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Dynamics of Polymer Solutions. 2. The Determination of Molecular Weight Distribution by Viscoelasticity

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ABSTRACT: Viscoelastic measurements on dilute solutions of large polymer molecules are useful for determining the molecular weight of the largest molecules in a solution. Here we show that it is also possible to get information about the distribution of molecular weights. This can be done either with strain-relaxation data, with stress-relaxation data, or both. We have described such instruments in the preceding paper and elsewhere. Such information is particularly useful for the study of very large DNA molecules, for which other physical techniques are not available.

When chromosome-sized DNA molecules are extracted from cells, there may be several species of different molecular weights present in the solution. This may be due to the natural distribution of DNA sizes in the cells, or it may be due to degradation. A change in the molecular weight distribution could result from natural biological processes, from extraction procedures or shear degradation, or from externally induced damage from sources such as radiation or harmful reagents. For these very large DNA molecules, it has only recently been possible to measure the molecular weight of the largest such molecules in solution.[1-3] This has been done by the measurement of viscosity and creep-recovery dynamics.[4] In the preceding paper, hereinafter referred to as paper 1,[5] we describe a modified instrument which can additionally measure stress-relaxation dynamics. Depending on which experiments or combination of experiments are performed on a DNA solution, we measure several parameters such as relaxation times and exponential amplitudes. Here we show how those parameters can be combined to give not only the molecular weight of the DNA, but also a few of the higher moments of the molecular weight distribution. We present the equations for strain relaxations alone, for stress relaxations alone, and for both together. The most detailed information, of course, should result from the combination.

Table I
Eigenvalues[a]

k	λ_k' ($h = \infty$)	λ_k^* ($h = 1.812$)
1	5.0076	2.3224
2	16.258	7.9142
3	31.343	15.897
4	49.719	26.072
5	70.832	38.239
6	94.599	52.366
7	120.56	68.287
S_1	2.211	2.041
S_2	1.143	1.126
S_3	1.035	1.035

[a] Note the λ_k^* are the eigenvalues for T2 DNA with partial draining. The λ_k' are eigenvalues for nondraining DNA, molecules much larger than T2 DNA.

Creep Recoveries (Strain Relaxations)

In a creep-recovery experiment, we apply a shear stress to the solution by applying torque to a rotor therein. The torque is then removed so that the rotor is allowed to recoil freely. In this way the elastic component of the strain is recovered. We follow the rotor angle, $\theta(t)$, which is a measure of the strain-relaxation dynamics, and is governed by[4,5]

$$\theta(t) = \frac{\omega\eta_{\mathrm{sp}}\nu}{S_1\langle\tau_1\rangle\eta_{\mathrm{rel}}} \sum_{i=1}^{P} f_i\tau^2{}_{i1} \sum_{k=1}^{N} \left(\frac{\lambda_1'}{\lambda_k'}\right)^2 e^{-\lambda_k' t/\lambda_1'\tau_{i1}}(1 - e^{-\lambda_k' t_{\mathrm{w}}/\lambda_1'\tau_{i1}}) \tag{1}$$

where the notation here and throughout is the same as that in paper 1. Note that prior to relaxation, the time during which this shear stress is applied is called the "windup", t_{w}. If the windup is long ($t_{\mathrm{w}} \to \infty$), it is called "steady state"; if it is short ($t_{\mathrm{w}} \to 0$), it is called a "twitch";

$$\Gamma = \theta(0) = \frac{\omega\eta_{\mathrm{sp}}\nu}{\eta_{\mathrm{rel}}S_1\langle\tau_1\rangle} \sum_i f_i\tau_{i1}{}^2 \sum_k \left(\frac{\lambda_1'}{\lambda_k'}\right)^2 [1 - e^{-\lambda_k' t_{\mathrm{w}}/\lambda_1'\tau_{i1}}] \tag{2}$$

which simplifies to

$$\Gamma = \frac{\omega\eta_{\mathrm{sp}}\langle\tau_1{}^2\rangle S_2}{\eta_{\mathrm{rel}}\langle\tau_1\rangle S_1} \tag{3}$$

