and $N_c = 3.75 \times 10^{18}$ chains/g of PE; the grafted chains were calculated from equation 10 to be an average of 2000 styrene units in length. So we are indeed talking about very few but long grafted chains which should have a negligible effect on the average polyethylene chain length available for crystallization. Hence the second thermogram provides a good estimate of the apparent graft in the sample.

If we assume that grafting a long polystyrene chain on the surface of a formed polyethylene lamella will strain the crystal, the heat of fusion of the grafted sample will be lower than the ungrafted polyethylene. After recrystallization, the long grafted branches will be excluded from the crystalline region and newly formed crystals will not have the surface strain they experienced before. Therefore, one would expect the heat of fusion determined from the first DSC run to be proportional to the covalent graft. However, the heat of fusion from the second DSC run, after the exclusion of the grafted chains from the crystalline region, will have decreasing values depending on the effect of branching on the average length of the polyethylene chains available for crystallization. If the concentration of branches is very low, as was the case in this research, the second DSC run should be expected to yield heat of fusion values, per gram of polyethylene, close to those obtained from the ungrafted samples.

The deviation of the present results from those predicted by Flory's equation requires some clarification. First of all Flory's model of a copolymer[17] is a completely random one composed mainly of one type of monomer units capable of crystallization and of a second type of monomer units which do not crystallize. Although the production of free radicals by radiation is a random process, these radicals migrate to the surface of the crystals[18] in order to interact with the styrene monomer. In addition, each one of these radicals will initiate a very long nonrandom polystyrene chain. In other words, this system is not a random copolymer. The grafted polystyrene is up to twice the weight of the original polyethylene sample, while the maximum concentration of chains is only 1/10,000 CH_2 units. It is mainly due to this nonrandomness that the depression of the copolymer melting point calculated by Flory's equation was much greater than the measured experimental values. Accordingly, Flory's equation is not applicable to the present system and can only be used in a qualitative manner. It is interesting to note, however, that the deviation of the present results from Flory's equation indicates that the number of branches rather than the mole fraction of the crystalline units seems to be the important factor affecting the depression of the melting point of this copolymer.

Acknowledgments. We gratefully acknowledge the National Science Foundation Departmental Development Grant and the Division of Isotope Development of the U. S. Atomic Energy Commission for the support of this research, the use of the γ radiation facility at the National Bureau of Standards, and the experimental assistance of Mr. M. Needleman.

---

(17) L. Mandelkern, "Crystallization of Polymers," McGraw-Hill, New York, N. Y., 1964, p 74.

(18) T. Kawai, A. Keller, A. Charlesby, and M. G. Ormerod, *Phil. Mag.*, **10**, 779 (1964); **12**, 657 (1965).

Comparison of the Effects of Polydispersity on the Rayleigh Line Width as Determined by Homodyning and Heterodyning Spectrometry

Wu-Nan Huang, Erik Vrancken, and J. E. Frederick*

Institute of Polymer Science, The University of Akron, Akron, Ohio 44325.

Received August 3, 1972

ABSTRACT: Numerical calculations have been made of the spectrum of the Rayleigh line in light scattered from dilute solutions of random-coil polymers having the Schulz distribution of molecular weights; the resulting spectra are comparable to heterodyning experiments. These calculated spectra have been convolved to give results comparable to homodyning experiments. As shown by Reed, the Rayleigh spectral width from heterodyning measurements can be used to calculate an average diffusion constant which is close to the diffusion constant of the species having molecular weight equal to the z-average molecular weight. Homodyning spectra are somewhat more than twice as broad as corresponding heterodyning spectra. Analogous numerical calculations are given for the autocorrelation function of the photocurrent for both homodyning and heterodyning experiments.

With the increasing use of Rayleigh line spectrometry for measuring diffusion coefficients of macromolecules in dilute solution, considerable attention has been given to the effects of polydispersity on such measurements. In principle, it is possible to determine the distribution function of molecular weights from the Rayleigh line spectrum, and mathematical techniques for doing this have been devised. However, extremely accurate spectral measurements are required for successful application of these methods, and determination of a molecular weight distribution using only experimental Rayleigh line data has not yet been demonstrated.

Although it is difficult to determine the distribution function of molecular weights from the Rayleigh line spectrum, it is relatively easy to determine the effect of polydispersity by calculating the spectra which would be observed using known distribution functions. Such calculations show how the shape and breadth of the spectrum depend on the distribution function and its characteristic parameters, typically an average molecular weight and a measure of the breadth of the distribution. The most extensive calculations of this type[1,2] have been made using the Schulz distribution function, and recently Reed[3] has done calculations for the Tung, two-exponent, and log-normal distributions.

A general result of these calculations is that unless the distribution function is very broad, the spectrum of the Rayleigh line is fairly well approximated by the single Lorentzian functional form characteristic of a monodisperse material. In addition, if either the molecular weight or the scattering angle is not too high, the spectral half-width is nearly proportional to the square of the scattering vector κ^2, as for a monodisperse material.[1] The spectral half-width for polydisperse materials has been related to the average molecular weight and polydispersity for several distribution functions, and the influence of the functional form of the distribution has been examined.[3]

Thus far, calculations have been made of the spectrum of scattered light. The results are comparable to data obtained in a heterodyning experiment,[4] where the dominant component of photocurrent spectrum is proportional to the light spectrum. Most experiments, however, utilize the homodyning technique, for which the measured photocurrent spectrum is proportional to the convolution of the light spectrum. Convolution of Lorentzian spectra observed for monodisperse materials merely doubles the spectral breadth without changing the spectral shape. Because the spectra for polydisperse materials are not exactly Lorentzian, convolution causes changes in the spectral shapes, and the half-widths of the convolved spectra are not exactly twice those of the corresponding light spectra.

We give here the results of computations which show the effect of convolution on light spectra calculated for polydisperse materials having the Schulz distribution of molecular weights.[5] Because of the increasing use of autocorrelation techniques, we also give the analogous calculations for autocorrelation, both for the homodyning and the heterodyning experiment. In the discussion that follows, only that part of the spectrum due to translational motion of the macromolecule is considered. The shot noise and dc portions of spectra and terms due to intramolecular motion have been omitted; these are discussed in detail elsewhere.[6,7]

Four quantities have been computed. These are $S(\omega)$, the spectrum of scattered light due to translational motion; $P(\omega)$, the power spectral density of the photocurrent in a homodyning experiment; $C_e(\tau)$, the autocorrelation function of the electric field at the photocathode surface; and $C_i(\tau)$, the autocorrelation function of the photocurrent in a homodyning experiment. The general relationships between these quantities are given in Figure 1. $S(\omega)$ and $C_e(\tau)$, which are each proportional to the results obtained in heterodyning, are a Fourier transform pair. $P(\omega)$ and $C_i(\tau)$, which are proportional to the results of homodyning experiments, are also a Fourier transform pair. $P(\omega)$ is the convolution of $S(\omega)$, and $C_i(\tau)$ is the square of $C_e(\tau)$.

$$\begin{tikzcd}
S(\omega) \arrow[r, "\text{convolution}"] \arrow[d, leftrightarrow, "\text{Fourier transform}"'] & P(\omega) \arrow[d, leftrightarrow, "\text{Fourier transform}"] \\
C_e(\tau) \arrow[r, "\text{square}"] & C_i(\tau)
\end{tikzcd}$$

Figure 1. Relationships between the spectra $S(\omega)$ and $P(\omega)$ and the autocorrelation functions $C_e(\tau)$ and $C_i(\tau)$.

