Viscoelasticity of biomolecular condensates conforms to the Jeffreys model

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ABSTRACT

Biomolecular condensates, largely by virtue of their material properties, are revolutionizing biology, and yet, the physical understanding of these properties is lagging. Here, I show that the viscoelasticity of condensates can be captured by a simple model, comprising a component where shear relaxation is an exponential function (with time constant τ_1) and a component with nearly instantaneous shear relaxation (time constant $\tau_0 \rightarrow 0$). Modulation of intermolecular interactions, e.g., by adding salt, can disparately affect the two components such that the τ_1 component may dominate at low salt, whereas the τ_0 component may dominate at high salt. Condensates have a tendency to fuse, with the dynamics accelerated by interfacial tension and impeded by viscosity. For fast-fusion condensates, shear relaxation on the τ_1 timescale may become rate-limiting such that the fusion speed is no longer in direction proportion to the interfacial tension. These insights help narrow the gap in understanding between the biology and physics of biomolecular condensates.

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Biomolecular condensates formed by phase separation are now recognized for mediating numerous cellular processes such as ribosome biogenesis¹ and implicated in neurodegeneration and cancer.² Condensates often appear as micro-sized droplets under a microscope. Much attention has been paid to the factors that determine the phase equilibrium.^{3–9} The material properties, in particular, interfacial tension and viscoelasticity, of condensates dictate their spatial organization,^{1,8} shape recovery,^{10,11} and fusion^{1,10,12,13}. The first set of viscoelasticity data has recently been reported for PGL-3 protein droplets.^{14,15} The corrected analysis of those data has been published.¹⁶ Here, I show that the Jeffreys model of linear viscoelasticity can be used to fit the data of PGL-3 and other biomolecular droplets. This finding makes it possible to not only resolve practical problems in the extraction of viscoelastic properties from experimental data but also gain deeper physical understanding of biomolecular condensates.

Viscosity contributes to stress, i.e., force per unit area, between different layers of a fluid. For a Newtonian fluid, this stress, $\tilde{\tau}_d$, is proportional to the symmetrized strain-rate tensor $\tilde{\epsilon}$,

$$\tilde{\tau}_d = \eta \tilde{\epsilon}, \quad (1)$$

where η is the viscosity and

$$\tilde{\epsilon} = \nabla \mathbf{v} + (\nabla \mathbf{v})^T. \quad (2)$$

Here, $\mathbf{v}$ is the velocity field in the fluid, ∇ denotes the gradient operator, and the subscript T denotes transpose. The hydrostatic pressure, p , also contributes to the stress, and hence, the total stress tensor is

$$\tilde{\tau} = -p\tilde{I} + \tilde{\tau}_d, \quad (3)$$

where $\tilde{I}$ is the unit tensor. Instead of responding instantaneously to the strain rate as in a Newtonian fluid, the stress in biomolecular condensates and other viscoelastic materials depends on the entire history of the strain rate. Limiting to small strain rates so that the relation between $\tilde{\tau}_d$ and $\tilde{\epsilon}$ is still linear, Eq. (1) is generalized to

$$\tilde{\tau}_d = \int_{-\infty}^t dt' G(t-t') \tilde{\epsilon}(t'). \quad (4a)$$

The function $G(t)$ introduced above is the shear relaxation modulus. Note that $\tilde{\epsilon}(t')$ at $t' > t$ cannot have any effect on $\tilde{\tau}_d(t)$. Hence, we must stipulate that

$$G(t) = 0 \text{ for } t < 0. \quad (4b)$$

Correspondingly, we can change the upper limit of the integral in Eq. (4a) to $+\infty$,

$$\tilde{\tau}_d = \int_{-\infty}^{+\infty} dt' G(t-t') \tilde{\varepsilon}(t'). \quad (4c)$$

The constitutive relation [Eq. (1)] for a Newtonian fluid corresponds to a delta function for the shear relaxation modulus,

$$G(t) = \eta \delta(t - 0^+), \quad (5)$$

where 0^+ indicates a peak position that is beyond $t = 0$ by an arbitrarily small positive amount.

