indicate that the barriers to conversion of the unstable dimeric species to the stable form is less than 60 cal.

CONCLUSION

The nitric oxide dimer has been shown to exist in three forms when trapped in a matrix at low temperatures. The most stable form is the *cis* O=N–N=O species ($\nu_{sym}=1870$ cm^{-1} and $\nu_{asym}=1776$ cm^{-1}) having C_{2v} symmetry, while the other two unstable forms appear to be the *trans* O=N–N=O ($\nu_{asym}=1764$ cm^{-1}), and possibly another *cis* form ($\nu_{sym}=1870$ cm^{-1} and $\nu_{asym}=1785$ cm^{-1}). The production of these dimeric species by limited diffusion and photochemical means indicates that these reactions occur with practically zero activation energies. The force constants calculated using a valence-bond potential indicate that the dimers retain a strong N=O bond and a relatively weak N–N bond. All attempts to observe the low frequencies of these dimers were unsuccessful.

Ultrasonic Absorption in Aqueous Solutions of Dextran*

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The ultrasonic absorption measured in aqueous solutions of dextran (1–6 polyanhydroglucose) as a function of molecular weight (3.4×10^3–3.7×10^6) and frequency (3–195 MHz) reveals a relaxational behavior related to the calculated viscoelastic properties of free-draining random coils.

INTRODUCTON

In recent years there have been a number of investigations involving aqueous solutions of biopolymers which employ ultrasonic spectoscopy as a method of analysis.[1] The particular interest in the acoustic properties of these substances is related both to biomedical applications of ultrasound as well as to the relevance of the propagation parameters to characterization of equilibrium kinetics of reactions involving biologically interesting marcomolecules.

It has been observed that in solutions of hemoglobin,[2] bovine serum albumin,[3] and poly-L-glutamic acid[4] the frequency dependence of the absorption coefficient α is compatible with a distribution of relaxation processes, viz.,

$$\frac{\alpha}{f^2} = \sum_{p=1}^{N} \frac{A_p}{1+(f/f_p)^2} + B, \qquad (1)$$

in which $f_p=1/2\pi\tau_p$ where τ_p is the characteristic relaxation time of the pth process and B represents nonrelaxing absorption contributions. Little information has been provided, however, which serves to reveal the molecular origins of acoustic-macromolecular interactions. Part of the difficulty resides in the complexity of the macromolecular species, in particular the uncertainties concerning dynamic viscoelastic interactions that may be present for a polyelectrolytic molecular species.

In this study, ultrasonic absorption measurements are presented for aqueous solutions of dextran, a quasi-linear uncharged polymer, as a function of molecular weight as well as frequency. Since there is available both experimental and theoretical information that delineates the shear viscoelastic behavior of linear polymer solutions, the choice of such a material for ultrasonic absorption measurements provides an opportunity to examine any interrelations that may exist.

EXPERIMENTAL

The dextran (1–6 polyanhydroglucose, 5% non 1–6 linkages) employed in this investigation is type NRRL B512 obtained in fractionated form.[5] Molecular-weight and intrinsic viscosity information provided by the manufacturer is listed in Table I. Solutions were prepared with distilled water and clarified via membrane filtering prior to use. Concentration was determined via optical rotation based on a measured $[\alpha]_D^{20}$ of $+199°$.

* This work represents in part, the Ph.D. thesis in biophysics of the first named author, University of Illinois, Urbana, Ill., 1967.
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[1] A detailed bibliography will be found in F. Dunn, P. D. Edmonds, and W. J. Fry, in *Bioelectronics*, H. P. Schwan, Ed. (McGraw-Hill Book Co., New York, 1968).
[2] P. Edmonds, Biochem. Biophys. Acta **63**, 216 (1962).
[3] S. A. Hawley and F. Dunn (unpublished).
[4] J. Burke, G. Hammes, and T. Lewis, J. Chem. Phys. **42**, 3250 (1965).