Calculation of Spectra

The translational spectrum S_{oz} of scattered light from random coils having the Schulz distribution of molecular weight is given by[8]

$$\omega S_{oz}(x,z,\alpha) = \frac{\delta(z+1)^{z+1}}{xz!\,\zeta^{z+1-\alpha}}\int_0^\infty \left\{\mathrm{erf}\left[\frac{1}{2}\left(\frac{tx}{\zeta}\right)^{1/2}\right]\right\}^2 \frac{t^{z+\alpha}e^{-t}}{[t^{2\alpha}+\delta^2\zeta^{2\alpha}]}\mathrm{d}t \quad (1)$$

where $\zeta = z + 1 + x/6$, $x = \kappa^2 nb^2/6 = \kappa^2\langle s\rangle_{\mathrm{w}}$, and $\delta = \kappa^2 D_{\mathrm{w}}/\omega$, and the other symbols are defined in ref 1. The Schulz weight distribution function[5] used in deriving eq 1 is

$$f(M) = (1/z)y^{z+1}M^z \exp(-yM) \quad (2)$$

where $y = (z+1)/M_{\mathrm{w}}$. The molecular weight and diffusion constant are assumed to be related by

$$D = CM^{-\alpha} \quad (3)$$

where C and α are constants.

Light spectra were computed according to eq 1 as described previously,[1,8] using a 24-point Gaussian–Laguerre quadrature formula. The range of x was $0.01 \leq x \leq 1.0$, and the range of z was $0.1 \leq z \leq 10$. Four values of α were used: 0.5, 0.55, 0.6, and 0.65; these values cover the range normally encountered with random-coil polymers in usual solvents.[9] For each spectrum the amplitude was computed at 100 frequencies, with the highest frequency ω_{m} being approximately 20 times the spectral half-width at half-maximum amplitude. The spectra were made symmetric about zero frequency in preparation for subsequent convolution by means of the relation $S(-\omega) = S(\omega)$.

The photocurrent spectrum $P(\omega)$ observed in the homodyning experiment is obtained from the light spectrum $S(\omega)$ by convolution according to the relationship[10]

$$P(\omega) = K\int_{-\infty}^{+\infty} S(\omega')S(\omega-\omega')\mathrm{d}\omega' \quad (4)$$

where K is a constant. An IBM 370/155 computer was used to calculate $P(\omega)$ from $S(\omega)$ according to the following procedure. The calculated light spectrum $S(\omega)$ was shifted by an amount $n\Delta\omega$, where $\Delta\omega = \omega_{\mathrm{m}}/50$, and the product of the shifted and unshifted curves was formed for all points where the two curves overlapped. The product function $S(\omega)\cdot S(\omega - n\Delta\omega)$ was then integrated numerically by Simpsons rule.[11] The procedure of shifting by $n\Delta\omega$, forming the product function and integrating, was repeat-

---

(1) J. E. Frederick, T. F. Reed, and O. Kramer, *Macromolecules*, **4**, 242 (1971).

(2) O. Kramer and J. E. Frederick, *Macromolecules*, **4**, 613 (1971).

(3) T. F. Reed, *Macromolecules*, **5**, 771 (1972).

(4) The term "heterodyning" refers here to the experiment in which the scattered light is mixed with a local oscillator, while "homodyning" refers to self-beating of the scattered light. Use of these terms is not consistent in the literature.

(5) G. V. Schulz, *Z. Physik. Chem.*, **43**, 25 (1935).

(6) H. Z. Cummins and H. L. Swinney, *Progr. Opt.*, **8**, 133 (1970).

(7) R. Pecora, *J. Chem. Phys.*, **49**, 1032 (1968).

(8) R. Pecora and Y. Tagami, *J. Chem. Phys.*, **51**, 3298 (1969).

(9) J. Brandrup and E. H. Immergut, Ed., "Polymer Handbook," Interscience, New York, N. Y. 1966, p IV-73ff.

(10) J. M. Stone, "Radiation and Optics," McGraw-Hill, New York, N. Y., 1963, p 323.

(11) M. Abramowitz and I. A. Stegun, "Handbook of Mathematical Functions," U. S. Government Printing Office, Washington, D. C., 1964, p 886.

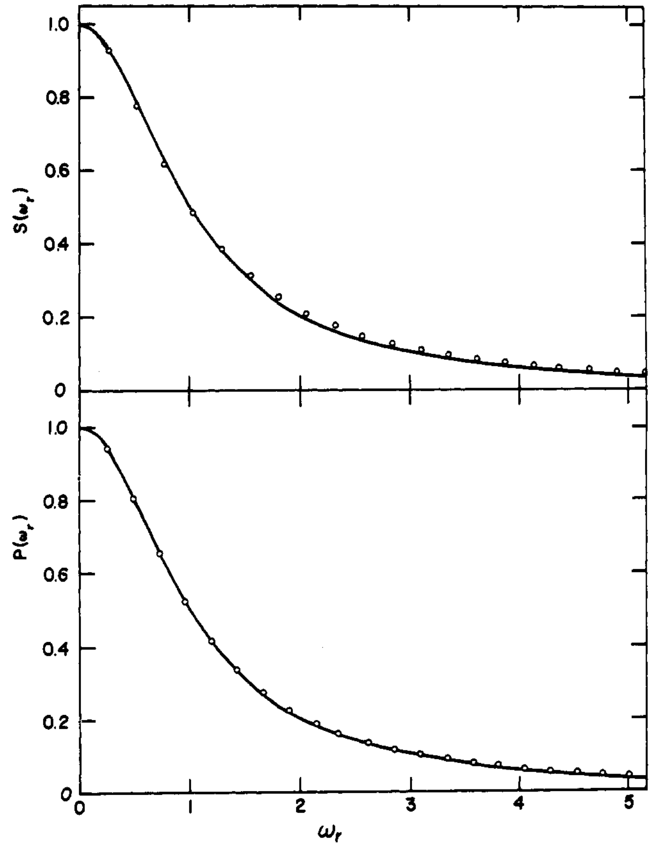

Figure 2. Reduced spectra $S(\omega_r)$ and $P(\omega_r)$ for $x = 0.1$, $z = 0.5$, and $\alpha = 0.5$. (O) Amplitude computed from eq 1; (—) Lorentzian of unit amplitude and half-width.