for steady-state windups. S_1 and S_2 are constants given in Table I. Under twitch conditions, eq 2 simplifies to

$$\Gamma = (\Delta\theta)\eta_{\mathrm{sp}}/\eta_{\mathrm{rel}} \tag{4}$$

where $\Delta\theta = \omega t_{\mathrm{w}}$. Therefore by rearranging eq 4, we can get η_{sp} directly from a twitch experiment:

$$\eta_{\mathrm{sp}} = \frac{\Gamma/\Delta\theta}{1 - \Gamma/\Delta\theta} \tag{5}$$

There are other parameters we can get easily from retardation curves. Because of the $\tau_{ik}{}^2$ weighting of the exponentials in eq 1, $\theta(t)$ will be predominantly comprised of the exponential of longest time constant, τ_{11}. Therefore a semilog plot of the curve yields a straight line at long times, $t > \tau$.[2,3] The slope of that line gives $-1/\tau_{11}$, and the

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intercept (extrapolated back to the $t = 0$ axis) will be

$$\Gamma_{11} = \frac{\omega\eta_{\mathrm{sp}}\nu f_1 \tau_{11}^2(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\eta_{\mathrm{rel}} S_1 \langle\tau_1\rangle} \tag{6}$$

from which we can form the useful dimensionless ratio

$$\frac{\Gamma_{11}}{\Gamma} = \frac{f_1\tau_{11}^2(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\sum\limits_{i,k} f_i\tau_{i1}^2\left(\dfrac{\lambda_1'}{\lambda_k'}\right)^2(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'})} \tag{7}$$

We can get the first moment equation for the C_i and M_i by using eq 7 and the empirical relation of Crothers and Zimm:[6]

$$[\eta]_i = KM_i^{\alpha} \tag{8}$$

where $\alpha = 0.665$ for large DNA molecules in 0.2 M Na^+ solutions. From the bead-spring theory,[7] we know that

$$\tau_{ik} = \frac{M_i\eta_0[\eta]_i\lambda_1'}{RTS_1\lambda_k'} \tag{9}$$

In the absence of more refined theory, it is known empirically that this expression accounts for excluded volume and ionic effects.[6] By substituting eq 8 into 9, we get

$$\tau_{i1}/\tau_{11} = \left(\frac{M_i}{M_1}\right)^{1+\alpha} \tag{10}$$

With this and the definition of the number fraction of molecules, f_i,

$$f_i = \frac{C_i/M_i}{\sum\limits_{j=1}^{P} C_j/M_j} = \frac{N_{\mathrm{a}}}{L}\left(\frac{C_i}{M_i}\right) \tag{11}$$

where L is the number of molecules per unit volume, eq 7 becomes

$$\sum_{i,k}\frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{1+2\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)^2(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \frac{\Gamma}{\Gamma_{11}}(1 - e^{-t_{\mathrm{w}}/\tau_{11}}) \tag{12}$$

which simplifies to the steady-state result

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{1+2\alpha} = \frac{\nu\Gamma}{S_2\Gamma_{11}} \tag{13}$$

and for twitches

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{\nu\Gamma}{S_1\Gamma_{11}} \tag{14}$$

Since it is impossible in practice to obtain infinitely short twitches, it may be better to retain the full generality of eq 12 than to use eq 14. In that case, the sum over k need not include more than a few eigenvalues, however, since it converges rapidly.

Using the same procedure as before, but now with $\Gamma_{11}\eta_{\mathrm{rel}}/\tau_{11}\eta_{\mathrm{sp}}$ rather than Γ_{11}/Γ, we get a differently weighted moment

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{\omega\eta_{\mathrm{sp}}\nu\tau_{11}(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{S_1\Gamma_{11}\eta_{\mathrm{rel}}} \tag{15}$$

which reduces to

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{\omega\eta_{\mathrm{sp}}\nu\tau_{11}}{S_1\Gamma_{11}\eta_{\mathrm{rel}}} \tag{16}$$

for steady states but which reduces again to eq 14 for twitches.