[5] Obtained from Pharmacia Fine Chemical Co., Piscataway, N.J.
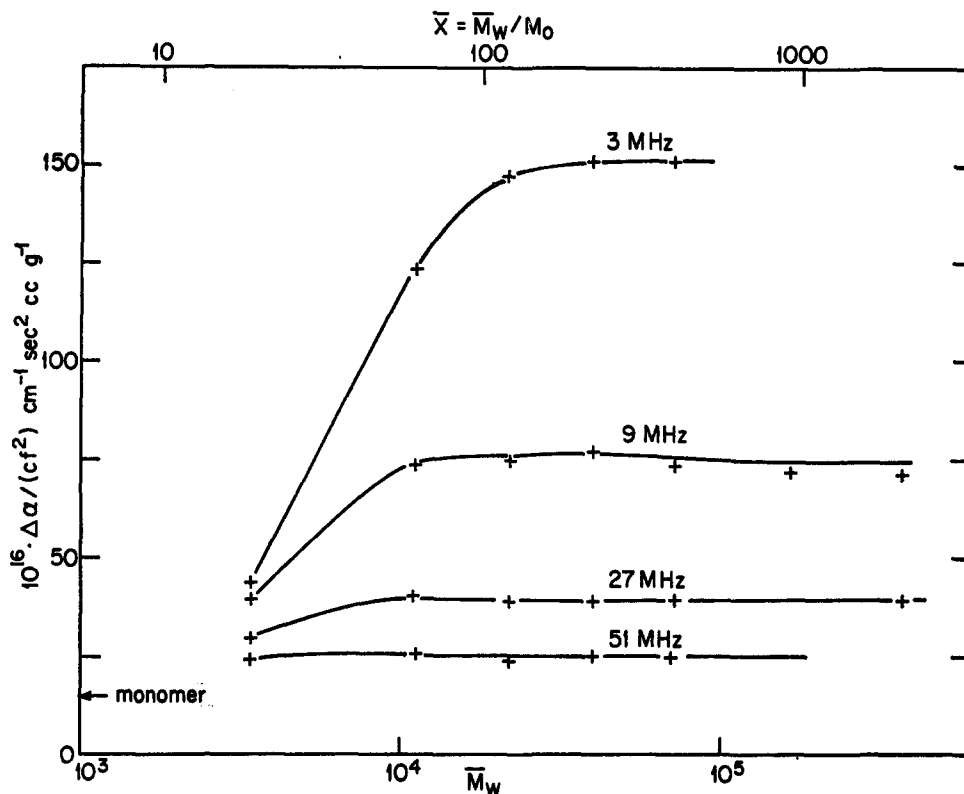
FIG. 1. $\Delta\alpha/(cf^2)$ as a function of weight average molecular weight at four frequencies, 20.0°C. The average degree of polymerization $\bar{X}$ is denoted on the superior abscissa.

A semiautomatic version of the conventional pulse technique was employed over the frequency range 3–195 MHz. The instrumental precision has been determined in the couse of several hundred determinations of α in distilled water conducted throughout the course of the investigation. Diffraction corrections are made at 3 and 9 MHz assuming that these losses are additive and noninteracting with the intrinsic absorption of the sample. The fractional standard deviation has been found to vary between 3.72% at 9 MHz to 18.0% at 195 MHz, with a minimum deviation of 2.46% observed at 21 MHz. Throughout the frequency range the mean values of α obtained in water agreed in all instances within 3% of accepted values.[1] Sample temperature was determined and maintained at 20°± 0.01°C.

The measurement of the speed of sound in the dextran solutions revealed that the presence of the solute decreases the over-all compressibility, with solutions having a slightly higher sound speed than water. For most solutions considered in this study, the net change is of the order of measurement error (0.3%) so that a systematic examination of this data will not be attempted here.

RESULTS

Ultrasonic absorption values have been determined for at least three concentrations at each molecular weight and frequency. Within the experimental error, the absorption was found to be a linear function of

concentration to about 10% throughout the molecular-weight and frequency range, extrapolating to the absorption coefficient for water at zero concentration.

The results are depicted in Figs. 1 and 2 in terms of the concentration-free relaxation parameter $\Delta\alpha/(cf^2)$
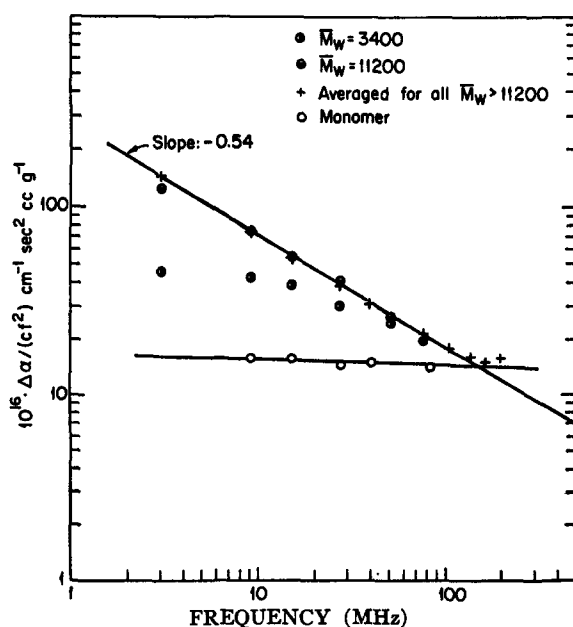

