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IMPROVEMENTS IN INSTRUMENTATION FOR VISCOELASTOMETRY OF DNA SOLUTIONS

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Improvements are described in the instrumentation for viscoelastometry of DNA solutions. The precision is improved by the order of a factor of ten.

1. Introduction

A study on the viscoelasticity of dilute DNA solutions was reported by us earlier in this journal [1]. In the course of that work we developed a number of improvements in our previously described methods [2,3]; in this paper we give a brief description of them. Details are given much more extensively in ref. [4].

Since the molecular theory of viscoelasticity from macromolecular solutions applies only to infinitely dilute solutions at vanishing stress, we found it important in our work [1] to measure as small signals as possible in order to reduce the errors of extrapolation. The minimum usable signal size is limited by the sensitivity and stability of the apparatus. The modifications described below made major improvements, of the order of a factor of ten, in these quantities, and at the same time they significantly improved the precision of measurements.

2. Brief description of apparatus

The apparatus is shown in figs. 1 and 2. It consists of several component systems: an optical system (fig. 1, (R) through (Z)); a chamber assembly (fig. 1, (A) through (C), and fig. 2) and servo system for control of rotor height (fig. 1, (J) through (M)), as described by Gill and Thompson [5]; a rotor drive system (fig. 1, (I1)) and centering coil (fig. 1, (I2)); and electronics for measuring the phase difference between rotor and reference signals. An earlier version of this instrument was described by Chapman et al. [2] and by Klotz and Zimm [3]. The more significant changes are discussed below.

The optical system senses the rotor's angular position by reflecting polarized light from the rotor cap, rather than by transmitting light through the whole rotor; otherwise the operation of the detecting system is essentially the same as described previously [3]. The rotor cap is a piece of Polaroid sheet laminated between Plexiglas sheets with a film of aluminum evaporated on the under side. Light from the lamp R and rotating polarizer S passes throught the rotor cap and is reflected back to the rotor-sensing photocell W. The intensity of this reflected beam is much stronger than that of the transmitted beam previously used, so that the sensing of the rotor's angular position is more precise. The precision of the angular measurement is about 0.01°. The reference beam is reflected from the upper window of the "pressure bar", C.

The chamber that holds the solution (fig. 2, D) has been made removable so that one solution can be prepared while another is being measured.

A coil of copper wire (I2 in fig. 1 and C in fig. 2) is arranged to allow centering of the rotor by means of a vertically oriented alternating magnetic field, frequency about 10 kHz.

The construction of the rotor is very similar to that described in ref. [3], except for two improvements: the under side of the Polaroid cap is coated with a reflecting film, mentioned above, and the metal ring is