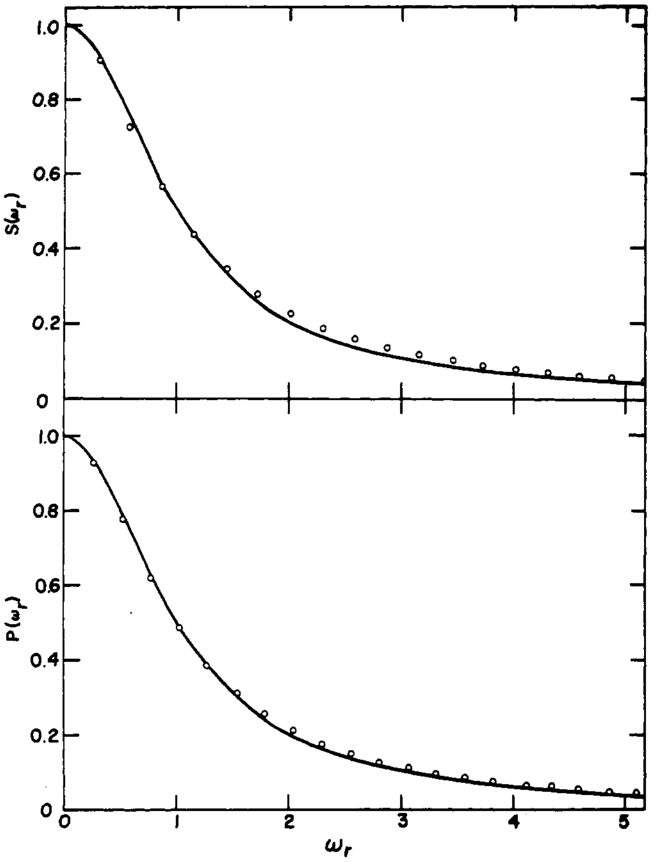

Figure 3. Reduced spectra $S(\omega_r)$ and $P(\omega_r)$ for $x = 0.1$, $z = 0.5$, and $\alpha = 0.65$. (O) Amplitude computed from eq 1; (—) Lorentzian of unit amplitude and half-width.

ed for $n = 0, 2, 4, \ldots, 100$ in order to obtain 50 points of the spectrum $P(\omega)$.

It was determined that for values of ω near the half-width of $P(\omega)$, the error in the amplitude of $P(\omega)$ due to use of finite limits of integration of the product function was negligible compared to the zero-frequency amplitude $P(\omega = 0)$. At high frequencies where the amplitude of $P(\omega)$ was about 1% of $P(\omega = 0)$, the error in the amplitude was less than 0.1% of the zero-frequency amplitude. It was also determined that introducing a finer spacing by dividing the frequency range into more than 100 intervals made a negligible difference in the calculated amplitude of $P(\omega)$ at all frequencies. Round-off errors due to summing a large number of terms according to Simpsons rule were found to be negligible by comparing single-precision and double-precision calculations. The results reported below are from the double precision calculations.

The shapes of the computed spectra depend upon all three of the parameters α, x, and z. The effect of each will be discussed in turn. The effect of α on the spectral shape can easily be seen by comparing Figures 2 and 3, where spectra have been plotted in a reduced form. In each case the amplitudes have been divided by the zero-frequency amplitude, and the frequencies have been divided by the true spectral half-width ω^* to give $\omega_r = \omega/\omega^*$. The solid lines are calculated Lorentzians having unit half-width and unit amplitude at zero frequency. Comparison of the upper panels of Figures 2 and 3 shows the effect on $S(\omega)$ of changing the parameter α. For $\alpha = 0.5$, as in Figure 2, the correspondence between the calculated points and a Lorentzian is fair; increasing α to 0.65, as in Figure 3, causes a larger discrepancy between the calculated spectrum and a Lorentzian.

Comparison of the upper and lower panels of either Figure 2 or 3 shows the effect of convolution. In each case, the convolved spectrum $P(\omega)$ approximates a single Lorentzian better than $S(\omega)$. This demonstrates that use of the homodyning technique for determining properties that depend upon deviations of the spectral shape from the Lorentzian form will be very difficult, since homodyning tends to decrease these deviations.

Close examination of a large number of computed spectra shows that the influence of x on the spectral shape is negligible for $0.01 \leq x \leq 1.0$. The effect of z is that as z decreases, the deviations of both $S(\omega)$ and $P(\omega)$ from a Lorentzian form increase. This is to be expected, since polydispersity increases with decreasing z. The curves shown in Figures 2 and 3 for $z = 0.5$ correspond to a fairly broad distribution of molecular weights. The non-Lorentzian character of the spectra is apparent, although the deviation is not large. For higher values of z, both $S(\omega)$ and $P(\omega)$ approach the Lorentzian form more closely, and the convolved spectra $P(\omega)$ are practically indistinguishable from Lorentzians.

The half-width h of each calculated spectrum $S(\omega)$ and the half-width h' of the corresponding convolved spectrum $P(\omega)$ were obtained by plotting the spectral amplitudes at frequencies near the half-width and determining by interpolation the frequencies at which the spectral amplitudes were one-half their zero-frequency values. The error in the half-width caused by interpolation was estimated to be a few tenths of a per cent or less. The ratios D_c/D_w were then calculated, where D_c is the diffusion constant based on the half-width of the computed spectrum and D_w is the half-width which would be observed in measurements on a monodisperse polymer with molecular weight equal