Analogous to Eq. (2) for the strain-rate tensor, we can define the shear-strain tensor

$$\tilde{\sigma} = \nabla \mathbf{u} + (\nabla \mathbf{u})^T \quad (6)$$

in terms of the displacement field $\mathbf{u}$. It is obvious that

$$\tilde{\varepsilon} = \frac{\partial}{\partial t} \tilde{\sigma}. \quad (7)$$

Now, consider a shear strain with a sinusoidal dependence on time,

$$\tilde{\sigma} = \tilde{\sigma}_0 e^{i\omega t}. \quad (8a)$$

The corresponding strain-rate tensor is

$$\tilde{\varepsilon} = i\omega \tilde{\sigma}_0 e^{i\omega t}. \quad (8b)$$

Substituting Eq. (8b) into Eq. (4c), we find

$$\tilde{\tau}_d = i\omega \tilde{\sigma}_0 e^{i\omega t} \int_{-\infty}^{+\infty} dt' G(t') e^{-i\omega t'} \equiv G^*(\omega) \tilde{\sigma}. \quad (9)$$

The last identity defines the complex shear modulus $G^*(\omega)$, which is related to the Fourier transform of the shear relaxation modulus,

$$G^*(\omega) = i\omega \int_{-\infty}^{+\infty} dt' G(t') e^{-i\omega t'}. \quad (10)$$

We denote the real and imaginary parts as $G'(\omega)$ and $G''(\omega)$, i.e.,

$$G^*(\omega) = G'(\omega) + iG''(\omega). \quad (11)$$

$G'(\omega)$ is called the elastic (or storage) modulus, whereas $G''(\omega)$ is the viscous (or loss) modulus. A Newtonian fluid [with $G(t)$ given by Eq. (5)] has

$$G^*(\omega) = i\omega\eta \quad (12)$$

with a purely viscous modulus that is proportional to the angular frequency.

In the so-called Maxwell model,¹⁷ shear relaxation is an exponential function of time, with a time constant τ ,

$$G(t) = \frac{\eta}{\tau} e^{-t/\tau} \text{ for } t > 0. \quad (13)$$

The corresponding complex shear modulus is

$$G^*(\omega) = \frac{i\omega\eta}{1 + i\omega\tau}. \quad (14a)$$

In the limit $\tau \rightarrow 0$, the Maxwell model reduces to a Newtonian fluid, consistent with the latter's instantaneous shear relaxation. The elastic and viscous moduli of the Maxwell model are

$$G'(\omega) = \frac{\omega^2\tau\eta}{1 + \omega^2\tau^2}, \quad (14b)$$

$$G''(\omega) = \frac{\omega\eta}{1 + \omega^2\tau^2}. \quad (14c)$$

In this case, $G'(\omega)$ is a monotonically increasing function of ω ; in contrast, $G''(\omega)$ reaches its maximum at $\omega\tau = 1$. $G''(\omega)$ is larger than $G'(\omega)$ at $\omega\tau < 1$, whereas the opposite is true at $\omega\tau > 1$. Thus, the Maxwell model behaves as a viscous liquid at low frequencies (or for slow motions) but as an elastic solid at high frequencies (or for fast motions). No real fluid can be truly Newtonian, where the viscous modulus increases indefinitely with the increasing frequency. Although glycerol and water are often treated as Newtonian, their shear relaxation moduli are actually qualitatively Maxwellian at very high frequencies (GHz to THz), with shear relaxation times on the ns and ps timescales, respectively.^{18,19} In general, fluids appear Newtonian when the dynamics involved is slower than shear relaxation.

