

Biomimetic spinning of spider silk

Problem presented by

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Problem statement

Kevlar fibres are made from liquid crystalline polymer (LCP). Since their creation there has been extensive research effort in the use of LCPs as structural and barrier materials. Spider silk is formed from a lyotropic LCP which undergoes structural transitions during spinning, resulting in a highly orientated structure. It is this structure that is thought to be essential for the exceptional tensile strength of spider silk. Biomimetic spinning of spider silk protein dope has yet to achieve the strength characteristics found in nature. Spinorx have designed and built a biomimetic rig into which protein dope is fed and from which spun fibre is drawn. They want to model the spinning process in the spider and compare it with the biomimetic rig.

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1 Problem specification

Kevlar fibres are made from liquid crystalline polymer (LCP). Since their creation there has been extensive research effort in the use of LCPs as structural and barrier materials. Spider silk is formed from a lyotropic LCP which undergoes structural transitions during spinning, resulting in a highly orientated structure. It is this structure that is thought to be essential for the exceptional tensile strength of spider silk. Biomimetic spinning of spider silk protein dope has yet to achieve the strength characteristics found in nature.

In a spider, the lyotropic LCP is secreted from the walls of the major ampullate gland and stored in an alkaline state. This feedstock passes down an S-shaped converging duct of length approximately 40 mm. For nearly the first half of the duct length the structure is a nematic discotic liquid crystal. Birefringence is not observed beyond the halfway point, indicating structural change on some scale. At 4 mm back from the end of the duct, there is a change in secondary structure from helix to sheet forms of crystal structure. This results in internal draw down and phase separation. The activation energy required to bring about this structural change is provided by the force pulling the thread from the spider.

The duct has a thin cuticle which acts as a dialysis membrane; it takes water and sodium ions out of the lumen and puts potassium, possibly surfactants, lubricants and promoters of phase separation into the lumen. Proton pumps secrete hydrogen ions to render the solution more acidic near the exit from the duct. The resulting gradients in pH, polymer concentration and potassium concentration are believed to contribute to the structural transitions that occur.

The pull rate of the thread from the duct is typically about 10 mm/sec. At the entrance of the feedstock from the gland, the diameter is approximately 100 microns and this reduces to approximately 20 microns at the location of the start of internal draw down. The final spun fibre diameter is approximately 4 microns.

Spinox have designed and built a biomimetic rig into which protein dope is fed and from which spun fibre is drawn. The biomimetic process at present typically suffers from severe die swell (there is no internal draw down) and thread obtained in this way is more brittle and less strong than spider silk.

Spinox want to model the spinning process in the spider and compare it with the biomimetic rig to help them remove die swell and achieve internal draw down.

1.1 Differences between spider and rig

There are various differences between the duct in a real spider and the duct in the biomimetic rig, some of which are listed in Table 1.

	Spider duct	Biomimetic rig duct
1	Circular cross-section	Semicircular cross-section
2	Chemical treatment on the whole boundary	Chemical treatment on one side only
3	Serocin secreted in the gland	No serocin present
4	Internal surface of the duct is tissue	Internal surface is epoxy/metal
5	Protein dope secreted into duct	Protein dope pushed by a piston
6	Convergent channel over entire length	Parallel-sided channel near exit
7	Duct diameter reduces to $20\ \mu\text{m}$ near exit	Minimum diameter is $50\ \mu\text{m}$

Table 1: Summary of differences between the spider duct and the biomimetic rig

2 Background: phase transition and rheology

The dope can be thought of as a nematic liquid crystal, and the important phase change is from a gel phase, where the protein is present as α -helices, to a crystalline phase where it is present as β -sheets, roughly as indicated in Figure 1. It is found experimentally that this change is accompanied by a stretch factor of about 2.3 in length.



Figure 1: Phase transition from α -helices to β -sheets

The dope is a shear-thinning fluid, and measurements indicate that $\sigma \propto \dot{\gamma}^m$ for m close to 0, *i.e.* approximately a constant stress material.¹ The flow of the material in a duct will therefore be almost a plug flow, with a region of high velocity gradient around the edge and approximately constant velocity within that.