Table I
D_c/D_z from the Spectrum of Scattered Light $S(\omega)$

		z								
x	α	0.1	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0
0.01	0.50	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	0.55	0.97	0.98	0.98	0.99	0.99	0.99	1.00	1.00	1.00
	0.60	0.96	0.97	0.98	0.98	0.99	0.99	0.99	0.99	0.99
	0.65	0.94	0.95	0.96	0.97	0.98	0.98	0.99	0.99	0.99
0.1	0.50	1.00	1.00	1.01	1.00	1.00	1.00	1.00	1.00	1.00
	0.55	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00
	0.60	0.98	0.98	0.98	0.99	0.99	0.99	0.99	1.00	1.00
	0.65	0.96	0.97	0.97	0.98	0.99	0.99	0.99	0.99	0.99
0.2	0.50	1.02	1.01	1.01	1.01	1.01	1.01	1.00	1.00	1.00
	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	0.60	0.99	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00
	0.65	0.98	0.98	0.98	0.99	0.99	0.99	0.99	0.99	0.99
0.3	0.50	1.03	1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.00
	0.55	1.02	1.01	1.01	1.01	1.00	1.01	1.00	1.01	1.00
	0.60	1.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	0.65	1.00	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00
0.4	0.50	1.04	1.03	1.02	1.02	1.02	1.01	1.01	1.01	1.01
	0.55	1.04	1.03	1.02	1.01	1.01	1.01	1.01	1.01	1.00
	0.60	1.02	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00
	0.65	1.01	1.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.5	0.50	1.06	1.04	1.03	1.02	1.02	1.01	1.01	1.01	1.01
	0.55	1.05	1.04	1.03	1.02	1.01	1.01	1.01	1.01	1.00
	0.60	1.04	1.03	1.02	1.02	1.01	1.01	1.01	1.01	1.00
	0.65	1.03	1.02	1.01	1.01	1.00	1.00	1.00	1.00	1.00
0.7	0.50	1.08	1.06	1.05	1.03	1.03	1.02	1.02	1.01	1.01
	0.55	1.08	1.06	1.04	1.03	1.02	1.02	1.02	1.01	1.01
	0.60	1.07	1.05	1.04	1.03	1.02	1.01	1.02	1.01	1.01
	0.65	1.06	1.04	1.03	1.02	1.02	1.01	1.01	1.01	1.00
1.0	0.50	1.12	1.09	1.07	1.05	1.04	1.03	1.03	1.02	1.02
	0.55	1.12	1.09	1.07	1.05	1.04	1.03	1.03	1.02	1.02
	0.60	1.11	1.08	1.06	1.04	1.03	1.03	1.02	1.02	1.01
	0.65	1.10	1.07	1.06	1.04	1.03	1.02	1.02	1.01	1.01

to the weight-average molecular weight, M_w. For the heterodyning case $D_c/D_w = h/\kappa^2 D_w$, while for homodyning $D_c'/D_w = h'/2\kappa^2 D_w$.

Calculations were carried out only for values of x up to 1.0. For higher x, both theory[7] and experiment[12,13] show that the spectrum contains significant contributions from intramolecular motion in addition to the dominant contribution from translational diffusion. The effects of polydispersity and intramolecular motion are difficult to separate when both occur simultaneously, because at higher values of x polydispersity causes broadening of the spectrum with increasing angle in excess of proportionality to κ^2; this is the same as the effect of intramolecular motion. The magnitude of excess broadening due to polydispersity has been calculated previously[13] for specific values of the parameters z and α over a large range of x.

Results of calculations of the breadth of the light spectrum are given in Table I. This table is comparable to Table I of ref 1, except that the ratio D_c/D_z is given here instead of D_c/D_w. The present form was chosen in order to emphasize the observation made by Reed[3] that for heterodyning experiments on samples for which the molecular weight distribution is not too broad, the average diffusion constant obtained is close to that of the species having molecular weight equal to the z-average molecular weight, M_z. The ratio D_c/D_z was in each case readily calculated from D_c/D_w, since $D_c/D_z = (D_w/D_z)(D_c/D_w)$. According to eq 3, $D_w/D_z = (M_w/M_z)^{-\alpha}$, and for the Schulz distribution function, $M_w/M_z = (z + 1)/(z + 2)$. The close correspondence between D_c and D_z is illustrated in Table I for remarkably wide ranges of the parameters z, α, and x; the ratio D_c/D_z deviates from unity by only a few per cent in most cases. As was noted previously,[1] the change in D_c/D_z with x shows the amount of deviation from proportionality of the half-width to κ^2. The ratio would remain constant if the half-width were proportional to κ^2.

Table II gives the ratio D_c'/D_z to be expected for homodyning measurements. Comparison of Tables I and II shows the difference to be expected between heterodyning and homodyning measurements. When the distribution of molecular weights is narrow (high z), the light spectra are very close to Lorentzian in form. Convolution nearly doubles the spectral width, leading to nearly identical values of D_c/D_z and D_c'/D_z. For higher polydispersity (lower z) or stronger dependence of the diffusion constant on molecular weight (higher α), the convolved spectra become appreciably more than twice as broad as the corresponding light spectra, and D_c'/D_z becomes larger than D_c/D_z.

---

(12) T. F. Reed and J. E. Frederick, *Macromolecules*, 4, 72 (1971).
(13) O. Kramer and J. E. Frederick, *Macromolecules*, 5, 69 (1972).

Table II
D_C'/D_Z from the Convolved Spectrum of Scattered Light $P(\omega)$

		z								
x	α	0.1	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0
0.01	0.50	1.09	1.07	1.06	1.05	1.04	1.03	1.03	1.02	1.02
	0.55	1.09	1.08	1.06	1.05	1.04	1.03	1.03	1.02	1.02
	0.60	1.09	1.08	1.07	1.05	1.04	1.04	1.03	1.03	1.02
	0.65	1.09	1.08	1.07	1.05	1.04	1.04	1.03	1.03	1.02
0.1	0.50	1.10	1.08	1.07	1.05	1.04	1.04	1.03	1.02	1.02
	0.55	1.11	1.09	1.07	1.06	1.04	1.04	1.03	1.03	1.02
	0.60	1.11	1.09	1.07	1.06	1.05	1.04	1.03	1.03	1.02
	0.65	1.11	1.09	1.08	1.06	1.05	1.04	1.03	1.03	1.02
0.2	0.50	1.12	1.10	1.08	1.06	1.05	1.04	1.03	1.02	1.02
	0.55	1.12	1.10	1.08	1.06	1.05	1.04	1.04	1.03	1.02
	0.60	1.13	1.10	1.09	1.06	1.05	1.04	1.04	1.03	1.02
	0.65	1.13	1.11	1.09	1.07	1.05	1.04	1.04	1.03	1.02
0.3	0.50	1.13	1.11	1.09	1.06	1.05	1.04	1.04	1.03	1.02
	0.55	1.14	1.11	1.09	1.07	1.05	1.04	1.04	1.03	1.02
	0.60	1.14	1.12	1.10	1.07	1.06	1.05	1.04	1.03	1.02
	0.65	1.15	1.13	1.10	1.07	1.06	1.05	1.04	1.03	1.02
0.4	0.50	1.15	1.12	1.09	1.07	1.06	1.04	1.04	1.03	1.02
	0.55	1.15	1.13	1.10	1.07	1.06	1.05	1.04	1.03	1.02
	0.60	1.16	1.13	1.11	1.08	1.06	1.05	1.04	1.03	1.03
	0.65	1.17	1.13	1.11	1.08	1.06	1.05	1.05	1.03	1.03
0.5	0.50	1.16	1.13	1.10	1.07	1.06	1.05	1.04	1.03	1.02
	0.55	1.17	1.14	1.11	1.08	1.06	1.05	1.04	1.03	1.02
	0.60	1.18	1.14	1.12	1.08	1.07	1.05	1.05	1.04	1.03
	0.65	1.19	1.15	1.12	1.09	1.07	1.06	1.05	1.04	1.03
0.7	0.50	1.19	1.15	1.12	1.08	1.07	1.05	1.04	1.03	1.02
	0.55	1.20	1.16	1.13	1.09	1.07	1.06	1.05	1.04	1.03
	0.60	1.21	1.17	1.13	1.09	1.07	1.06	1.05	1.04	1.03
	0.65	1.22	1.18	1.14	1.10	1.08	1.07	1.06	1.04	1.03
1.0	0.50	1.23	1.18	1.14	1.10	1.08	1.06	1.05	1.04	1.03
	0.55	1.25	1.19	1.15	1.10	1.08	1.07	1.06	1.04	1.03
	0.60	1.26	1.21	1.16	1.11	1.09	1.07	1.06	1.05	1.04
	0.65	1.28	1.22	1.17	1.12	1.09	1.08	1.07	1.05	1.04

