On the other hand, the refractive index increments of DNA preparations under a variety of conditions do not vary by more than ±5%,[20] and similar results would be expected for proteins. Therefore, the intensity B_1 associated with the chemical reaction, according to eq 26a, would be quite small under most circumstances, and the deviation from pure diffusion behavior consequently difficult to detect.

When $D_1 = 6 \times 10^{-7}$ cm^2/sec, $D_2 = 3 \times 10^{-7}$ cm^2/sec, and the scattering angle is 90°, it may be seen from Figure 2 that the half-width at half-height in frequency units ($\omega_{1/2}/2\pi$) varies from 2.09 kHz at $\tau^{-1} = 1$ sec^{-1}, to 2.27 kHz at $\tau^{-1} = 10^4$ sec^{-1}. Thus it is required to detect a frequency shift of 180 Hz to distinguish the rapidly reacting mixture from the slowly reacting one. This shift of 8.6% is easily measurable with current apparatus. When $D_1 = 6 \times 10^{-8}$ cm^2/sec and $D_2 = 3 \times 10^{-8}$ cm^2/sec, the comparable half-widths are 209 Hz for $\tau^{-1} = 1$ sec^{-1}, and 247 Hz for $\tau^{-1} = 10^4$ sec^{-1}. This shift of 38 Hz, or 18%, should also be measurable with adequate precision.

In these calculations we have neglected nonideality effects by setting the coefficients $A_{kj} = 0$. Nonzero values of these coefficients will produce two effects. First, the diffusion coefficients will be functions of the concentrations of the macromolecular and small molecular components of the solution. Therefore, the diffusion coefficients D_A and D_B appearing in the expression for μ, eq 24, should be those determined under the same conditions of ionic strength, temperature, pH, and concentration as those under which the light-scattering measurements on the reacting mixtures are performed. Second, the A_{kj}'s enter into the intensities of the various Lorentzian components, eq 20. The relative intensities of these components will thus be different from the ideal case, but the differences can be minimized by working at low macromolecular concentrations.

From the fact that the half-widths and intensities are identical functions of the diffusion coefficients, chemical relaxation times, and suitably defined equilibrium constants for both the isomerization and dimerization reactions, it may be conjectured that the inelastic light-scattering spectrum of a reacting solution depends only on the number of species and the number of reactions among them. This conjecture will be examined in future work.

Acknowledgments. This research was supported in part by grants from the National Institutes of Health, the A. P. Sloan Foundation, and the Graduate School of the University of Minnesota.

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[20] E. P. Geiduschek and A. Holtzer, *Advan. Biol. Med. Phys.*, **6**, 431 (1958).

Rayleigh Line-Width Measurements of Diffusion Constants of Polydisperse Random-Coil Macromolecules

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ABSTRACT: Measurements of the concentration dependence of the apparent diffusion constant of the polydisperse polystyrene NBS-706 in 2-butanone at 25.0° have been made by Rayleigh line-width spectroscopy over the concentration range 0.0007–0.0046 g cm^{-3}. Extrapolation of these data yields a value of 3.88×10^{-7} cm^2 sec $^{-1}$ for D_c, the apparent diffusion constant at zero concentration for this polymer. Rayleigh line-width measurements of the concentration dependence of two polystyrenes with narrow molecular weight distributions were made in order to establish the relation between the zero concentration diffusion constant D_0 and molecular weight over a limited range. The data for the narrow-distribution polymers allowed determination of D_w, the diffusion coefficient of the species having molecular weight equal to the weight-average molecular weight of NBS-706. The experimental value of D_c/D_w is in good agreement with predictions based on the theory of Pecora for the spectrum of the Rayleigh line in light scattered from solutions of polydisperse random-coil macromolecules. Experiments are also reported which show that very small amounts of dust in solutions can lead to serious error in diffusion constants determined by Rayleigh line-width spectroscopy.

Since its inception in 1964, the technique of Rayleigh line-width spectroscopy has found increasing application to determination of diffusion constants of both synthetic and naturally occurring macromolecules in dilute solution. Several significant advantages of this method have become apparent.[1] Only a small amount of solution is required. A direct measurement of the diffusion constant at a given concentration is obtained from a solution in macroscopic equilibrium; the need for tedious corrections or extrapolations to eliminate effects due to macroscopic concentration gradients is eliminated. Line-width measurements are rapid compared to conventional methods, and the speed of measurement is independent of the diffusion constant; determinations of diffusion constants of slowly diffusing species for which conventional measurements are difficult or impractical may thus be made with acceptable precision. Because of these many advantages, the Rayleigh line-width technique seems likely to gain increasing use in routine measurements of translational diffusion of high-molecular-weight substances in solution.

One complication frequently encountered in such measurements, however, is the effect of sample polydispersity on the shape and width of the Rayleigh line. The effects of polydispersity on the Rayleigh line spectrum have been treated theoretically by Pecora and Tagami[2,3] for both random-coil and rigid-rod macromolecules, and detailed numerical calcu-

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[1] N. C. Ford, Jr., F. E. Karasz, and J. E. M. Owen, *Discuss. Faraday Soc.*, No. **49**, 228 (1970).