FIG. 2. $\Delta\alpha/(cf^2)$ vs frequency for the monomer glucose and dextran of molecular weights listed in Table I.
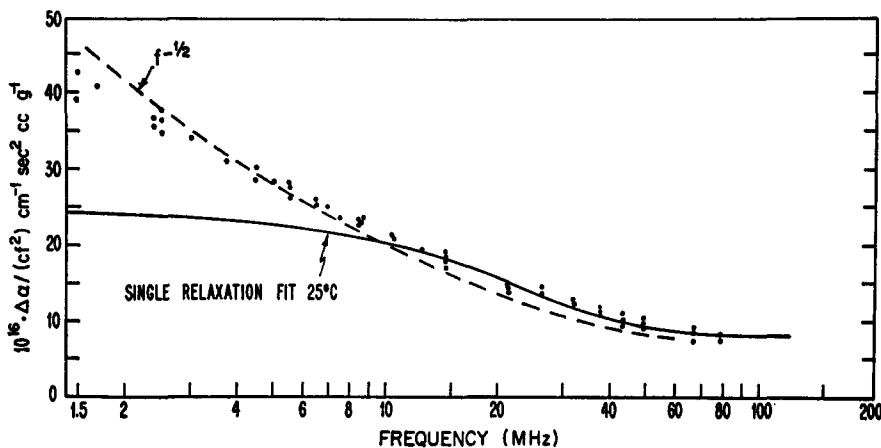
FIG. 3. $\Delta\alpha/(cf^2)$ vs frequency for polyethylene glycol, molecular weight = 20 000. Solid line represents authors' (Ref. 10) single relaxation fit to their data in frequency range 10–185 MHz, 25°C; points correspond to measurements in frequency range 1.5–80 MHz, 20°C (Ref. 11).

in which $\Delta\alpha$ represents the absorption difference between that of solution and of solvent and c is the concentration. The important features to note are: (1) except at the highest frequencies of observation there is an absorption contribution associated with polymerization, (2) there is a molecular-weight dependence of $\Delta\alpha/(cf^2)$ for molecular weights less than about 25×10^3 which becomes less pronounced as the frequency is increased; (3) at molecular weights above 25×10^3 the absorption is independent of molecular weight within the frequency range considered; and (4) there is an apparent limiting dependence corresponding to $\Delta\alpha/(cf^2) \propto f^{-1/2}$.

These observations are suggestive of results obtained for shear viscoelastic properties of polymer solutions,[6] and one can proceed along this line of analysis by considering the anticipated viscous losses, viz.,

$$\alpha/f^2 = (2\pi^2/\rho V^3)(\eta_v' + \tfrac{4}{3}\eta_s'),\qquad(2)$$

where ρ is the density, V is the sound velocity, and η_v' and η_s' are the dynamic volume and shear viscosities, respectively. Static hydrodynamic and viscosity measurements in Pharmacia NRRL B512 dextran have been

TABLE I. Molecular weights and intrinsic viscosities of dextran samples employed in this investigation, 20°C.

$\bar{M}_w \times 10^{-3}$	$\bar{M}_n \times 10^{-3}$	$[\eta]_0$ (cc/g)	$\bar{M}_w/\bar{M}_n$
3.4	2.0	5.4	1.7
11.2	5.7	9.8	1.97
21.8	14.5	14.7	1.50
39.8	25.6	19.8	1.55
72.0	40.5	25.6	1.77
167	105	38	1.59
370	185	50	2.00

observed to be compatible with random-coil behavior with a small excluded-volume effect attributable to branching.[7] If this behavior is maintained under dynamic conditions, one may reasonably formulate an expression for η_s'. For this purpose the limiting slope suggests that the Rouse formulation[8] for the free-draining coil, which predicts a limiting viscosity proportional to $f^{-1/2}$, may be appropriate. One can then easily show that if the concentration dependence of the speed of sound and of the density are neglected, an assumption which introduces an uncertainty of the order of the experimental error, the excess concentration-free absorption attributable to shear viscosity, $\Delta\alpha_s/(cf^2)$, in terms of the free-draining coil becomes

$$\frac{\Delta\alpha_s}{cf^2} = \frac{4\pi RT}{3M\rho V^3} \sum_{p=1}^{N} \frac{1/f_p}{1+(f/f_p)^2}.\qquad(3)$$

One would expect, on the basis of the random coil model, that[9] for $f < f_1 \approx \pi RT/(12M\eta_{s,0}[\eta]_0)$, the shear viscosity, and consequently $\Delta\alpha/cf^2$, will be independent of frequency, passing to an $f^{-1/2}$ dependence in the region above f_1. Employing the above relation for f_1 to the two lowest molecular weights ($\bar{M}_w = 3.4\times10^3$ and 11.2×10^3) considered in this investigation, one expects the transitions to occur at about 35 and 6 MHz, respectively. Transitions of the correct form are in fact observed in both cases which suggest that the absorption may be intimately related to the normal modes involved in shear viscosity (see Fig. 2).