The deviation from proportionality of the half-width to κ^2, reflected by the increase in D_C'/D_Z with x, is more pronounced for the convolved spectra than for the light spectra. Differences between the diffusion coefficients calculated from experimental homodyning and heterodyning spectra have been observed by Herbert and Carlson[14] for myosin in dilute solution, and these differences have been used as supporting evidence for polydisperity due to association.

Because the computed spectra $S(\omega)$ and $P(\omega)$ are not exactly Lorentzian, the exact procedure used to find the spectral half-width influences the value obtained. In the present treatment, the "true" half-widths h or h' were used to calculate D_C/D_W or D_C'/D_W. The true half-width may be determined unambiguously for computed spectra. It would have been possible instead to determine the computed spectral half-widths from a two-parameter least-squares fit to a single Lorentzian, where one parameter is the half-width and the other is the zero-frequency amplitude. The half-widths determined in this way are greater than the "true" half-widths used above. The "true" and least-squares half-widths have been compared previously[2] for the case of $x = 0.1$, $\alpha = 0.5$, and $z = 1$ (corresponding to the "most probable" distribution of molecular weights). The ratio D_C/D_W was found to be 0.83 using the "true" half-width and 0.87 using the least-squares half-width. The same percentage variation would be found in D_C/D_Z.

Least-squares fitting introduces some additional ambiguity in that the half-width obtained depends somewhat upon the range and spacing of frequencies for which the spectra amplitude is calculated. A variation in the half-width of much less than a per cent is typically obtained if the maximum frequency is at least five times the half-width.

The possibility of obtaining numerical results which depend upon the exact procedure used for analysis should be kept in mind when experimental data are analyzed, since experimental spectra are quite likely to be fitted by a least-square method. If high-precision experimental data are very sensitive to the fitting procedure used, a method of analysis other than the one given above should be considered.

Calculation of Autocorrelation Functions

The alternative to determination of photocurrent spectra in either a heterodyning or homodyning experiment is autocorrelation of the photocurrent. Because of its speed and precision, this method is probably to be preferred in most instances. In the case of monodisperse material for which the light spectrum is Lorentzian, Fourier transfor-

---

(14) T. J. Herbert and F. D. Carlson, *Biopolymers*, 10, 2231 (1971).

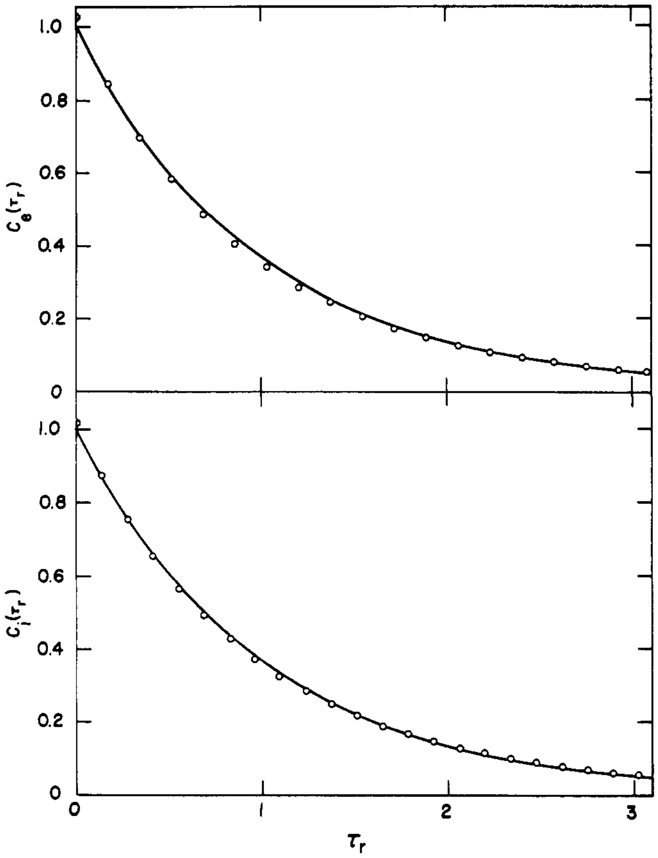

Figure 4. Reduced autocorrelation functions $C_e(\tau_r)$ and $C_i(\tau)$ for $x = 0.1$, $z = 0.5$, and $\alpha = 0.5$. (O) Amplitude computed from eq 5; (—) exponential with unit decay constant.

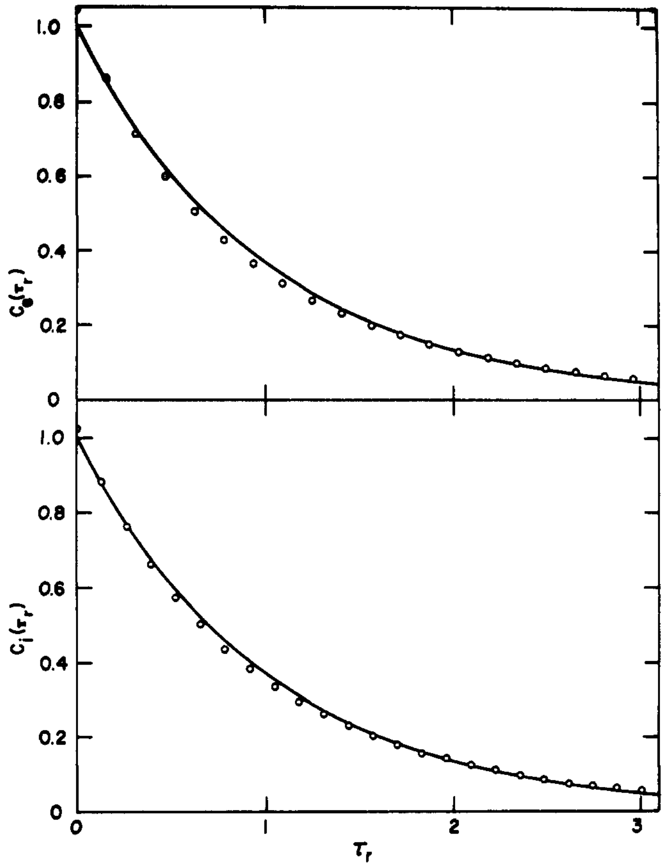

Figure 5. Reduced autocorrelation functions $C_e(\tau_r)$ and $C_i(\tau_r)$ for $x = 0.1$, $z = 0.5$, and $\alpha = 0.65$. (O) Amplitude computed from eq 5; (—) exponential with unit decay constant.