¹Although this is the best information we have to go on, it should be borne in mind that it is based on measurements made in a rheometer designed for Newtonian fluids: the flow of a shear-thinning fluid in the rheometer will be different, and therefore the correct interpretation of the measurements will also be different.

A (nonequilibrium) thermodynamic model of the polymer solution would give a consistent link between the phase transition and the stress-strain behaviour. Although there was discussion of this area, it remains one of great uncertainty, and in fact such an approach was not developed at the Study Group. Instead we shall propose here effectively independent models of the phase transition and of the viscoelastic properties.

3 A mechanically driven phase transition

A typical spider weighs 2 grams, and is supported by a thread $10\ \mu\text{m}$ in radius. The resulting stress in the thread is thus

$$\frac{mg}{\pi r^2} \approx \frac{0.002\ \text{kg} \times 10\ \text{ms}^{-2}}{\pi 10^{-10}\ \text{m}^2} \approx 6 \times 10^7\ \text{Nm}^{-2}. \quad (1)$$

By contrast, the chemical energy necessary to break the hydrogen bonds (represented by the dotted lines on the α -helix in Figure 1) is $10\ \text{kJ/mol}$. There are 3×10^{27} bonds per cubic metre of dope. Thus the necessary stress is

$$\frac{3 \times 10^{27}\ \text{m}^3}{6 \times 10^{23}\ \text{mol}^{-1}} \times 10\ \text{kJ/mol} = 5 \times 10^7\ \text{Nm}^{-2}, \quad (2)$$

where 6×10^{23} is Avogadro's number, the number of particles in a mole. Since these two stresses are comparable, we assumed that the phase transition from dope to thread was mechanically driven. Both stresses may be converted into rates of working (or power densities) by multiplying them by a strain rate equal to the extrusion rate. For comparison, these stresses are equal in magnitude to pressures of 500 and 600 atmospheres (1 atmosphere is 10^5 Pascals or $10^5\ \text{Nm}^{-2}$), but unlike pressures they are directed almost entirely *along* the thread.

3.1 Simple kinetic model

In a simplified model, we consider steady flow along the duct, and assume that gelling occurs at some axial position $x = x_g$. At some point x_* downstream of x_g , the phase transition begins, with release of water, and we think of the phase transition as a 2-stage process, consisting of breaking of hydrogen bonds within the helices, followed by cross-linking of newly available hydrogen bonds to form the sheets. Defining

$$\psi = \text{fraction of polymer with broken hydrogen bonds}, \quad (3)$$

$$\varphi = \text{fraction of polymer with new cross-links formed}, \quad (4)$$

the fraction of polymer still in the helices is $1 - \psi - \varphi$. We shall consider ψ , φ and the other variables to be functions of x only, neglecting variations over the cross-section. Then the rates of change following a particular fluid element then are given by, for instance, $D\psi/Dt = u\partial\psi/\partial x$. We shall let ϵ_e denote the axial extensional strain, *i.e.* $\epsilon_e = \lambda - 1$

where λ is the ratio of the axial separation of two nearby material particles to their separation when they passed the position x_g in the duct at which gelling occurs. The rate at which the tension force does work on the material is proportional to the rate of strain $D\epsilon_e/Dt = u\partial\epsilon_e/\partial x$, so we model the rate of breaking of hydrogen bonds as being proportional to this, and the rate of formation of cross-links as being $k\psi^2$. This leads to the simple model

$$u\frac{\partial\psi}{\partial x} = \alpha(1 - \psi - \varphi)u\frac{\partial\epsilon_e}{\partial x} - k\psi^2, \quad (5)$$

$$u\frac{\partial\varphi}{\partial x} = k\psi^2. \quad (6)$$

In the limit of fast kinetics in the cross-linking stage, $k \rightarrow \infty$ and $\psi \rightarrow 0$, equations (5–6) simplify to

$$\frac{\partial\varphi}{\partial x} = \alpha(1 - \varphi)\frac{\partial\epsilon_e}{\partial x}, \quad (7)$$

which may be integrated to give

$$\varphi = 1 - \exp[-\alpha(\epsilon_e - \epsilon_\star)], \quad (8)$$

where ϵ_\star is the critical strain (relative to gelation) at which the phase transition begins. It is this simplified form that we shall use.

