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 prouct 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_i}	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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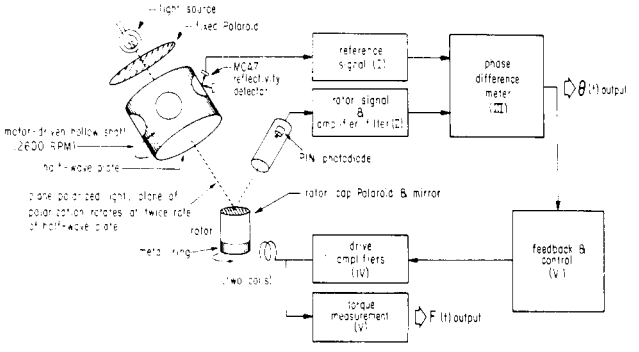

Figure 1. Instrument diagram. The spinning hollow shaft produces two signals. The reference signal is produced by a Monsanto computer card reader, which responds to four areas of high reflectivity on the shaft. The plane of the polarized beam is rotated by the rotation of the half-wave plate mounted in the shaft. This beam, passing through a polaroid on the rotor, is reflected to a photodiode, providing a signal whose phase relative to the reference signal indicates the rotor's angular position. The torque that affects the rotor position is provided by a set of coils which generate eddy currents in a metal ring.

creep-recovery experiment, and it allows determination of the molecular weight and concentration of the largest DNA molecules in the solution. In this paper, we explain how the viscoelastometer can be modified to also perform a second type of experiment, stress relaxation, which is complementary to the strain-relaxation experiment. When the instrument is used to perform an experiment of both types on the same DNA solution, it is easier to get information about the distribution of molecular weights of the DNA in solution, as discussed in the preceding paper.[5]

General Description

As this device was built as an addition to a previously described instrument,[4,7] only a brief description of the common features will be presented. The sample (usually a DNA solution), in a thermostated cylindrical glass chamber, is partially confined to an annular region by a floating cylindrical plastic rotor. The rotor has a metal ring at the bottom and a piece of mirror-coated polaroid at the top. An external electromagnetic field produces eddy currents in the ring which turn the rotor and thus apply a shear stress to the solution. This "windup" of the rotor is required for either the strain- or the stress-relaxation kind of experiment. Some of the applied energy is stored in the "elastic" entropy of the molecules as they are stretched from their equilibrium configuration. Then, at the time we call $t = 0$, the molecules are allowed to relax, allowing either of the following experiments:

(a) Relaxation of the Strain. This (also called a creep-recovery experiment) is performed by turning off the externally-applied drive field, allowing the rotor to be governed by the solution dynamics alone. The rotor, whose position we measure with an optical system (see Figure 1 and ref 4), reverses direction, and its angular velocity decays exponentially.

(b) Relaxation of the Shear Stress. In this case (a stress-relaxation experiment) the rotor angular position, as monitored by the optical system, is held fixed by servoelectronics which apply a restoring torque to the rotor to maintain its angular position. As the molecules relax, the applied torque, which balances the relaxing shear stress, decays exponentially to zero. We measure the relaxation of the applied torque.

The mechanical details of the instrument and the geometry of the reflecting optical system are the same as those previously described.[4] We shall describe only the novel features that allow us to perform relaxations of stress.

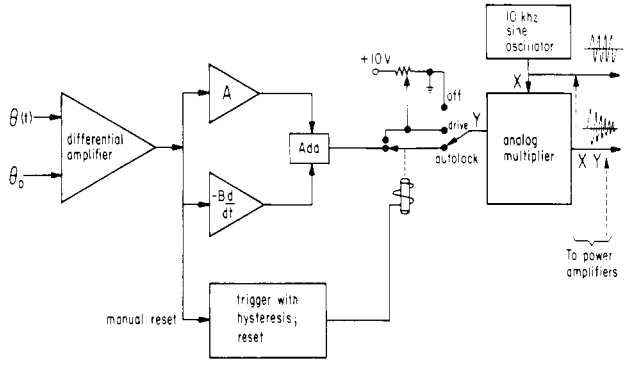

Figure 2. The feedback and control design. See text.

The general diagram is shown in Figure 1.

Drive System with Feedback

The strategy of stress-relaxation experiments involves applying a constant torque for a specified time and then holding the rotor in a fixed position. To accomplish this, the electronic system supplying current to the drive coils has two distinct modes corresponding to the two stages of the experiment. A relay switches from the constant-torque mode to the constant-position mode, the switching being triggered by the rotor reaching a preset position. The triggering circuit has electronic hysteresis to prevent deviations of the position of the rotor from the holding position, which would cause an accidental return to the constant-torque mode. The relay is manually reset before each experiment.

Once the relay has switched over to the constant-position mode, the amplitude of the sinusoidal voltage applied to one of the drive coils is controlled by a typical servomechanism loop. As seen in Figure 2, the constant position is maintained by amplifying deviations from the holding position and applying an appropriate restoring torque. This torque is the sum of two terms: a signal proportional to the difference between the holding position and the actual rotor position, and one proportional to the negative of the velocity of the rotor. (The negative velocity feedback, intended to improve transient response by minimizing overshoots, has been unnecessary for the fairly viscous solutions used so far.) This correction signal, which is zero when the rotor is stationary at the holding position, is used to modulate the amplitude of a 10 kHz sine wave. The signal is applied to one input terminal of an analog multiplier; a constant amplitude sine wave is applied to the other, and their product is sent to a power amplifier. This amplifier powers one set of electromagnetic coils. Another set of coils, perpendicular to the first, is driven by a constant amplitude sine wave with the same frequency as the first but 90° out of phase. The net effect is to produce a torque proportional to the amplitude of the modulated signal, and thus proportional to the position error.

