimens tested. The thermal expansion data suggest that the highest intrinsic stiffness of MAS is realised at spinning rates used in web construction. When the spider must elude a predator, it spins MAS that is more compliant. © 1998 Elsevier Science B.V.

Keywords: Silk; Spider; Thermal expansion; Dynamic mechanical analysis; Differential scanning calorimetry

1. Introduction

Major ampullate silk (MAS) is found in the radial threads that support a spider's web and in the drag line used by the spider to make rapid vertical descents. From the viewpoint of materials science, MAS fibre exhibits an impressive portfolio of properties still unrivalled by synthetic polymers. These properties, described in detail elsewhere [1–3], include (1) combined high stiffness, breaking strength and toughness, (2) spinnability of a water-insoluble product from aqueous solution at ambient temperatures, (3) controlled biodegradability and (4) independence from petrochemical feedstocks. Much of the molecular, microstructural and property characterisation of MAS has been performed on the fibre spun by *Nephila clavipes* (golden orb weaver) spiders and it is on that material that we conducted the studies described below.

A mature *N. clavipes* may produce MAS fibre at approximately 1 cm s^{-1} during web construction [4] and up to ten times faster during a rapid descent to elude a predator. In both cases, the spider

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[1]Presented at TAC 97, Oxford, UK, 14–15 April 1997.

PII S0040-6031(98)00277-9

must produce a fibre having dependable tensile properties [5]. However, given the order of magnitude difference in processing rates, it would be surprising if the properties remained constant – and indeed there is no reason to suppose that the same properties would provide the optimal response for both applications.

Stress-strain curves obtained from MAS samples spun at a constant rate (e.g. 6.1 cm s^{-1} [6] or 10.3 cm s^{-1} [7]) exhibit significant variability. If curves for MAS spun at a constant rate (e.g. 1.5 cm s^{-1}) are averaged and compared to the averaged tensile test data for MAS spun at another constant rate (e.g. 12.2 cm s^{-1}), the difference is just as marked [6]. The highly non-uniform and difficult-to-measure cross-sectional area of MAS [8] is a significant source of the variability in all such comparisons. Standard tensile tests, therefore, do not provide an unambiguous, ready means of finding correlations between spinning rate and mechanical properties.

Transmission electron microscopy has been used [9] to investigate the microstructures of MAS spun at rates ranging from 0.5 to 10.0 cm s^{-1}. Individual ordered regions formed at the fastest spinning rate are significantly smaller and their collective volume fraction in the microstructure is reduced slightly, compared to those formed at the slowest rate. It is because the crystals depend on statistical rather than perfect matches between neighbouring polymer chains [9,10] that their overall capacity to form is not highly sensitive to the processing conditions; the same reason lies behind the greater sensitivity of microstructural *scale* to the spinning rate.

Given that the spinning rate has some impact on microstructure (not necessarily limited to those effects just described), we expect properties to be affected as well. However, the above-mentioned problems of reproducibility, as well as limitations of available load cell sensitivity, attend conventional tensile testing of MAS. We therefore sought alternative ways of characterising MAS properties and turned to thermal analysis as a possible solution. DMA was seen as a particularly attractive technique, because it enables the measurement of a thermomechanical response (expansion coefficient) that is not affected significantly by the sample cross-section.

2. Experimental details

2.1. Collecting spider MAS

MAS was acquired from live *N. clavipes* spiders at forced spinning rates of 0.5, 1, 2, 5 and 10 cm s^{-1}. Our apparatus for 'silking' spiders at controlled rates is described in detail elsewhere [11,12]. For the present experiments, the silk from both major ampullate glands was wound concurrently onto a rotating but non-translating teflon mandrel (2.54 cm diameter). After 50 rotations of the mandrel, the 'silking' apparatus was stopped and the fibre was secured with adhesive tape and cut as shown in Fig. 1. Each piece of the tape was then lifted carefully from the mandrel with forceps and folded in half, the tape sticking to itself. These processes create a bundle of 100 closely parallel and tightly packed fibres, approximately 8 cm long. Bundles were placed individually in dry glass vials, capped with airtight lids and stored in a dark location.

2.2. Differential scanning calorimetry

Sample masses were approximately 1 mg. Taking 3 μm as a representative diameter [8] and 1350 kg m^{-3} as a typical density [1] for *N. clavipes* MAS, it is easily shown that 1 mg corresponds to approximately 105 m of fibre. A single bundle contained approximately 8 m of silk, so silk from 13–14 bundles was combined in each DSC sample. Preparation of larger DSC samples would require a proportionate but prohibitive increase in the effort required to collect material. To make the silk easier to place into DSC pans, it was chopped into short lengths with a razor blade cleaned in ethanol.