A linear combination of a Newtonian fluid and the Maxwell model,

$$G^*(\omega) = i\omega\eta_0 + \frac{i\omega\eta_1}{1 + i\omega\tau_1}, \quad (15)$$

is the Jeffreys model, which can also be seen as the linearized version of the Oldroyd B model. As the preceding paragraph suggests, the Newtonian component should really be viewed as an approximation of a Maxwellian component with a very short relaxation time. The resulting sum of two Maxwellian components,

$$G^*(\omega) = \frac{i\omega\eta_0}{1 + i\omega\tau_0} + \frac{i\omega\eta_1}{1 + i\omega\tau_1}, \quad (16)$$

with $\tau_0 \ll \tau_1$, is the Burgers model. Dilute polymer solutions conform to this model, where the two terms represent the contributions of the solvent and the polymer solute, respectively. A main aim of this paper is to demonstrate that the Jeffreys model or its extension to the Burgers model can be used to fit the viscoelasticity data of dense biomolecular condensates.

The first set of such viscoelasticity data was reported by Jawerth *et al.*,¹⁴ who used optical tweezers to deform PGL-3 droplets over a range of frequencies and determined the effective spring constant $\chi(\omega)$ of the droplets (Fig. 1). The experiment involves optically trapping two beads (with radius a) at the opposite poles of an otherwise spherical droplet (with radius R). To analyze their data, Jawerth *et al.* modeled the droplet deformation dynamics by the Navier–Stokes equations, thereby expressing $\chi(\omega)$ in terms of $G^*(\omega)$ and the interfacial tension, γ , of the droplet. The expression contains an infinite sum that Jawerth *et al.* truncated at the fifth term because the expressions for the terms grow rapidly in complexity. I have obtained a compact expression for $\chi(\omega)$ that allows for the summation to be evaluated to any order,¹⁶

$$\chi(\omega) = \frac{\pi R(1 - \cos^2\theta_0)/2}{\sum_{l \geq 2, l \text{ even}} \frac{P_{l-1}(\cos\theta_0) - P_{l+1}(\cos\theta_0)}{2G^*(\omega) \frac{(l-1)(2l^2+4l+3)}{l(2l+1)} + \frac{\gamma}{R} (l-1)(l+2)}}, \quad (17)$$

FIG. 1. Experimental setup for determining the effective spring constant $\chi(\omega)$ of biomolecular droplets. (a) Side view: two optically trapped polystyrene beads pull at the poles of a droplet. Trap 2 is fixed at the origin, while the position of trap 1 undergoes harmonic oscillation. (b) Top view: ellipse (circle) with solid (dashed) boundary shows the deformed (unperturbed) droplet. F_{t1} and F_{t2} are the forces exerted by the traps on the beads; X_{t1} is the position of trap 1; X_1 and X_2 are the positions of the beads. (c) The deformation of the droplet is equivalent to the oscillation of a spring. (d) The effective spring constant.

where $\theta_0 = a/R$ and is small, at 0.1 or less, and $P_l(x)$ are Legendre polynomials.²⁰ This summation turns out to converge very slowly, requiring l up to a range of 40 000–100 000. Truncation at the fifth term (i.e., $l = 10$) thus incurs severe numerical errors and leads to

qualitatively incorrect asymptotic formulas at $\theta_0 \rightarrow 0$. In addition to the full expression for $\chi(\omega)$, Ref. 16 also presented a numerical procedure for the complete extraction of $G^*(\omega)$ from $\chi(\omega)$ data, which is essentially an inversion of Eq. (17). The extraction procedure was illustrated using simulated $\chi(\omega)$ data, generated by using a Jeffreys model for $G^*(\omega)$ in Eq. (17).