We can make use of the area under the curve to get a third independent equation for steady states or a second independent one for twitches. The area, A_θ, is

$$A_\theta = \int_0^\infty \theta(t)\,\mathrm{d}t = \frac{\omega\eta_{\mathrm{sp}}\nu}{\eta_{\mathrm{rel}}S_1\langle\tau_1\rangle}\sum_i f_i\tau_{i1}^3\sum_k\left(\frac{\lambda_1'}{\lambda_k'}\right)^3(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) \tag{17}$$

which we transform to

$$\sum_{i,k}\frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{2+3\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)^3(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \frac{A_\theta(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\Gamma_{11}\tau_{11}} \tag{18}$$

so as $t_{\mathrm{w}} \rightarrow \infty$

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{2+3\alpha} = \frac{\nu A_\theta}{S_3\Gamma_{11}\tau_{11}} \tag{19}$$

and as $t_{\mathrm{w}} \rightarrow 0$

$$\sum \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{1+2\alpha} = \frac{\nu A_\theta}{S_2\Gamma_{11}\tau_{11}} \tag{20}$$

In order to get a third independent equation for twitches, we can get an even higher moment if the data are good enough.

$$M_\theta = \int_0^\infty t\theta(t)\,\mathrm{d}t \tag{21}$$

In this case

$$\sum_{i,k}\frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{3+4\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)^4(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \frac{M_\theta(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\Gamma_{11}\tau_{11}^2} \tag{22}$$

which, for twitches, becomes

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{2+3\alpha} = \frac{\nu M_\theta}{S_3\Gamma_{11}\tau_{11}^2} \tag{23}$$

Depending on the windup conditions, the appropriate combination of eq 12, 15, 18, and 22 gives us a system of equations which apply to a single retardation experiment. At the end of this section, we discuss the solution of these equations. However, at this point we note that all of them favor—to different degrees—the largest molecular weights. In that sense stress-relaxation experiments are complementary to strain relaxations, because from them we can get estimates more biased in favor of lower molecular weight material.

Stress Relaxations

Stress-relaxation conditions require that we hold the rotor at a fixed angle, $\theta(t) = \theta_0$, after the shear stress has been applied to the solution. We measure the torque required to hold the rotor in this manner. These dynamics are described by[5]

$$F(t) = \gamma\omega k_{\mathrm{B}}T\nu L\sum_{i=1}^{P} f_i\tau_{i1}\sum_{k=1}^{N}\left(\frac{\lambda_1'}{\lambda_k'}\right)e^{-\lambda_k't/\lambda_1'\tau_{i1}}(1 - e^{-\lambda_k't_{\mathrm{w}}/\lambda_1'\tau_{i1}}) \tag{24}$$

Therefore, we find the total amplitude of the exponentials in a stress-relaxation curve to be

$$F(0) - F(\infty) = F(0) = \gamma\omega k_{\mathrm{B}}T\nu L \sum_i f_i\tau_{i1} \sum_k \left(\frac{\lambda_1'}{\lambda_k'}\right)(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) \quad (25)$$

which is analogous to Γ for strain-relaxation curves. Corresponding to the longest relaxation time (τ_{11}) is the amplitude

$$F_{11}(0) = \gamma\omega k_{\mathrm{B}}T\nu L f_1\tau_{11}(1 - e^{-t_{\mathrm{w}}/\tau_{11}}) \quad (26)$$

and the area under the curve is

$$A_{\mathrm{F}} = \int_0^\infty F(t)\,\mathrm{d}t = \gamma\omega k_{\mathrm{B}}T\nu L \sum_i f_i\tau_{i1}^2 \sum_k \left(\frac{\lambda_1'}{\lambda_k'}\right)^2 (1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) \quad (27)$$