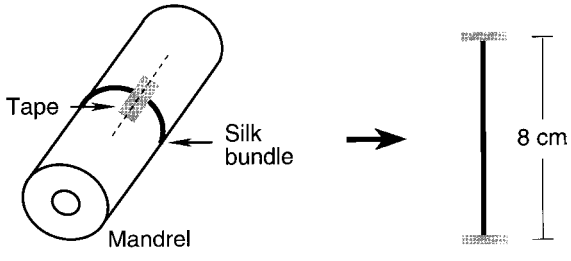

Fig. 1. Retrieving a bundle of 100 MAS fibres from the apparatus used to 'silk' spiders at controlled rates.

A Perkin Elmer DSC7 was used. The calorimeter was calibrated with indium reference material according to the manufacturer's instructions. Samples were placed into standard aluminium pans, kept in a desiccator for at least 24 h and sealed non-hermetically with a crimping press. They were heated from 30°C to 200°C at either 5, 10 or 50°C min^{-1}. The purge gas was dry nitrogen, flowing at 30 ml min^{-1}. Data were analysed with the manufacturer's software.

2.3. Dynamic mechanical analysis

A Perkin Elmer DMA7 was used, with bundles loaded in tension. A bundle for analysis was removed from its storage vial and a piece of tape was attached approximately 1.5 cm from one end. The bundle was cut at the middle of this tape, to yield bundles approximately 1.5 and 6.5 cm long. The 1.5 cm bundle was mounted vertically in the DMA, while the remainder was returned to its storage vial to provide material for diameter characterisation and for backup in case the sample in the DMA was damaged.

The DMA was operated in the temperature scan mode, with the following parameters: heating from 30°C to 200°C at 5°C min^{-1}; 1 Hz oscillation frequency; 102% tension (static control); 0.03% strain (dynamic control). Dry helium flowing at 50 ml min^{-1} was used as the purge gas. Data were analysed with the manufacturer's software.

2.4. Measuring fibre diameter

To obtain quantitative values of storage and loss moduli from the DMA data, it was necessary to know the cross-sectional area of each silk bundle tested. Three fibres were removed from the leftover part of the bundle, i.e. the part not used in the DMA. The diameter of individual fibres was characterised at three separate positions by laser diffraction, using a previously published protocol [8], to give a total of nine diameter measurements. The average of these values was multiplied by 100 (the number of fibres in the corresponding bundle), to obtain a measure of the cross-sectional area of the entire bundle.

3. Results and discussion

3.1. Differential scanning calorimetry

Fig. 2 shows DSC traces for MAS spun at 2 cm s^{-1}. The only clearly visible transition is a broad endotherm that becomes more pronounced and is displaced towards higher temperatures as the heating rate increases. No additional detail was observed in scans conducted on MAS collected at the other four spinning rates. DSC studies of *Bombyx mori* (silkworm) silk [13] have revealed the presence of a glass transition in amorphous material at around 175°C. Our DMA work (below) shows that a corresponding transition also exists in *N. clavipes* MAS, so its failure to register in DSC traces must be due to our practically limited sample sizes. While the spinning rate is unpredictably variable, *B. mori* silk is readily available in larger quantities because it is produced in the convenient form of cocoons that contain several hundred meters of fibre.

We associate the broad endotherm with water loss, consistent with previous interpretation [13] of DSC scans of amorphous silkworm silk. The fact that the MAS samples retain water, even after drying in a desiccator, is significant in our interpretation of DMA results below.

3.2. Dynamic mechanical analysis

For samples collected at a fixed silking rate, plots of E' (storage modulus) or E'' (loss modulus) as a func-

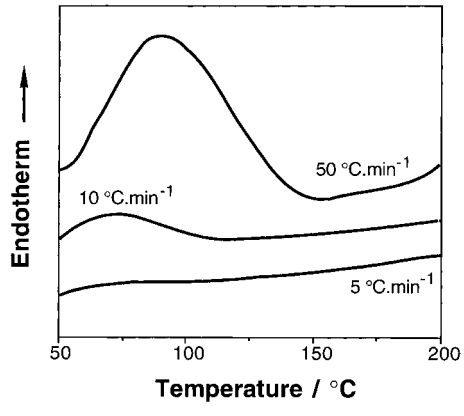

Fig. 2. DSC traces for MAS spun at 2 cm s^{-1}.