This approach is not without frailty, however, inasmuch as it does not explain the magnitude of the absorption. Consider, for example, the predicted behavior of the highest-molecular-weight solute employed ($M_w = 3.7\times10^5$) which will yield the greatest theoretical shear viscosity. For a 5% solution at 9 MHz ($f/f_1 \approx 270$), the observed excess absorption will be

[6] W. Philippoff, in *Physical Acoustics*, W. P. Mason, Ed. (Academic Press Inc., New York, 1965), Vol. II, Part B, pp. 1–90.

[7] K. Granath, J. Colloid. Sci. **13**, 308 (1958).
[8] P. Rouse, J. Chem. Phys. **21**, 1272 (1953).
[9] $\bar{M}_w$ is employed in all relevant computation. $\eta_{s,0}$ represents the viscosity of the solvent.

approximately 25×10^{-17} cm^{-1} sec^2 or nearly that of water. The excess attenuation anticipated from the model is less than 2×10^{-17} cm^{-1} sec^2 so that it becomes evident from Eq. (2) that a disproportionately high volume viscosity ($\eta_v'/\eta_s' > 10$) would be required for adherence to the simple approach presented here.

DISCUSSION

At this juncture it is worthwhile to compare the results obtained in dextran to measurements obtained by others in aqueous solutions of another linear polymer, viz., polyethylene glycol. Measurements[10] over the frequency range 10–185 MHz, obtained for molecular weights of 7500 and 20 000 have been interpreted in terms of a single relaxation, i.e., $N=1$ in Eq. (1). While this formulation does provide a somewhat reasonable fit to the data, the total relaxation occurring over this frequency range is somewhat less than that usually necessary to distinguish a single process. That this procedure can be somewhat perilous may be seen in Fig. 3 in which the solid line depicts the single relaxation fit to these data[10] for $M_w = 20\,000$ at 25°C, and the solid points correspond to more recent measurements[11] at 20°C from 1.5 to 80 MHz in the same material, suggesting the presence of multiple relaxations. The relaxation frequency corresponding to the primary mode at this molecular weight, calculated as above for dextran, is 0.95 MHz and it is evident from Fig. 3 that for polyethylene glycol, as well as for dextran (Fig. 2), the absorption behavior above this frequency is in reasonable agreement with the $f^{-1/2}$ dependence.

The absorption behavior in solutions of polyethylene glycol has been measured as a function of molecular weight to a lower limit of 600 by Hammes and Schimmel,[12] and a single relaxation analysis was again employed to characterize the relaxation behavior in the frequency range 14–175 MHz. Although the complete absorption spectra are not presented, they observe that the apparent relaxation frequency decreases with increasing molecular weight to a molecular weight of

about 7000 and is thereafter independent of molecular size. This may be interpreted in the following way: At high molecular weight (above 7000), the primary relaxation frequency is below the frequency range of observation and single relaxation analysis will be insensitive to variations of the primary relaxation frequency. As the molecular weight is lowered, however, the relaxation frequency increases and becomes important in determining the apparent single relaxation parameters as it approaches and enters the experimental range and thus provides a useful qualitative indication of the relaxational properties. Their observations are in this sense compatible with those indicated above, together suggesting the possibility that, in solutions of linear polymers, the normal modes involved in shear viscoelastic behavior may become intimately involved in the ultrasonic relaxation.

In general, the origin of a volume viscosity in associated liquids such as water is believed to be closely related to that of shear viscosity,[13] both involving structural rearrangements of the liquid lattice. In addition, for simple hydrogen-bonded liquids the ratio η_v'/η_s' is usually of the order of 3 or less. For polyethylene glycol, and as indicated above for dextran, the magnitude of the observed absorption would require a substantially higher volume contribution than would be warranted by straightforward predictions from the random-coil model. Thus it appears likely that normal modes of the molecule merely determine the mode of coupling to some other relaxation process involving the solvent. One may speculate, for example, that acoustic perturbation of normal modes accommodates rearrangement of water molecules engaged in weak bond interactions along the polymer backbone. More substantial delineation, however, is not presently available and remains the subject of work in progress.

[10] G. Hammes and T. Lewis, J. Phys. Chem. **70,** 1610 (1966).
[11] W. O'Brien, M. S. thesis in electrical engineering, University of Illinois, 1968.
[12] G. Hammes and P. Schimmel, J. Am. Chem. Soc. **89,** 442 (1967).

[13] T. Litovitz and C. Davis, in *Physical Acoustics,* W. P. Mason, Ed. (Academic Press Inc., New York, 1965), Vol. II, Part A, pp. 281–349.