We have previously noted that the amplitudes of stress-relaxation exponentials are one order lower in τ_{ik} than are the amplitudes of strain-relaxation exponentials.[5] Also, for either kind of experiment, a very short windup time (twitch) will reduce the amplitude of the exponentials by one order of τ_{ik}, when compared to a steady-state experiment. Therefore, the amplitudes of a steady-state creep-recovery curve are weighted as $f_i\tau_{ik}^2$, whereas for a twitched creep-recovery or steady-state stress relaxation they are weighed as $f_i\tau_{ik}$, and finally for a twitched stress-relaxation curve they depend only on f_i. The amplitudes for the twitched stress-relaxation exponentials are weighted only by the number fraction of molecules of that size and do not depend on the eigenvalues:

$$F(t) = \gamma(\Delta\theta)k_{\mathrm{B}}T\nu L \sum_{i,k} f_i e^{-t\lambda_k'/\tau_{i1}\lambda_1'} \quad (28)$$

In analogy with the creep-recovery moment expressions involving Γ_{11}/Γ, we could now get stress-relaxation moment expressions involving $F_{11}(0)/F(0)$. However, in practice $F(0)$ is hard to measure because of the competing torque impulse at $t = 0$ which must counteract the rotor inertia. It is easier to measure the total driving force, F_0, applied prior to time $t = 0$. That is calculated by using eq 7 of part 1, subject to initial conditions eq 9 and 10 of paper 1.

$$F_0 = \gamma\omega[\eta_0 + \sum_{i,k} \eta_{ik}(1 - e^{-t_{\mathrm{w}}/\tau_{ik}})] \quad (29)$$

For steady-state windups, this of course reduces to the simple expression relating the applied shear force, solution viscosity, and rate of shear:

$$F_0 = \gamma\omega\eta_0(1 + \eta_{\mathrm{sp}}) = \gamma\omega\eta_0\eta_{\mathrm{rel}} \quad (30)$$

We can form the dimensionless ratio

$$\frac{F_{11}(0)}{F_0} = \frac{\nu k_{\mathrm{B}}TLf_1\tau_{11}(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\eta_0 + \nu k_{\mathrm{B}}TL \sum_{i,k} f_i\tau_{i1}\left(\frac{\lambda_1'}{\lambda_k'}\right)(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'})} \quad (31)$$

As before, we can rearrange this by using eq 10 and 11 to get the moment equation

$$\sum_{i,k} \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \left(\frac{\eta_{\mathrm{sp}}}{\eta_{\mathrm{rel}}}\right)\left(\frac{F_0}{F_{11}(0)}\right)(1 - e^{-t_{\mathrm{w}}/\tau_{11}}) \quad (32)$$

where we have also used

$$F_0 = \frac{\eta_{\mathrm{rel}}}{\eta_{\mathrm{sp}}}F(0) = F(0) + \gamma\omega\eta_0 \quad (33)$$

Under steady-state conditions, we have

$$\frac{F_{11}(0)}{F_0} = \frac{\eta_{\mathrm{sp}_1}\nu}{S_1(1 + \eta_{\mathrm{sp}})} \quad (34)$$

where $\eta_{\mathrm{sp}_1} = C_1[\eta]_1$, so that

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{\nu F_0\eta_{\mathrm{sp}}}{S_1F_{11}(0)\eta_{\mathrm{rel}}} \quad (35)$$

and for twitches

$$\frac{F_{11}(0)}{F_0} = \frac{t_{\mathrm{w}}\nu k_{\mathrm{B}}Tf_1L}{\eta_0 + t_{\mathrm{w}}\nu k_{\mathrm{B}}TL} \quad (36)$$

so that

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{-1} = \frac{\eta_{\mathrm{sp}}F_0}{\eta_{\mathrm{rel}}F_{11}(0)} \quad (37)$$

From the area under the curve, A_F, we get

$$\sum_{i,k} \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{1+2\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)^2(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \frac{A_F(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{\tau_{11}F_{11}(0)} \quad (38)$$

so for the steady state

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{1+2\alpha} = \frac{\nu A_F}{S_2\tau_{11}F_{11}(0)} \quad (39)$$

and twitches

$$\sum_i \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{\nu A_F}{S_1\tau_{11}F_{11}(0)} \quad (40)$$