The major modification in the drive system from that described previously[6] is the change to the higher frequency of the sine wave, which allowed use of smaller air-core coils (2.5-cm diameter, 50 turns of No. 22 gauge copper wire) in place of the more cumbersome iron-core coils. The smallness of the magnetic field produced is more than compensated for by the higher rate of change of the field, as the torque is proportional to the product of both quantities.

The power amplifier for both sets of coils is a commercial "stereoamplifier" (Dynaco Model ST-80), which has a rated power of 40 W per channel and handles frequencies as high as 20 kHz without difficulty.

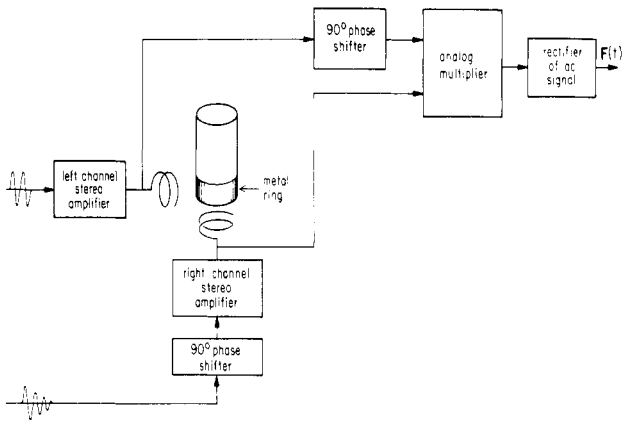

Figure 3. The torque-producing and -measuring system. See text.

Torque Measurement

As shown in Figure 3, the torque is measured by sampling the voltages applied to the drive coils. After phase shifting them back into register, they are multiplied by an analog multiplier (Motorola, MC1595). The output from this stage is a 20 kHz signal whose amplitude is proportional to the product of the input amplitudes. This amplitude is measured by rectifying the alternating current component of the 20-kHz signal (the DC level of the multiplier output is capacitively blocked). The output of the rectifying circuit is filtered with a fourth-order Butterworth circuit and applied to a strip-chart recorder, which displays the torque.

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Dynamics of Polymer Solutions. 4. Shear-Stress-Relaxation Experiments on Solutions of DNA from Bacteriophage T2

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ABSTRACT: We have previously described a stress-relaxation viscoelastometer which applies very small shear stresses to dilute solutions of large polymer molecules. Here we present experimental results. We show that in the limit of zero applied shear stress, and zero concentration, the primary retardation time is equal to the primary stress-relaxation time. We show that various stress-relaxation parameters are more sensitive to smaller molecules in the solution than are strain-relaxation parameters. One stress-relaxation parameter, a dimensionless measure of the primary relaxation amplitude, is found to be sensitive to the heterogeneity of the distribution of molecular weights, even at nonzero shear stress and concentration.

Dilute solutions of very large polymer molecules demonstrate remarkable effects due to their properties of viscoelasticity. For example, suppose a rotor is suspended in such a solution and is rotated in one direction by an external force. Then upon removal of the force there is enough energy stored in the elasticity to impart a recoil to the rotor—it reverses its direction of motion, even when the polymer concentration is only a few parts per million.[1,2] The angular velocity of the rotor decays exponentially to zero in that direction, and the time constant of this decay can be related directly to the molecular weight of the largest polymer molecules in solution.[1,2] This technique has been successfully applied to the measurement of chromosomal-sized DNA molecules in sizes ranging from 20 million to 40 billion daltons, a difficult size range for other techniques. The experimental procedure is called "creep recovery", and the decay times are known as "retardation" times. The instrument is a modified Couette viscometer and is capable of measuring intrinsic viscosities as well as these retardation times.[1,2] In parts 1 and 3,[3,4] we have described the theory and design for a substantially modified instrument which can measure shear-stress relaxation as well as creep-recovery dynamics. In this paper, we present experimental data from this instrument on solutions of DNA from bacteriophage T2. The advantage of having this additional stress-relaxation capability is in the potential for getting simple information about the molecular weight distribution. Creep-recovery dynamics are insensitive to all but the largest such molecules, while stress relaxations give data more biased toward the lower molecular weight components in the solution. Here, we demonstrate that effect. Also we present a stress-relaxation parameter, F_{11}/F_0, which is simple to get from the experimental curves and which appears to be a reasonably good measure of molecular weight heterogeneity, even without extrapolation to zero concentration and zero applied shear. With our measured values of this parameter and of the primary stress-relaxation time, we have confirmed the results from several other studies. First, the theory of Chapman et al.[1] predicts that the primary retardation time and the primary relaxation time are the same at zero concentration. We find this to be true so long as these decay times are also extrapolated to zero applied shear stress. In those limits, our stress-relaxation times are the same as the retardation times of Bowen and Zimm, who predict a molecular weight of 1.26×10^8 daltons for T2 DNA.[5] Using our parameter which is more sensitive to heterogeneity than the ones used by Bowen and Zimm,

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