However, with actual experimental $\chi(\omega)$ data, the extraction of $G^*(\omega)$ faces serious problems. The first problem is that, without an independently determined value for the interfacial tension, $\chi(\omega)$ data alone are insufficient to extract both γ and $G^*(\omega)$. Both $\chi(\omega)$ and $G^*(\omega)$ have real and imaginary parts. In essence, one is trying to determine three quantities [γ , $G'(\omega)$, and $G''(\omega)$] from two knowns [$\chi'(\omega)$ and $\chi''(\omega)$, denoting the real and imaginary parts of $\chi(\omega)$]. Jawerth *et al.* used the average of $\chi'(\omega)$ at the lowest three frequencies studied ($\omega/2\pi$ at 0.04 Hz, 0.1 Hz, and 0.2 Hz) to set γ , assuming a negligible contribution from $G^*(\omega)$ at such low frequencies. Following this recipe and using Eq. (17), I obtain $\gamma = 6.2 \mu\text{N/m}$ for a set of $\chi(\omega)$ data reported by Jawerth *et al.* at 75 mM KCl [presented in their Fig. 3(a)]. However, γ cannot be uniquely determined. For a given γ , a $G^*(\omega)$ can be found together with γ that reproduces the $\chi(\omega)$ data according to Eq. (17). Figure 2(a) presents $G^*(\omega)$ results corresponding to four different γ values: 6.2 $\mu\text{N/m}$, 4.91 $\mu\text{N/m}$, 4.0 $\mu\text{N/m}$, and 0 $\mu\text{N/m}$; all four γ - $G^*(\omega)$ combinations, including the extreme case with 0 interfacial tension, reproduce the same set of $\chi(\omega)$ data, which is reproduced in Fig. 2(b) as circles.

The second problem in the extraction of $G^*(\omega)$ is that some of the extracted $G'(\omega)$ values at low frequencies are negative [see the inset of Fig. 2(a)] and hence unphysical. For example, with $\gamma = 6.2 \mu\text{N/m}$, $G'(\omega)$ is -1.8 Pa at 1 Hz. The negative $G'(\omega)$ values might be taken as an indication that the choice of γ is too high, as $G'(\omega)$ is no longer negative when γ is reduced to 4.0 $\mu\text{N/m}$. However, the deeper reason is that $G'(\omega)$ at low frequencies is particularly sensitive to errors in the $\chi(\omega)$ data, as illustrated by the large differences of the low-frequency $G'(\omega)$ values extracted with the three non-zero γ choices. As $G'(\omega)$ is expected to approach 0 rapidly at low frequencies, errors in $\chi(\omega)$ may inevitably lead to negative values for the extracted $G'(\omega)$.

FIG. 2. Problems with extraction of γ and $G^*(\omega)$ and a solution. (a) Four sets of $G^*(\omega)$, corresponding to four choices of γ , all reproduce the same set of $\chi(\omega)$ data. Extraction of $G^*(\omega)$ at a given γ is according to Ref. 16, with $l = 40\,000$. Solid and dashed curves display the real and imaginary parts of $G^*(\omega)$, respectively; the legend displays the γ values. Pieces of $G'(\omega)$ missing on the ordinate log scale (yellow shading) correspond to negative values, which are shown on a linear scale in the inset. Filled and open circles display the Jeffreys model for $G'(\omega)$ and $G''(\omega)$, respectively. (b) The $\chi(\omega)$ data of Jawerth *et al.* at 75 mM KCl, shown as filled and open circles, are accurately reproduced by the Jeffreys model (curves).

To solve both of these problems, I propose choosing an appropriate model for $G^*(\omega)$ and using the corresponding $\chi(\omega)$ to fit experimental data. Reference 16 already gave an indication that the shape of $\chi(\omega)$ produced by the Jeffreys model looks similar to the experimental data of Jawerth *et al.* [circles in Fig. 2(b)]. By scanning over the values of four parameters, γ , η_0 , η_1 , and τ_1 , the $\chi(\omega)$ data of Jawerth *et al.* can be accurately reproduced [curves in Fig. 2(b)], with $\gamma = 4.91 \mu\text{N/m}$, $\eta_0 = 0.659 \text{ Pa s}$, $\eta_1 = 1.42 \text{ Pa s}$, and $\tau_1 = 6.03 \text{ ms}$. This solution is unique as all other solutions lead to larger deviations from the experimental data for $\chi(\omega)$. Positivity of $G'(\omega)$ is guaranteed in the Jeffreys model. The $G^*(\omega)$ with the above parameter values is displayed as circles in Fig. 2(a). It is closest to the $G^*(\omega)$ [red curves in Fig. 2(a)] extracted from the $\chi(\omega)$ data by the numerical procedure when γ is set to the optimal fitting value, i.e., $4.91 \mu\text{N/m}$ but avoids the negative values of the extracted $G'(\omega)$. Note that the inverse of $\tau_1 = 6.03 \text{ ms}$ corresponds to a frequency of 26 Hz ; shear relaxation at a τ_0 much shorter than this τ_1 would not have a significant effect even at the highest frequency (40 Hz) studied. This explains why the Jeffreys model, not the full Burgers model, is needed for fitting the viscoelasticity data of PGL-3 droplets. Also importantly, η_0 and η_1 are both three orders of magnitude higher than the viscosity of water, and hence, both components of the shear relaxation modulus are dictated by the interactions and dynamics of PGL-3 molecules inside droplets. Our preliminary experimental data for the viscoelasticity of a variety of biomolecular droplets fit the Jeffreys or Burgers model.²¹