The higher moment

$$M_F = \int_0^\infty tF(t)\,\mathrm{d}t \quad (41)$$

gives

$$\sum_{i,k} \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{2+3\alpha}\left(\frac{\lambda_1'}{\lambda_k'}\right)^3(1 - e^{-t_{\mathrm{w}}\lambda_k'/\tau_{i1}\lambda_1'}) = \frac{M_F(1 - e^{-t_{\mathrm{w}}/\tau_{11}})}{F_{11}(0)\tau_{11}^2} \quad (42)$$

We now have a series of moment equations which are linear in C_i/C_1 and which depend on powers of M_i/M_1. For a single stress- or strain-relaxation curve, typically two or three equations apply. If we perform several experiments on the same solution, and they differ either in the mode of relaxation or in the amount of windup prior to relaxation, then more of these equations will apply. Therefore, we have a nonlinear system of equations which can, in principle, be solved for C_i/C_1 and M_i/M_1, for $i = 1, 2, ..., P$. We can take either of the following approaches. For a given set of experimental curves, and a given P, we choose just enough equations to specify a unique solution. We give an example below. The other alternative, if the number of data curves is large, or P is small, is to solve the overspecified system of equations by best-fit or least-squares type procedures. Either a nonlinear least-squares method could be used to solve for both the C_i/C_1 and M_i/M_1, or if we can measure or assume the M_i/M_1, then we could perform a much simpler linear least squares to solve for the C_i/C_1.

Suppose we perform a steady-state strain relaxation, from which we get τ_{11}, τ_{21}, τ_{31}, Γ_{11}, Γ. From a steady-state stress-relaxation curve, we get additionally $F_{11}(0)$, F_0. From

Table II
Heterogeneity Parameters[a]

HP based on	HP[b]	exp of HP $\Sigma \frac{C_i}{C_1}\left(\frac{M_i}{M_1}\right)^{\exp}$	HP for soln with 50% whole, 50% half molecules
	A. Strain Relaxation		
M_θ (tw)	$\frac{\nu M_\theta}{S_3 \Gamma_{11} \tau_{11}^2} - 1$	$2 + 3\alpha$	0.03125
A_θ (ss)	$\frac{\nu A_\theta}{S_3 \Gamma_{11} \tau_{11}} - 1$	$2 + 3\alpha$	0.03125
A_θ (tw)	$\frac{\nu A_\theta}{S_2 \Gamma_{11} \tau_{11}} - 1$	$1 + 2\alpha$	0.100
$\frac{\Gamma_{11} \eta_{\text{rel}}}{\tau_{11} \eta_{\text{sp}}}$ (ss)	$\frac{\omega \eta_{\text{sp}} \nu \tau_{11}}{S_1 \Gamma_{11} \eta_{\text{rel}}} - 1$	α	0.315
$\frac{\Gamma}{\Gamma_{11}}$ (ss)	$\frac{\nu \Gamma}{S_2 \Gamma_{11}} - 1$	$1 + 2\alpha$	0.100
$\frac{\Gamma}{\Gamma_{11}}$ (tw)	$\frac{\nu \Gamma}{S_1 \Gamma_{11}} - 1$	α	0.315
	B. Stress Relaxation		
A_F (ss)	$\frac{\nu A_F}{S_2 \tau_{11} F_{11}(0)} - 1$	$1 + 2\alpha$	0.100
A_F (tw)	$\frac{\nu A_F}{S_1 \tau_{11} F_{11}(0)} - 1$	α	0.315
$\frac{F_{11}(0)}{F_0}$ (ss)	$\frac{\nu F_0 \eta_{\text{sp}}}{S_1 F_{11}(0) \eta_{\text{rel}}} - 1$	α	0.315
$\frac{F_{11}(0)}{F_0}$ (tw)	$\frac{F_0 \eta_{\text{sp}}}{F_{11}(0) \eta_{\text{rel}}} - 1$	-1	1.00