mation of the spectrum shows that the autocorrelation function $C_e(\tau)$ is exponential, with the decay time being equal to the inverse of the half-width (in radians) of the Lorentzian spectrum. If the spectrum is nearly Lorentzian, as for a polydisperse material, then $C_e(\tau)$ will be nearly exponential, and an approximate decay time d may be determined. For the homodyning case the autocorrelation function $C_i(\tau)$ is obtained; $C_i(\tau)$ is proportional to the square of $C_e(\tau)$ and will also be exponential if $C_e(\tau)$ is exponential. Squaring the nearly exponential function $C_e(\tau)$ expected for polydisperse materials should give a function $C_i(\tau)$ which is also close to a single exponential, and again an approximate decay time d' for this function can be determined.

Because autocorrelation weights information in the signal in a different way from spectral analysis, $1/d$ and $1/d'$ would not be expected to exactly equal h and h'. In order to estimate these differences, computations of autocorrelation functions were made in such a way that results could be compared to Tables I and II. As shown in Figure 1, the autocorrelation function $C_e(\tau)$ is the Fourier transform of the spectrum $S(\omega)$. The transform of the spectrum given by eq 1 for a polydisperse sample is given in eq 5.

$$C_{ez}(\tau) = \frac{\pi(z+1)^{z+1}}{2xz!\zeta^{z+1}} \times$$

$$\int_0^\infty \left\{\mathrm{erf}\left[\frac{1}{2}\left(\frac{tx}{\zeta}\right)^{1/2}\right]\right\}^2 t^2[\exp(-\kappa^2 D_w \zeta^\alpha \tau/t^\alpha)]e^{-t}\mathrm{d}t \quad (5)$$

This equation was used to compute $C_{ez}(\tau)$ for the same values of z, α, and x used in calculating spectra. Again computation was performed by means of a 24-point Gaussian-Laguerre quadrature formula. The spectrum $C_{iz}(\tau)$, which is comparable to the results of homodyning experiments, was computed by squaring $C_{ez}(\tau)$. Both $C_{ez}(\tau)$ and $C_{iz}(\tau)$ were then fit to a single exponential by a least-squares program which utilized both the initial amplitude and the decay time as fitting parameters.

The decay time was found to depend slightly on the upper limit of the range of τ over which the least-squares fit was made, although a limiting value of the decay time was approached as the upper limit $\tau_{\max}$ was increased. Typically, $\tau_{\max}$ was taken such that $C(\tau_{\max})$ was a few per cent of $C(\tau = 0)$, since it was determined that the decay time is within 0.1% of its asymptotic value for this condition. An additional fortunate advantage of choosing this range for fitting is that it corresponds to the typical range over which reliable experimental data may be collected.

Figures 4 and 5 show the autocorrelation functions $C_{ez}(\tau)$ and $C_{iz}(\tau)$ for the same values of x, z, and α for which spectra were given in Figures 2 and 3. As would be expected, the calculated functions are satisfactorily approximated by two-parameter single exponential fits. Other analogies with the spectral calculations hold. The deviation between the calculated autocorrelation functions and the least-squares exponential decreases with increasing z and decreasing α, while the effect of x on the shape of the curves is negligible. The function $C_i(\tau)$, corresponding to homodyning, fits an exponential more closely than does $C_e(\tau)$.

The ratios D_c/D_w were calculated for the heterodyning case from the relation $D_c/D_w = 1/d\kappa^2 D_w$, and for the homodyning case the corresponding ratios D_c'/D_w were given by $D_c'/D_w = 1/2d\kappa^2 D_w$. The parameters d and d'

Table III
D_c/D_z from Autocorrelation of the Scattered Field $C_e(\tau)$

		z								
x	α	0.1	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0
0.01	0.50	1.02	1.03	1.03	1.03	1.02	1.02	1.02	1.01	1.01
	0.55	1.00	1.02	1.03	1.03	1.02	1.02	1.02	1.01	1.01
	0.60	0.98	1.01	1.02	1.02	1.02	1.02	1.02	1.01	1.01
	0.65	0.97	1.00	1.01	1.02	1.02	1.02	1.02	1.01	1.01
0.1	0.50	1.04	1.04	1.04	1.03	1.03	1.02	1.02	1.02	1.01
	0.55	1.02	1.03	1.04	1.03	1.03	1.02	1.02	1.02	1.01
	0.60	1.01	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.01
	0.65	1.00	1.02	1.03	1.03	1.03	1.02	1.02	1.02	1.01
0.2	0.50	1.06	1.05	1.05	1.04	1.03	1.03	1.02	1.02	1.01
	0.55	1.05	1.05	1.05	1.04	1.03	1.03	1.02	1.02	1.01
	0.60	1.04	1.05	1.05	1.04	1.03	1.03	1.02	1.02	1.01
	0.65	1.03	1.04	1.04	1.04	1.03	1.03	1.02	1.02	1.01
0.3	0.50	1.08	1.07	1.06	1.05	1.04	1.03	1.03	1.02	1.02
	0.55	1.07	1.07	1.06	1.05	1.04	1.03	1.03	1.02	1.02
	0.60	1.06	1.06	1.06	1.05	1.04	1.03	1.03	1.02	1.02
	0.65	1.05	1.06	1.05	1.04	1.04	1.03	1.03	1.02	1.02
0.4	0.50	1.10	1.08	1.07	1.05	1.04	1.03	1.03	1.02	1.02
	0.55	1.09	1.08	1.07	1.05	1.04	1.04	1.03	1.02	1.02
	0.60	1.09	1.08	1.07	1.05	1.04	1.04	1.03	1.02	1.02
	0.65	1.08	1.08	1.07	1.05	1.04	1.04	1.03	1.02	1.02
0.5	0.50	1.11	1.10	1.08	1.06	1.05	1.04	1.03	1.02	1.02
	0.55	1.11	1.10	1.08	1.06	1.05	1.04	1.03	1.03	1.02
	0.60	1.11	1.10	1.08	1.06	1.05	1.04	1.03	1.03	1.02
	0.65	1.11	1.09	1.08	1.06	1.05	1.04	1.04	1.03	1.02
0.7	0.50	1.15	1.12	1.10	1.07	1.05	1.04	1.04	1.03	1.02
	0.55	1.15	1.12	1.10	1.07	1.06	1.05	1.04	1.03	1.02
	0.60	1.16	1.13	1.10	1.08	1.06	1.05	1.04	1.03	1.02
	0.65	1.16	1.13	1.11	1.08	1.06	1.05	1.04	1.03	1.02
1.0	0.50	1.20	1.15	1.12	1.09	1.07	1.05	1.05	1.04	1.03
	0.55	1.21	1.16	1.13	1.09	1.07	1.06	1.05	1.04	1.03
	0.60	1.22	1.17	1.14	1.10	1.07	1.06	1.05	1.04	1.03
	0.65	1.23	1.18	1.14	1.10	1.08	1.06	1.05	1.04	1.03