Applying the same fitting procedure to the $\chi(\omega)$ data of Jawerth *et al.* for PGL-3 droplets at other KCl concentrations, I obtain the salt dependences of the interfacial tension and the parameters, η_0 , η_1 , and τ_1 , of the Jeffreys model. Figure 3(a) displays the γ values at four KCl concentrations: 75 mM , 115 mM , 150 mM , and 180 mM . γ

spans the range of $1 \mu\text{N/m}$ – $5 \mu\text{N/m}$ and decreases with increasing salt concentration, as expected from the salt screening of electrostatic attraction between PGL-3 molecules inside droplets. Similar to the effect of raising temperature, as the salt concentration increases, the droplet and bulk phases become more and more similar, and hence, the interfacial tension becomes smaller and smaller. At the critical salt concentration, there is no more difference between the two phases, i.e., the protein no longer phase separates, and correspondingly, the interfacial tension must be 0. The salt dependence can be represented by a scaling law $\gamma = A([\text{KCl}]_c - [\text{KCl}])^\alpha$,^{22,23} with $\alpha = 3/2$ as the critical exponent and $[\text{KCl}]_c = 241 \text{ mM}$ as the critical salt concentration.

Over the same range of salt concentrations, η_0 decreases from 0.66 Pa s to 0.10 Pa s , and the salt dependence can be fit to the same scaling law [solid circles and solid curve in Fig. 3(b)]. Meanwhile, η_1 decreases from 1.42 Pa s to 0.057 Pa s and thus shows a much stronger salt dependence, requiring a rise of the critical exponent to 3.5 [open circles and dashed curve in Fig. 3(b)]. As a result, the viscosity of PGL-3 droplets switches from being dominated by η_1 at low salt to being dominated by η_0 at high salt. While viscosity is expected to decrease with increasing salt concentration due to weakened electrostatic attraction, it is not clear why the η_0 and η_1 components show different salt sensitivities. In contrast to γ , there is no theoretical justification for applying a scaling law to the salt dependences of η_0 and η_1 . Indeed, the effects of salt on the viscosities of dense polyelectrolyte coacervates or protein condensates are very complex, and no simple theory is available. The fits, although purely empirical, do highlight the disparate salt dependences of η_0 and η_1 . The shear relaxation time τ_1 decreases from 6.03 ms to 3.0 ms , and the salt dependence also appears to follow the scaling law with the regular critical exponent $3/2$ [Fig. 3(c)].

FIG. 3. Salt dependences of interfacial tension and the three parameters in the Jeffreys model of viscoelasticity. Circles display data obtained from the fitting procedure to the $\chi(\omega)$ data of Jawerth *et al.* at four KCl concentrations; curves are fits to a scaling law, $\gamma = A([\text{KCl}]_c - [\text{KCl}])^\alpha$; $[\text{KCl}]_c = 241 \text{ mM}$ and α is set to $3/2$, except for η_1 . (a) γ . (b) η_0 (filled circles and solid curve) and η_1 (open circles and dashed curve). For η_1 , the critical exponent is increased to 3.5 . (c) τ_R . (d) $(\eta_0 + \eta_1)/\gamma$.