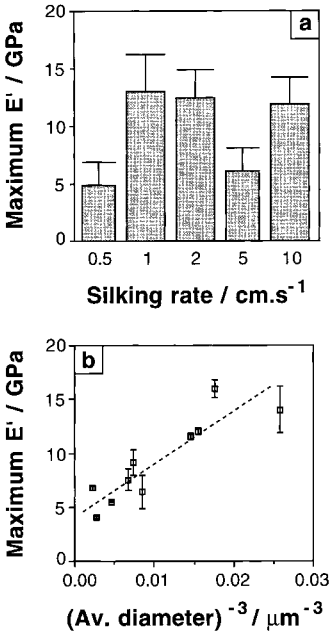

Fig. 3. (a) Maximum storage modulus of MAS, obtained by averaging results from two specimens at each spinning rate. The 'nails' indicate the higher of the two values recorded for each spinning rate. (b) Maximum storage modulus for the same 10 specimens used in (a), re-plotted versus the cubed reciprocal average diameter of the fibre in each bundle. Error bars show how modulus is affected when one standard deviation is added to or subtracted from the mean fibre diameter associated with each data point.

tion of T (temperature) are noisy and unreproducible. In all cases, however, E' reached a maximum between 64°C and 127°C, a temperature range encompassed by the water loss endotherm in DSC traces. An association of moisture loss with an increase in E' for MAS has been noted previously [6]. The onset of a rapid decrease in modulus was detected in all samples at approximately 170°C; accurate determination of this value was made difficult by the noisy profile of the experimental E' versus T plots.

Fig. 3(a) shows maximum E' (average for two specimens and also the higher of the two values) as a function of spinning rate. The averages pertaining to different spinning rates vary by an amount comparable to the variability at any given spinning rate. There is no conclusive evidence for a correlation between maximum E' and spinning rate from this limited data set. To reduce the variability in data at each spinning rate would require a prohibitively large number of samples.

However, a more informative picture emerges if the data are re-plotted as in Fig. 3(b). Here maximum E' is shown as a function of the cubed reciprocal average fibre diameter in the bundles. Error bars indicate the uncertainty in maximum E' arising from the uncertainty in fibre diameter. The rationale behind our choice of abscissa is as follows:

Release of bound water from the sample on heating allows an increase in modulus, to the extent that the water loss occurs quickly on the timescale needed for the sample to reach temperatures at which the intrinsic stiffness begins to fall. Thinner fibres will lose water more rapidly than thick fibres, for two reasons. First, the average diffusion distance to the surface is smaller for thin fibres. An approximate solution to Fick's second law of diffusion states that diffusion time is proportional to the square of diffusion distance. In the present context (a linear dependence of maximum E' on the reciprocal of diffusion time), this solution implies that maximum E' is a linear function of $(\text{radius})^{-2}$. Second, thinner fibres have a surface-to-volume ratio that is more favourable with regard to water loss, so there is a further factor of $(\text{radius})^{-1}$ in the relationship between maximum E' and fibre thickness. Taken together, both factors predict a linear relationship between maximum E' and $(\text{radius})^{-3}$.

The impact of the non-uniform cross-section of spider silk on accurate measurement of stress and therefore on the accurate determination of strength and stiffness, has been noted previously [8]. The results shown in Fig. 3 demonstrate that the variability in thickness also affects the maximum storage modulus (through the effect of thickness on the ability of the fibre to lose bound moisture), masking any dependence of maximum storage modulus on spinning rate.

3.3. Thermal expansion coefficient

Plots of bundle length versus temperature are significantly less noisy and more reproducible than those recording E' or E''. A typical plot of bundle length versus temperature is shown in Fig. 4. A marked change in gradient (thermal expansion coefficient, α) at around 160°C corresponds to the glass transition. The glass transition onset temperature is independent of the spinning rate, suggesting that the free volume of

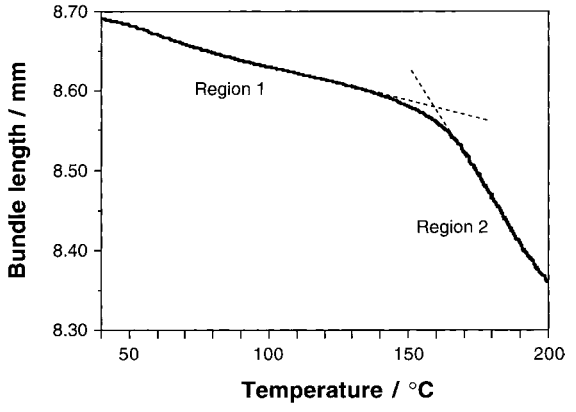

Fig. 4. Typical plot of bundle length versus temperature, showing the construction used to determine the glass transition. This sample was spun at 10 cm s^{-1}.

chains is similar in all the microstructures. Given that microstructure is affected by the spinning rate (see Introduction and [9]), we deduce that chain free volume is not sensitive to the distribution of reinforcing domains in the solid fibre. In turn, this deduction is consistent with the evidence [10] that the local composition, symmetry and perfection of order vary over distances that are small compared to the size of 'crystalline' and 'amorphous' regions in MAS.