are the least-squares single-exponential decay times for $C_{ez}(\tau)$ and $C_{iz}(\tau)$, respectively. The ratios D_c/D_z and D_c'/D_z were then found in the same way as for the spectral calculations above. Results are given in Tables III and IV, respectively. Inspection of Tables III and IV shows that the influence of z, α, and x on the autocorrelation functions is qualitatively the same as for the spectra in most instances. The most striking difference between autocorrelation and spectral results is in the influence of x. The variation of the ratios D_c/D_z and D_c'/D_z is considerably greater for autocorrelation, showing that deviation of the dependence of the halfwidth from proportionality to κ is expected to be more pronounced in autocorrelation measurements. It should be noted that an experimental homodyning autocorrelation function may be converted to the equivalent heterodyning autocorrelation function by taking the square root. Subsequent analysis can then be performed using Table III.

Conclusions

The method used here for treating Rayleigh line measurements on polydisperse random-coil polymers involves at the outset the approximation that the spectrum is close to Lorentzian in form, or equivalently, that the observed autocorrelation function is nearly exponential. Examination of the computed heterodyning spectra and autocorrelation functions show that this assumption is justified for a wide variety of commonly encountered experimental conditions. With data of currently attainable precision, the heterodyning spectrum or autocorrelation function is quite likely to be indistinguishable from a Lorentzian or an exponential, respectively, unless the molecular weight distribution is very broad or the dependence of D on M is unusually strong. In the more common homodyning measurement, deviations from the Lorentzian and exponential forms are further obscured.

Because the shape of the Rayleigh spectrum is not very sensitive to the shape or breadth of the molecular weight distribution function, it does not seem practical to use the technique as a general tool for high-precision characterization. However, line-width measurements may be used in conjunction with Tables I–IV as a very rapid method of determining approximate molecular weights in situations where good estimates of the system polydispersity and the parameter α are already available.

The computed results summarized in Tables I–IV are also very useful for making polydispersity corrections in high-precision studies of random-coil diffusion utilizing samples for which characterization data is already available. Rayleigh line measurements can presently establish the experimental value of the diffusion coefficient with a precision of 1% or less. The tables can then be used to convert the measured value D_c to the value D_z (or alternatively D_w) characteristic of the monodisperse species

Table IV
D_c'/D_z from Autocorrelation of the Self-Beating Photocurrent $C_i(\tau)$

		z								
x	α	0.1	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0
0.01	0.50	1.10	1.10	1.08	1.07	1.05	1.04	1.04	1.03	1.02
	0.55	1.11	1.10	1.09	1.07	1.06	1.05	1.04	1.03	1.02
	0.60	1.12	1.11	1.10	1.07	1.06	1.05	1.04	1.03	1.03
	0.65	1.13	1.12	1.11	1.08	1.07	1.06	1.05	1.04	1.03
0.1	0.50	1.12	1.11	1.09	1.07	1.06	1.05	1.04	1.03	1.02
	0.55	1.13	1.12	1.10	1.08	1.06	1.05	1.04	1.03	1.03
	0.60	1.14	1.13	1.11	1.08	1.07	1.06	1.05	1.04	1.03
	0.65	1.15	1.14	1.12	1.09	1.07	1.06	1.05	1.04	1.03
0.2	0.50	1.15	1.12	1.10	1.08	1.06	1.05	1.04	1.03	1.02
	0.55	1.16	1.13	1.11	1.09	1.07	1.06	1.05	1.04	1.03
	0.60	1.17	1.15	1.12	1.09	1.07	1.06	1.05	1.04	1.03
	0.65	1.18	1.16	1.13	1.10	1.08	1.07	1.06	1.04	1.03
0.3	0.50	1.16	1.14	1.11	1.08	1.07	1.05	1.05	1.04	1.03
	0.55	1.18	1.15	1.12	1.09	1.07	1.06	1.05	1.04	1.03
	0.60	1.19	1.16	1.13	1.10	1.08	1.07	1.05	1.04	1.03
	0.65	1.21	1.17	1.14	1.11	1.09	1.07	1.06	1.05	1.03
0.4	0.50	1.18	1.15	1.12	1.09	1.07	1.06	1.05	1.04	1.03
	0.55	1.20	1.16	1.13	1.10	1.08	1.06	1.05	1.04	1.03
	0.60	1.22	1.18	1.15	1.11	1.08	1.07	1.06	1.04	1.03
	0.65	1.23	1.19	1.16	1.12	1.09	1.08	1.06	1.05	1.04
0.5	0.50	1.20	1.16	1.13	1.10	1.07	1.06	1.05	1.04	1.03
	0.55	1.22	1.18	1.15	1.10	1.08	1.07	1.06	1.04	1.03
	0.60	1.24	1.20	1.16	1.11	1.09	1.07	1.06	1.05	1.04
	0.65	1.26	1.21	1.17	1.13	1.10	1.08	1.07	1.05	1.04
0.7	0.50	1.24	1.19	1.15	1.11	1.08	1.07	1.06	1.04	1.03
	0.55	1.26	1.21	1.17	1.12	1.09	1.07	1.06	1.05	1.04
	0.60	1.29	1.23	1.18	1.13	1.10	1.08	1.07	1.05	1.04
	0.65	1.31	1.25	1.20	1.14	1.11	1.09	1.07	1.06	1.04
1.0	0.50	1.29	1.23	1.18	1.12	1.10	1.08	1.07	1.05	1.04
	0.55	1.32	1.25	1.20	1.14	1.11	1.09	1.07	1.05	1.04
	0.60	1.35	1.28	1.21	1.15	1.11	1.09	1.08	1.06	1.04
	0.65	1.39	1.30	1.23	1.16	1.13	1.10	1.09	1.07	1.05

having molecular weight M_z (or M_w). Such a procedure assumes a Schulz molecular weight distribution, but for samples of low polydispersity the result would be very insensitive to the form of the distribution.