[a] Note that for DNA in 0.2 M Na$^+$, $\alpha = 0.665$.[6] [b] Heterogeneity parameter.

the steady-state windup of either one we get η_{rel}. If we choose $P = 3$, then from eq 13

$$\frac{C_2}{C_1}\left(\frac{M_2}{M_1}\right)^{1+2\alpha} + \frac{C_3}{C_1}\left(\frac{M_3}{M_1}\right)^{1+2\alpha} = \frac{\nu \Gamma}{S_2 \Gamma_{11}} - 1 \quad (43)$$

and from eq 35

$$\frac{C_2}{C_1}\left(\frac{M_2}{M_1}\right)^{\alpha} + \frac{C_3}{C_1}\left(\frac{M_3}{M_1}\right)^{\alpha} = \frac{\nu F_0 \eta_{\text{sp}}}{S_1 F_{11}(0) \eta_{\text{rel}}} - 1 \quad (44)$$

We know that

$$\frac{M_2}{M_1} = \left(\frac{\tau_{21}}{\tau_{11}}\right)^{1/(1+\alpha)} \quad (45)$$

and

$$\frac{M_3}{M_1} = \left(\frac{\tau_{31}}{\tau_{11}}\right)^{1/(1+\alpha)} \quad (46)$$

so we can solve eq 43 and 44 for C_2/C_1 and C_3/C_1. Now to get the absolute concentrations, we can simply use

$$C_1 = \frac{C}{\sum\limits_i C_i/C_1} \quad (47)$$

where C is the total polymer concentration, which we assume is known. To get the absolute molecular weights, we combine eq 9 with any applicable moment expression to get, for example,

$$M_1 = \frac{\nu R T C_1 \tau_{11}}{\eta_0 \eta_{\text{rel}}}\left(\frac{F_0}{F_{11}(0)}\right) = \frac{\nu R T C_1 \tau_{11}}{\eta_0 \eta_{\text{rel}}}\left(\frac{\omega \tau_{11}}{\Gamma_{11}}\right) \quad (48)$$

Finally, it is also possible to get the number fraction of largest molecules from a creep-recovery curve

$$f_1 L = L_1 = \frac{\eta_0 \eta_{\text{rel}} \Gamma_{11}}{k_B T \omega \nu \tau_{11}^2 (1 - e^{-t_w/\tau_{11}})} \quad (49)$$

or from a steady-state relaxation curve

$$L_1 = \frac{\eta_0 \eta_{\text{rel}} F_{11}(0)}{k_B T \nu \tau_{11} F_0} \quad (50)$$

It is clear from eq 43 and 44 that the expressions on the right-hand side of those equations are useful estimators of the molecular-weight heterogeneity in the solution. If the solution contains only a single molecular-weight species then

$$\frac{\nu \Gamma}{S_2 \Gamma_{11}} - 1 = \frac{\nu F_0 \eta_{\text{sp}}}{S_1 F_{11}(0) \eta_{\text{rel}}} - 1 = 0 \quad (51)$$

This is also true for all the similar moment expressions which are given in Table II. We are interested in which such parameter is most sensitive to heterogeneity. The value which each of these parameters takes is calculated for the case in which 50% of the molecules are of the primary size and 50% are half-sized, and this is shown in Table II. It is evident that the most sensitive parameter comes from a twitched stress-relaxation experiment.

These parameters are affected by the DNA concentration of the solution and the shear rate at which the experiment was performed, as are the relaxation times and amplitudes themselves. Therefore, to get reasonable estimates of heterogeneity with these parameters, or to get good molecular-weight distributions from equations such as 43 and 44, it should be necessary to extrapolate these values to zero shear and zero concentration. There are

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other considerations too which will affect the quality of the predicted molecular-weight distribution. We have assumed a discrete distribution of species, and this may not be valid. Also, it may be difficult to extract a single relaxation time from a twitched stress-relaxation curve, and a semilog plot may yield an average of 2 or more relaxation times.