4 Rheology

4.1 Lubrication theory

Both in the spider and in the biomimetic rig, we have slow flow along a narrow duct, and so lubrication theory for a slowly varying channel is the appropriate flow model. In the spider the duct is of diameter $20\ \mu\text{m}$, and is gradually narrowing, whereas in the rig the duct is of constant diameter $50\ \mu\text{m}$. This narrowing of the spider duct is an important difference from the rig. In both spider and rig, the tension in the fibre can be resisted by viscous forces, but for a very shear-thinning material the viscous resistance to flow will be small, with effectively the stress taken in a narrow circumferential layer and the dope just sliding down the middle. However, in the spider the narrowing of the duct means that, even for inviscid flow, the pressure on the walls has a component in the axial direction, and so provides a further means to resist the tension in the fibre — a means that is not present in the rig where the duct diameter is constant.

4.2 Rôle of expelled water

Water is released by the phase transition, and treating the material in the duct as a porous medium it is found that the water can easily cross the distance (of order $20\ \mu\text{m}$)

to escape. We assume the spider cells on the duct wall easily absorb water, so there is a zero pressure boundary condition on the free surface. (The spider needs to reabsorb this water in order to avoid excessive water loss.)

Our picture of the nucleation process is that the fibre detaches from the wall as soon as phase transition first begins, roughly as in Figure 2. This detachment point x_* is also

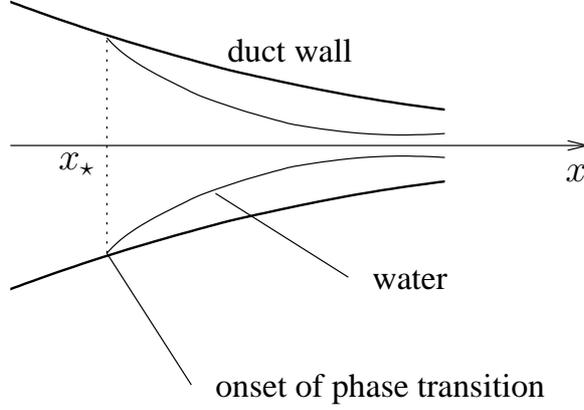


Figure 2: Schematic of the drawing of thread inside the spider duct.

the point at which free water is first released. The tension T is uniform along the fibre downstream of the detachment point since there is no viscous drag. The stress will vary inversely with area, but the area of the fibre is no longer prescribed by being equal to the area of the channel. Instead we calculate the amount of water expelled (and hence the shrinkage) from the fractional completion of phase transition. We find there must be a nasty singularity at the detachment point, owing to the change of boundary condition from no slip to free slip and zero pressure. At this point the flow changes from being almost a plug flow (due to the extreme shear thinning) to being a plug flow (due to the lubricated, zero stress, boundary).

4.3 Constitutive model

We now wish to construct a simple constitutive equation for the material, allowing for both viscosity and the nonlinear aspects of the behaviour. For the elastic aspect of the behaviour, a typical “finite extension nonlinear elasticity” (FENE) model is

$$\tau = \frac{E_0 \epsilon}{1 - \epsilon/\epsilon_{\max}}, \quad (\epsilon < \epsilon_{\max}) \quad (9)$$

possibly with different powers in the denominator. (See for instance [1].) The denominator is designed to make the stress τ tend to infinity as the strain ϵ approaches the limit ϵ_{\max} , thereby keeping the strain ϵ below ϵ_{\max} , hence the name “finite extension”.