The plots of bundle length versus temperature are especially reproducible at temperatures below T_g. In contrast to measurements of E' and E'', measurements of α do not depend on our ability to characterise sample cross section. Also, α is not sensitive to energy loss arising from friction between individual fibres in a bundle. Table 1 lists the value of α below T_g for two bundles collected at each spinning rate (the same ten bundles from which the data in Fig. 3 were obtained).

Table 1
Thermal expansion coefficient of MAS below T_g (two bundles were collected at each spinning rate)

Draw rate/cm.s^{-1}	Thermal expansion coefficient, α/°C^{-1}	
	First sample	Second sample
0.5	-8.3×10^{-4}	-8.2×10^{-4}
1.0	-10.6×10^{-4}	-11.0×10^{-4}
2.0	-4.2×10^{-4}	-4.2×10^{-4}
5.0	-5.9×10^{-4}	-5.9×10^{-4}
10.0	-5.2×10^{-4}	-5.2×10^{-4}

Expansion coefficients pertaining to a fixed spinning rate are reproducible, while comparatively large changes in expansion coefficient accompany changes in the spinning rate. The observation that the near-ambient expansion coefficient is highest in the material spun at 1 cm s^{-1} is therefore unlikely to be an artifact of specimen thickness or moisture content.

The magnitude of α is an increasing function of the overall degree of molecular alignment in a fibre. In turn, the latter quantity increases with (a) the retained volume fraction of aligned material (greatest for low spinning rates, where molecules are better able to develop statistical matches with their neighbours [9]) and (b) the local order parameter for aligned material (greatest for high spinning rates, where extended conformations are afforded less opportunity to randomise before the fibre solidifies). A maximum in the overall degree of molecular alignment, detectable as a high value of α, is therefore to be expected at some intermediate spinning rate. Because tensile modulus is also an increasing function of overall molecular alignment, the measurements of expansion coefficient provide strong evidence that stiffness is maximised at the 1 cm s^{-1} spinning rate typically used in web construction. By the same argument, the stiffness is significantly reduced at spinning rates used by spiders making a quick getaway.

In the context of a spider's web, the radial threads provide support for the capture thread, carry the static weight of the spider and transmit vibrations signalling that the prey has been caught [4]. For these purposes, a high modulus is useful. The kinetic energy of flying or falling prey is dissipated in the threads of the capture spiral. However, when a spider uses its drag line to arrest a fall (which may sometimes be preceded by a running jump), it has to rely on the drag line alone to dissipate the kinetic energy. In such circumstances, a more compliant fibre is preferable. The qualitative difference in stiffness for MAS spun at 1 cm s^{-1} and 10 cm s^{-1}, as deduced from thermal expansion measurements, therefore caters to divergent design criteria. Whereas a spider may produce as many as six different types of silk to perform distinct functions [14], MAS apparently is multifunctional. This observation both extends and limits the principle of 'design is cheaper than material' [15], applied by Nature in products as diverse as nut shells and hedgehog spines.

4. Conclusions

Differential scanning calorimetry shows that MAS retains bound water even after drying at ambient temperature in a desiccator. Attempts to correlate the maximum storage modulus (determined with a dynamic mechanical analyser) to silk spinning rate are inconclusive. Any correlation is masked by consequences of the non-constant cross-sectional area of the silk fibre collected from spiders. Fibre thickness affects the ability of samples to lose bound moisture and also the accuracy to which the measured values of load can be used to calculate stress and thence modulus.

The characterisation of thermal expansion coefficient with a dynamic mechanical analyser provides a sensitive way of detecting the glass transition (occurring at approximately 160°C) in small samples of MAS. Thermal expansion data are especially reproducible below the glass transition; they do not depend on accurate measurement of sample cross section, they are insensitive to the moisture content of samples and they are not affected significantly by energy loss arising from friction between individual fibres in a bundle.

Thermal expansion data, interpreted qualitatively in terms of the overall degree of molecular alignment within samples, suggest that the highest intrinsic stiffness of MAS is realised at spinning rates used in web construction. MAS spun at a factor-of-ten greater rate, used when the spider must elude a predator, is more compliant. The rate at which MAS is spun affects its microstructure and properties in a way that optimises its response in more than one application.

Acknowledgements

Support from the National Science Foundation (grant #BCS-9202007, jointly sponsored by the Division of Biological and Critical Systems and the Division of Materials Research), is acknowledged gratefully.

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