The dynamics of the shape change of biomolecular droplets depends on their interfacial tension and viscosity, with the former driving the relaxation toward a spherical shape while the latter impeding the process. When droplets are modeled as Newtonian fluids, the combination, $\eta R/\gamma$, determines the timescale of the shape recovery or fusion dynamics. In particular, the fusion time for two droplets with an equal radius R is $\tau_f = 1.97\eta R/\gamma$.¹³ When the Jeffreys and Burgers models are applied, we expect $(\eta_0 + \eta_1)R/\gamma$ to play a similar role. For PGL-3 droplets, the inverse speed, $(\eta_0 + \eta_1)/\gamma$, decreases from 423 ms/ μm at 75 mM KCl to 147 ms/ μm at 180 mM KCl, and the salt dependence also fits the scaling law with the regular critical exponent 3/2 [Fig. 3(d)]. At $R = 5\ \mu\text{m}$, the predicted fusion times decrease from 4170 ms at 75 mM KCl to 1450 ms at 180 mM KCl. Three comments are in order. First, the predicted fusion times can be directly tested by experiments. Second, these fusion times are three orders of magnitude longer than the corresponding shear relaxation times. Therefore, during the fusion of PGL-3 droplets, shear relaxation inside the droplets is effectively instantaneous and the droplets can be treated as a Newtonian fluid, justifying such treatment in previous studies¹³ and the present use of $(\eta_0 + \eta_1)R/\gamma$ as the time scale of droplet shape dynamics when invoking the Jeffreys or Burgers model. Third, the predicted threefold speedup in PGL-3 droplet fusion dynamics is due to the different rates at which the interfacial tension and viscosity decrease with increasing KCl concentration. The decrease in viscosity occurs at a higher rate, and as noted above [Fig. 3(b)], this is especially true for the η_1 component. A fourfold speedup in fusion dynamics was actually observed for droplets formed by the binary mixture of a pentameric construct of proline-rich motifs and a polymer, heparin, upon increasing the KCl concentration from 150 mM to 400 mM.¹³

The fusion times of different biomolecular droplets span several orders of magnitudes.^{12,13} For example, droplets formed by the mixture of polylysine and heparin fuse within a few ms, whereas the fusion of droplets formed by the mixture of lysozyme and a pentameric construct of SH3 domains takes as long as 10 000 ms. For the slow-fusion droplets, similar to the PGL-3 droplets, shear relaxation may be too fast to manifest itself. However, for the fast-fusion droplets, shear relaxation on the τ_1 timescale may be slow enough to affect the fusion speed, which then would no longer be in direct proportion to the interfacial tension. Our preliminary experimental data suggest that polylysine:heparin droplets may present such a case.²¹ In a recent study on droplet shape recovery dynamics in polyelectrolyte coacervates, Ali and Prabhu¹¹ showed an early stage where shear relaxation appears to play an important role. In the extreme case where $\tau_1 \gg \eta R/\gamma$, an interesting prediction is that droplet shape recovery or fusion may contain a late stage where the time constant is dominated by shear relaxation and hence is independent of the interfacial tension.

The biology of biomolecular condensates as a result of recent intense studies is far outstripping the physical understanding of the underlying material properties. The conformality to the Jeffreys and Burgers models of viscoelasticity will facilitate both the experimental determination of viscoelastic properties and the comparison of these properties under different solvent conditions and among different condensates. The present work helps with mechanistic interpretation regarding the dynamics of biomolecular condensates but also raises even deeper questions on the parameters of the

Jeffreys or Burgers model. In particular, what are the molecular motions that dominate shear relaxation, which can potentially rate-limit droplet shape recovery or fusion? Progress in these directions will narrow the gap in understanding between the biology and physics of biomolecular condensates.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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- 17 Some readers may be familiar with electrical-circuit representations for the models of linear viscoelasticity. For example, a Newtonian fluid is represented by a purely viscous damper (or dashpot, equivalent to a resistor), whereas an elastic solid is represented by a spring (equivalent to a capacitor). A Maxwell model is equivalent to a dashpot and a spring connected in series. A Jeffreys model is a dashpot connected in parallel to a Maxwell model, whereas a Burgers model is two Maxwell models connected in parallel.
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²⁰Note that Eq. (17) differs from the formula presented in Ref. 16 by a factor of 2, using the definition of the effective spring constant in Fig. 1.

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