To make a nonlinear model for the material analogous to a linear “spring and

dashpot” model it is convenient to rewrite the elastic part (9) as

$$\epsilon(\tau) = \frac{\tau}{E} = \frac{\tau}{E_0 + \tau/\epsilon_{\max}}. \quad (10)$$

Then when we include a viscous part we obtain the combined viscoelastic constitutive equation

$$u_x = \frac{\tau}{\mu} + \frac{u[(1-\varphi)\tau]_x}{E}, \quad (11)$$

where $\mu = \mu_0/(1-\varphi)$ is an extensional viscosity depending on φ , tending to infinity as the phase transition to sheets tends to completion ($\varphi \rightarrow 1$). The viscosity μ_0 of the dope may also depend on u_x . In this, $\tau = \sigma_{xx}$, the axial stress.

5 One-dimensional model

We now construct a one-dimensional model for the flow in the duct, based on these models for the phase transition kinetics and constitutive equation. Since the phase transition eliminates water, we define a function $f(\varphi)$ to be the ratio of the polymer volume (when a fraction φ has converted to sheets) to the initial volume of dope. We shall also let $A = A(x)$ denote the cross-sectional area of the polymer thread. Then if Q is the volumetric flow rate of dope, polymer mass conservation is represented by

$$Au = Qf(\varphi). \quad (12)$$

Upstream of the onset of the phase transition $\varphi = 0$ and $f(\varphi) = 1$, so this is simply conservation of volume.

If we write the axial force balance in terms of the stress tensor σ , for slow steady flow in a slowly-varying duct, and integrate over the duct cross-section, it takes the form

$$\frac{d}{dx} \left(\int_A \sigma_{xx} dA \right) = A_x(\sigma_{xx})_{\text{wall}} - 2\pi r(\sigma_{xr})_{\text{wall}}. \quad (13)$$

In this, we shall write $\sigma_{ij} = -p\delta_{ij} + \tau_{ij}$, so that τ_{ij} is the deviatoric part of the stress, and then we let

$$\bar{\tau} = \frac{1}{A} \int_A \tau_{xx} dA, \quad (14)$$

so $\bar{\tau}$ is the cross-sectionally averaged extensional stress, which we expect to be the quantity that occurs in the FENE model. The force balance equation for steady slow flow then takes the form

$$(A\bar{\tau})_x = Ap_x - 2\pi r(\tau_{xn})_{\text{wall}}, \quad (15)$$

where n denotes the normal direction to the channel wall, so $(\tau_{nx})_{\text{wall}}$ is the axial component of the traction at the wall.

In the region before the detachment point, $A = A_{\text{wall}}$, the given cross-sectional area of the duct. Beyond the detachment point, we assume perfect lubrication by the water

layer, which is assumed at zero pressure, so $p = 0$ and $(\tau_{xn})_{\text{wall}} = 0$, *i.e.* $A\bar{\tau} = T$, the constant applied tension. From now on we shall denote this $\bar{\tau}$ simply by τ .

The boundary conditions

$$A = A_{\text{wall}} \text{ for } x < x_*, \quad p = 0 \text{ for } x > x_*, \quad (16)$$

may be elegantly rewritten in complementarity form as

$$(A - A_{\text{wall}})p = 0. \quad (17)$$

5.1 Upstream of onset

The flow field is determined completely by the volume flux Q and the imposed shape $A_{\text{wall}}(x)$ of the channel:

$$u(x) = \frac{Q}{A_{\text{wall}}(x)}, \quad \text{for } x < x_*. \quad (18)$$

The constitutive equation (11) is then a first-order ODE for $\tau(x)$,

$$u_x = \frac{\tau}{\mu_0} + \frac{u\tau_x}{E_0 + \tau/\epsilon_{\text{max}}}, \quad (19)$$

on setting $\varphi = 0$. This rearranges into a Riccati equation for τ ,

$$\frac{d\tau}{dx} = - \left(E_0 + \frac{\tau}{\epsilon_{\text{max}}} \right) \left(\tau \frac{A_{\text{wall}}(x)}{Q\mu_0} + \frac{d \log A_{\text{wall}}(x)}{dx} \right), \quad (20)$$

which may be solved using the integrating factor

$$\frac{1}{(E_0\epsilon_{\text{max}} + \tau)^2} \left(\frac{A_{\text{wall}}(x)}{A_{\text{wall}}(x_*)} \right)^{-1/\epsilon_{\text{max}}} \exp \left(\frac{E_0}{Q\mu_0} \int_{x_*}^x A_{\text{wall}}(x') dx' \right). \quad (21)$$

(A Riccati equation can always be reduced to two first-order linear equations, but not always to a complete solution by an integrating factor like this. The reason an explicit integrating factor occurs in this case is that the Riccati equation (20) has the obvious constant solution $\tau = -E_0\epsilon_{\text{max}}$, and the method of [2, Art.167] reduces the solution to quadratures when one particular solution is known.)