Acknowledgments. This work was supported by the National Science Foundation through Grant GP-8328. We are grateful to the Computer Center of the University of Akron for a grant of IBM 370/155 computer time.

Appendix

It would be desirable to obtain analytically a simple expression which shows exactly what average over molecular weight is obtained by Rayleigh line spectrometry. Although general expressions may be written, it is illuminating to compute numerically the relationship between the spectrum and various averages over the molecular weight distribution function, as has been done in this paper and elsewhere.[1-3,8] The important result demonstrated by Reed[3] that the average molecular weight from line-width measurements is close to the z average for heterodyning experiments on low molecular weight materials at low angles can be rationalized by making two approximations. (1) The measurements are made over a low enough range of x so that the form factor $P(x)$ is essentially constant. (2) The distribution of molecular weights is not too broad.

The expression for the scattered intensity due to translational motion which includes the spectral distribution is[15]

$$S(\kappa,\omega) = \frac{1}{M_w}\int_0^\infty \left(\frac{1}{2\pi}\right)P(x)\frac{2\kappa^2 D}{\omega^2 + [2\kappa^2 D]^2}Mf(M)dM \tag{A-1}$$

Here (M) is the distribution function of molecular weights based on the weight fraction of species of molecular weight M, and a properly normalized Lorentzian spectrum is taken for each scattering species. If x is small, the form factor $P(x)$ is constant and may be removed from the integrand. The Lorentzian distribution remaining in the integrand may be approximated in the vicinity of its half-maximum by a straight line obtained by Taylor series expansion about the half-maximum point; this yields the relation

$$\kappa^2 D/[\omega^2 + (\kappa^2 D)^2] \simeq (1/\kappa^2 D)(1 - \omega/2\kappa^2 D) \tag{A-2}$$

For a narrow enough distribution of molecular weights, the amplitude of the composite spectrum near its half-maximum may be approximated by the integral of the linearized Lorentzians. Then

---

(15) Y. Tagami and R. Pecora, *J. Chem. Phys.*, 51, 3293 (1969).

$$S(\kappa,\omega) \simeq B\int_0^\infty (1/\kappa^2 CM^{-\alpha})(1 - \omega/2\kappa^2 CM^{-\alpha}) Mf(M)dM \quad \text{(A-3)}$$

Here the substitution $D = CM^{-\alpha}$ has been made, and B is a group of constants.

The maximum spectral amplitude at zero frequency is

$$S(\omega=0) = (B/2\kappa^2 C)\int_0^\infty M^{1+\alpha}f(M)dM \quad \text{(A-4)}$$

The frequency $\omega_{1/2}$ at which the composite spectrum attains this half-maximum value is found by setting $S(\kappa,\omega)$ as given by eq A-3 equal to one-half the maximum value given by eq A-4 and solving for ω. The result is

$$\omega_{1/2} = \kappa^2 C\left[\frac{\int_0^\infty M^{1+\alpha}f(M)dM}{\int_0^\infty M^{1+2\alpha}f(M)dM}\right] \quad \text{(A-5)}$$

By analogy with the procedure used in defining the viscosity-average molecular weight, we may define a "Rayleigh line-width average" molecular weight by

$$M_c = \left(\frac{\int_0^\infty M^{1+2\alpha}f(M)dM}{\int_0^\infty M^{1+\alpha}f(M)dM}\right)^{1/\alpha} \quad \text{(A-6)}$$

Then

$$\omega_{1/2} = \kappa^2 C M_c^{-\alpha} \simeq \kappa^2 D_c \quad \text{(A-7)}$$

The average M_c defined by eq A-6 does not correspond exactly to any conventional average. However, just as numerical calculation using common distribution functions shows the viscosity-average molecular weight to be close to the weight average, here similar calculations show that M_c is often close to M_z, the z-average molecular weight. (This may be readily verified using a distribution consisting of equal weights of two polymers which differ in molecular weight.)

The circumstances under which the approximations made in deriving eq A-6 will fail to be valid can be determined by numerical calculation. Examination of Tables I and III shows that because D_c/D_z does not deviate greatly from unity in a variety of circumstances, the approximation that in heterodyning experiments $M_c \simeq M_z$ is better than would be expected. Apparently the errors introduced by approximations compensate one another to some extent when an exact numerical calculation including $P(x)$ is made.[16]

---

(16) We are indebted to the referee for pointing out to us that an alternative means of considering the average obtained which leads to the z-average has been given by P. N. Pusey *et al.* (*J. Phys. (Paris) Colloq., Suppl. 2-3,* **33,** 163 (1972).

Electric Dichroism Studies of Macromolecules in Solutions. II. Measurements of Linear Dichroism and Birefringence of Deoxyribonucleic Acid in Orienting Electric Fields

Kiwamu Yamaoka† and Elliot Charney*

Molecular Biophysics Section, Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014.

Received June 19, 1972

ABSTRACT: A method and apparatus for measuring both optical dichroism and birefringence are described. The method is applied to two systems of biological interest, DNA and a DNA-10-Methylacridine Orange complex. Using native calf thymus DNA, these systems are observed experimentally to follow an almost linear rather than quadratic dependence on field strength at low field strengths, as would be predicted for rigid macromolecules with the dielectric properties of DNA if the orienting mechanism is restricted to the torque exerted by the applied electric field on the permanent and induced moments. Since, at the fields used in these studies, saturation of the orientation was not observed, the precise angle which the planes of the base pairs and of the 10-Methylacridine Orange moieties make with the helical symmetry axis of DNA has not been determined; the data show that the 10-Methylacridine Orange planes are approximately parallel to the planes of the bases and both make an angle larger than 60° with the helix axis. The wavelength dependence of the dichroism of DNA reveals the presence of more than one electronic transition moment with different orientations in the region between 225 and 300 nm. The effect of dichroism on birefringence measurements has been analyzed and applied to the observed birefringence of DNA in the 260-nm band.

Precise information on the intramolecular structure of localized or extended segments of such biologically important molecules, such as proteins, nucleic acids, and their synthetic analogs, has been sought by indirect or derivative methods such as chiral activity, nmr, and a host of others, and by direct methods such as X-ray diffraction. The latter, though a powerful tool for unraveling the structure of biological macromolecules which can be crystallized or drawn into fibers, is of little assistance for studying the solution properties. However, these molecules, which frequently incorporate chromophoric groups

---

†Department of Chemistry, Faculty of Science, Hiroshima University, Hiroshima 730, Japan.