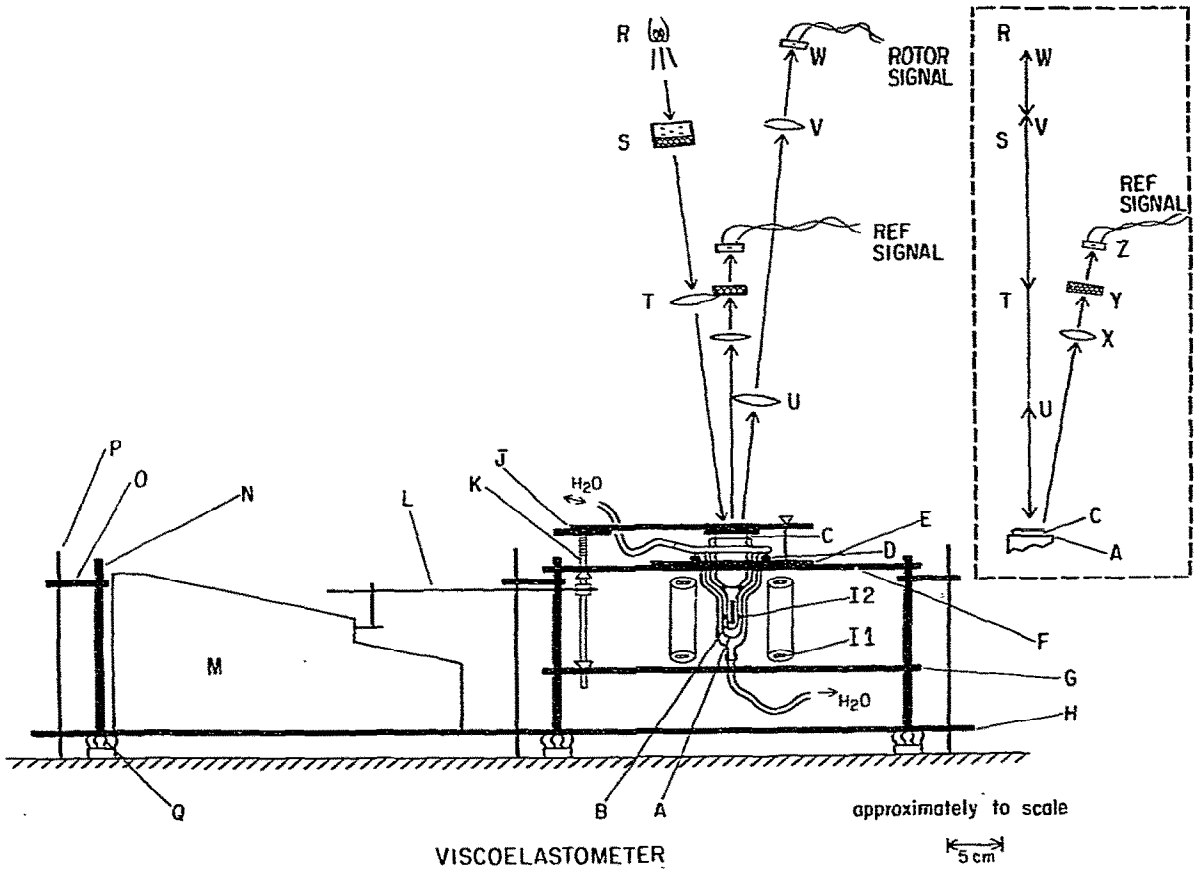

Fig. 1. Schematic diagram of viscoelastometer. (A) Glass thermostated temperature jacket with glass interchangeable inner chamber (see fig. 2 for detailed drawing). (B) Cartesian-diver rotor (see fig. 2) with Polaroid cap (rotor analyzer). (C) Pressure bar (see fig. 2). (D) Plaster. (E) Phenolic plate. (F) Top aluminum plate. (G) Middle aluminum plate. (H) Bottom aluminum plate. (I1) Copper-wire drive coils (in cross-section). (I2) Copper-wire centering coil (in cross-section). (J) Servo lever arm and gimbal (see fig. 3). (K) Brass servo rod with conical bushings seated in holes in (F) and)G) threaded (64 threads per inch) at the top. (L) Aluminum servo arm. (M) Heathkit Servo-Recorder (model EU-20B). (N) Stainless-steel threaded (1/2–13) rods. (O) Aluminum brackets. (P) Stainless-steel threaded (10–32) rods. (Q) Foam-rubber tubing on wood block. (R) Light source. (S) Rotating polarizer. (T) Focusing lens. (U) Rotor collimating lens. (V) Rotor detection lens. (W) Rotor photodetector. (X) Reference detection lens. (Y) Stationary reference analyzer. (Z) Reference photodetector. All parts are drawn as viewed from the front, except (I1) and (I2) which are drawn in cross-section. Diagram to the right is a side view of the optical system only. Approximate distances (in cm) and angles are: RS = 12, ST = 20, TA = 27, AU = 16, UV = 30, VW = 9, AX = 22, XY = 5, YZ = 5, RAW = 12°, RAZ = 8°.

gold-plated sterling silver instead of aluminum to avoid corrosion. Silver was chosen because of its high electrical conductivity.