Finally, we note that many DNA molecules occur naturally as covalently closed circular molecules. However, none of the viscoelastic parameters—stress or strain relaxation—are of much help distinguishing circles of molecular weight M from linear molecules of size $M/2$. Since half-sized linears are the most abundant shear-breakage proucts of full-sized molecules,[9] it is just this kind of heterogeneity that makes circle detection difficult. At best, the higher moment eigenvalue ratios in M_F and M_θ show only about a 20% difference between half-linears and full circles. However, with the help of endonuclease enzymes, viscoelastometry can be made to be quite sensitive to a small fraction of circular DNA molecules. We could first perform a stress relaxation, for example, and measure the area under the curve A_F. Then we enzymatically introduce an average of one double-strand break per DNA molecule and remeasure the solution. If circles were originally present, they would now be full length linear molecules, so A_F would increase by a factor of 8. If, on the other hand, full-length linear molecules were originally present, they would now be half-sized linears, and A_F would decrease by about that same factor. Thus in that way, A_F and higher moments should be highly sensitive to the presence of circular DNA in the solution.

Notation

A_F	area under the stress-relaxation curve
A_θ	area under the strain-relaxation curve
C_i	weight concentration of the ith species
$F(t)$	the relaxation of force for fixed strain
$F_{11}(t)$	primary component (longest relaxation time) of the force relaxation
F_0	applied driving force prior to relaxation
f_i	number fraction of molecules of species i
h	hydrodynamic draining parameter
i	index over molecular weight species
k	index over internal relaxation modes
k_B	Boltzmann's constant
K	Crothers–Zimm coefficient (see eq 8)
L	total number of molecules per unit volume
L_i	number fraction of molecules of species i per unit volume
M_i	molecular weight of species i
M_F	integral of area under the curve (stress relaxation)
M_θ	integral of area under the curve (strain relaxation)
N_a	Avogadro's number
RT	gas constant times absolute temperature
S_1	constant given by $\nu\sum_{k=1}^{\infty}(\lambda_1'/\lambda_k')$
S_2	constant given by $\nu\sum_{k=1}^{\infty}(\lambda_1'/\lambda_k')^2$
t_w	time duration of "windup", prior to relaxation
α	Crothers–Zimm exponent given by eq 8
γ	geometric constant, defined in the preceding paper for that instrument
Γ	total elastic recoil (strain relaxation)
Γ_{11}	primary component of elastic recoil
η_0	solvent viscosity
η_{sp}	specific viscosity of solution
η_{sp_1}	specific viscosity of the ith species
η_{rel}	relative viscosity
$[\eta]_i$	intrinsic viscosity of the ith species
$\theta(t)$	the relaxation of strain, after an applied stress has been removed
$\Delta\theta$	total windup angle
λ_k'	eigenvalues from the bead-spring theory
ν	degeneracy constant (1 if chains are linear, 2 if chains are covalently closed circles)
τ_{ik}	relaxation and retardation times (assumed to be equal at zero concentration and zero applied shear)
$\langle\tau_1\rangle$	average relaxation time, $\sum_{i=1}^{p} f_i\tau_{i1}$
ω	angular velocity of windup.

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Dynamics of Polymer Solutions. 3. An Instrument for Stress Relaxations on Dilute Solutions of Large Polymer Molecules

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ABSTRACT: We have previously described an instrument with which it is possible to measure strain relaxations of dilute solutions of very large DNA molecules. Herein we describe substantial modifications of that instrument, so that we can additionally measure stress relaxations. Thus the combined instrument can measure the solution viscosity, the creep recovery, and the stress relaxation of a single sample of a dilute solution of large polymer molecules.

The viscoelastic behavior of dilute polymer solutions gives much information about the sizes of the polymer molecules and their distribution.[1–3] An instrument in which these measurements can be made is the viscoelastometer; a recent version, designed for DNA solutions, is described in ref 4. With this device, we apply a shear stress to the solution and measure the relaxation of the strain after the stress is removed. This is referred to as a

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