Note that in the purely elastic case, $\mu_0 \rightarrow \infty$, the solution simplifies to

$$\tau(x) = C[A_{\text{wall}}(x)]^{-1/\epsilon_{\text{max}}} - E_0\epsilon_{\text{max}}. \quad (22)$$

We then cannot have $\tau \rightarrow 0$ far upstream unless $A_{\text{wall}}(x)$ tends to a constant. In practice we would expect $A_{\text{wall}}(x) \rightarrow \infty$ far upstream.

Note finally that the wall pressure p may be determined from the known stress field $\tau(x)$ using the momentum equation.

5.2 Downstream of onset

The fractional conversion of dope to thread is given by equation (8),

$$\varphi = 1 - \exp[-\alpha(\epsilon_e - \epsilon_*)]. \quad (23)$$

Mass conservation for the polymer gives

$$u = \frac{Q}{A}f(\varphi), \quad (24)$$

and momentum conservation gives

$$u = Qf(\varphi)\frac{\tau}{T}, \quad (25)$$

which may be combined with the constitutive law (11) to give an ODE for $\tau(x)$.

5.3 Singularity at onset

Preliminary attempts to solve the ODEs in the two regions suggest that dA/dx is singular at the detachment point, with the fibre trying to come out perpendicular to the wall. This suggests that a one-dimensional lubrication model is inadequate near the detachment point.

6 Gelling of the dope

We have obviously taken a somewhat naive point of view. If the yield stress of the dope is less than the critical stress for the phase transition there is a serious difficulty in how the process ever starts. An adult spider always retains a portion of fibre under tension within itself by clamping the exit point. How young spiders spin their first thread is an open question. (There is an analogy with drawing polymers by pulling — the first bit soon breaks off, but can be used to pull another bit, and so on.)

An area that was not looked at is the difference between the spider duct, where the chemistry is controlled all the way round the circumference, and the rig, where the dope is pushed by a piston, and there is diffusion of chemical agents through a semipermeable membrane only on the flat side of the semi-cylindrical duct. However, a quick calculation indicated that the ion diffusion rate would be enough to reach the whole cross-section evenly from the one-sided treatment. This is why we consider the gelling to take place at a particular point x_g in the duct, rather than occurring gradually over an interval.

7 Conclusion

Discussions during the week raised several modelling questions, notably about the rheology of the dope and gel phases, the kinetics of the phase transition, and the reabsorption of water inside the spider. In this respect the details of the specific model that emerged over the week are perhaps not crucial, but some general points can be made:

- Spiders work in a certain regime of parameters, notably channel shape, applied tension, and wall permeability, such that the detachment point is inside the spider.

The main conclusion is that the spider's converging channel is probably crucial, both to provide a form drag to restrain the dope, and to allow internal adjustment to an imposed tension or extrusion rate by moving the location, and hence the cross-section, of the detachment point. In a rig without a correctly designed converging channel, the flow can too easily become a plug flow, with the dope just sliding down the middle, lubricated by wall layers in this extremely shear-thinning material, and the phase transition not properly completed.

- Spinox need to make their machine work in the same regime.
- We could identify this regime more precisely, and in particular investigate the water layer close to the detachment point to refine the very crude zero-pressure assumption above, but this would require further resources.

References

- [1] Dynamics of Polymeric Liquids: Volume 2, Kinetic Theory. R. B. Bird, C. F. Curtiss, R. B. Armstrong, and O. Hassager. Wiley, second edition, 1987.
- [2] An Elementary Treatise on Differential Equations. H. T. H. Piaggio. Bell, London, 1937.