3. Baseline drift

In our experience the main difficulty in measure-

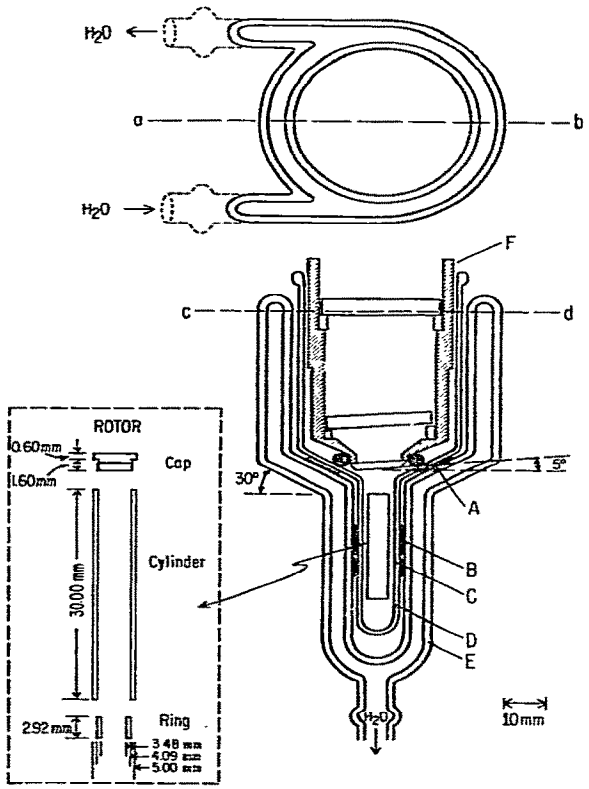

Fig. 2. Viscoelastometer chamber assembly. The drawing in the center is a front, cross-sectional view (section ab) of the chamber assembly: (A) Polyethylene gasket. (B) Plexiglas sleeve. (C) Copper-wire centering coil (30 turns), attached to temperature jacket (E). (D) Glass interchangeable inner chamber. (E) Glass temperature jacket, with constant-temperature water flowing in the directions indicated. (F) Plexiglas pressure bar with three Plexiglas windows. The upper drawing is a top, cross-sectional view (section cd) of the temperature jacket only. The inlet and outlet tubes are inclined to the horizontal (as shown) about 20°. The drawing to the left is an exploded, cross-sectional view of the rotor, which includes the Plexiglas-laminated Polaroid cap, Kel-F cylinder, and gold-plated silver ring.

ments of viscoelastic recoveries over periods of more than a few minutes with apparatus of the type described is caused by the presence of slow rotor motions that apparently have nothing to do with the viscoelastic effects being measured. We call these motions "baseline drift", and we have been able to identify three sources of this drift: vibration of the instrument, thermal convection of the fluid in the chamber, and interaction of ferromagnetic impurities in the rotor with static magnetic fields.

A steady drift of several degrees of rotor angle per hour can appear if the chamber is not isolated from building vibrations and from vibrations induced by the rotating polarizer and the motor that drives it. To avoid the latter, the motor is suspended from rubber strips and connected to the polarizer by a rubber belt, while the polarizer itself is mounted on two good-quality ball bearings which are attached to the wall of the building separately from the rest of the instrument. To reduce building vibrations we have adapted a familiar form of galvanometer suspension [6]. The frame of the instrument (N in fig. 1) is suspended from flexible rods, P, that rest on the laboratory bench. The period of natural oscillation of this arrangement is about 0.5 s; this oscillation is damped by pieces of foam rubber, Q.

A substantial source of erratic drift is thermal convection of the fluid in the chamber. This is particularly obvious with elevated temperatures, e.g. 50°C. Even though the jacket, E in fig. 2, is controlled by a thermostat, enough temperature gradients occur to cause slow convection. This kind of drift can be practically eliminated if the fluid in the chamber contains a density gradient. For example, a 0—4% sucrose gradient limits drift to less than 0.4°/hr at a temperature of 50°C. Unfortunately, it is dangerous to use a conventional gradient maker with solutions of large DNA molecules because of the possibility of breaking the molecules in the usual mixing chamber. We have found, however, that the apparatus shown in fig. 3 is effective. The gradient is constructed by adding 0.5 ml of a 16% sucrose solution, G, to 3.5 ml of the DNA solution, B, which already contains the rotor. As G is released from a motor-driven syringe through the micropipette tip, at a rate of about 0.1 ml/min, the chamber is steadily rotated at about one rpm by a small clock motor. When the flow rate and the rotation speed are properly adjusted, G is dispersed evenly as it sediments through B. Diffusion soon smooths out any irregularities. In a 0—4% sucrose gradient the average viscosity is about 5% greater than with sucrose absent, so for relative viscosity measurements one must determine rotation times of the rotor in the solvent with the standard gradient present.

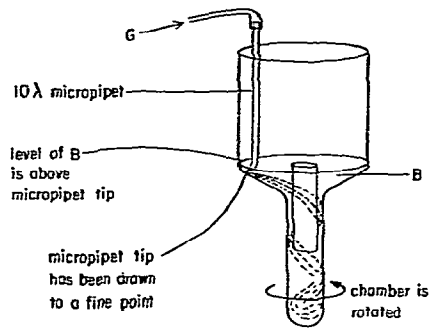

Fig. 3. Forming a density gradient in the viscoelastometer chamber.

We have also found it possible to make a convection-inhibiting gradient by allowing a DNA solution containing 1% sucrose and 0.2 M buffer to evaporate slowly while in the sample chamber. Five hours at 50°C with the chamber loosely covered with Parafilm gave satisfactory results.

The third source of drift, ferromagnetic impurities interacting with steady magnetic fields, is less troublesome than the other two. For one thing, the drift is steady and reproducible, so it can be taken into account during data analysis. In effect, the rotor is attempting to act as a magnetic compass and point toward the local magnetic field. Sometimes soaking the rotor in concentrated hydrochloric acid to destroy ferromagnetic particles produces a dramatic reduction in drift rate.

Occasionally air bubbles on the rotor cause drift as their size changes when the air dissolves; care should be taken to avoid these. Drifts sometimes occur if the height-control serve is oscillating or unsteady; it should be adjusted to hold the rotor at a constant height.

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