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

[3] R. Pecora and Y. Tagami, *ibid.*, **51**, 3298 (1969).

lations based on this theory have been made for the random-coil case by Frederick, Reed, and Kramer.[4] The latter calculations showed that the Rayleigh spectrum from a sample having a fairly broad unimodal distribution of molecular weights would appear very similar to the spectrum from a monodisperse material. The calculated spectra were closely fit by the single Lorentzian form predicted for a monodisperse material, and the breadth was shown to be approximately proportional to the square of the magnitude of the scattering vector κ^2. These predictions of theory were verified by experimental measurements on 0.5 wt % solutions of NBS-706 polystyrene in cyclohexane at 35.0° and in 2-butanone at 25.0°. Numerical calculations also allowed prediction of the ratio between the apparent diffusion constant D_c of a polydisperse material and the diffusion constant D_w of the species having molecular weight equal to the weight-average molecular weight of the polydisperse material.

It was not possible to utilize earlier experimental results to quantitatively confirm the theoretical prediction of D_c/D_w because results were available only for solutions of finite concentration.[4] Quantitative comparison with theory requires the value of the apparent diffusion constant D_c of the polydisperse samples extrapolated to zero concentration. In addition, the zero concentration values of the diffusion constants of at least two other monodisperse samples of known molecular weight must be found in order to establish the dependence of D on molecular weight and thus allow determination of D_w. We present here the concentration dependence and zero concentration value of the diffusion constant D_c for polystyrene NBS-706, which has a most probable distribution of molecular weights, and corresponding results for two narrow-distribution polystyrenes, NBS-705 and W-61970, which have lower and higher molecular weights, respectively, than NBS-706. The results are in good agreement with the theoretical prediction of D_c/D_w. In addition, we point out the large effects of dust on Rayleigh spectra, particularly spectra of solutions having low solute concentration.

Experimental Section

Details of the homodyning spectrometer and the method of collecting data have been given previously.[5] Spectra reported here were obtained in the same way as described before. However, a significant refinement of the method of analyzing data was achieved by least-squares fitting of the power spectral density of the photocurrent to a Lorentzian functional form. Prior to least-squares fitting of spectral data the standard deviation of the amplitude at each frequency was estimated using an experimental relationship described previously.[5]

$$\sigma_L = R(A_T^2 + A_S^2)^{1/2} \tag{1}$$

Here σ_L is the standard deviation of the amplitude of that portion of the signal due to self-beating of spectrally distributed light, A_T is the total (squared) signal amplitude, and A_S is the squared amplitude of the shot noise. The value of R, the experimentally determined proportionality constant between the standard deviation and the squared amplitude A_T, was taken as 0.03 for the 10-Hz bandwidth used for collecting data reported below. The variances of all amplitudes were obtained by squaring the standard deviations σ_L, and each point was then weighted during least-squares fitting in inverse proportion to its variance. This procedure was applied uniformly to all experimental data. Error bars having magnitude $\pm\sigma_L$ have been placed on amplitudes in plots of experimental spectra wherever the bars are large enough to make graphical presentation practical. Least-squares curves are shown as solid lines.

It should be noted that this least-squares procedure is a two-parameter fit. The zero-frequency spectral amplitude is not experimentally determinable with high accuracy because of errors at frequencies below 20–30 Hz due to several causes, including mechanical vibrations[5] and poorer spectrum analyzer performance. It is therefore necessary to utilize only data taken above these frequencies and to regard the zero-frequency amplitude as a fitting parameter to be obtained along with the desired half-width by use of the least-squares procedure. This has been done for all spectra presented below, and the amplitudes have been reduced by dividing by the least-squares value of the zero-frequency amplitude.

It was necessary to give particular attention in this series of experiments to the possible effects of dust in the sample solutions, since the solvent used, 2-butanone, is polar and difficult to clean. Also, it was desired to work at low solute concentrations where the relative magnitude of the scattering from dust is greater than at high concentrations. It was determined by preliminary experiments that simple filtration of 2-butanone solutions was not likely to be adequate for removal of dust. When the solvent was filtered through either 450- or 200-nm Flotronics filters and placed in the unattenuated beam of the Spectra-Physics 125 laser, a considerable quantity of fine dust was readily visible. (Similar filtration of the nonpolar solvent cyclohexane removed virtually all visible dust.) The effect of residual dust was determined by placing the filtered 2-butanone in the spectrometer as if for a spectral determination. Significant highly erratic spectral contributions were observed at frequencies up to 200 Hz, with occasional smaller contributions discernible at frequencies up to 800 Hz. No appreciable spectral contributions were seen with similarly prepared cyclohexane.

All effects of dust could be completely eliminated by high-speed centrifugation of the solvent or solution upon which measurements were to be made. The 2-butanone solvent was filtered through a Flotronics filter having either a 450- or 200-nm pore size into a cylindrical Pyrex cell having a bottom compartment. The bottom compartment was connected through a 1-mm constriction to the top portion of the cell from which scattering was observed. This cell containing solvent was centrifuged for about 2 hr at 1000*g*. After centrifugation no dust was visible when the cell was placed in the unattenuated laser beam, and no spectral contributions other than shot noise could be observed using the maximum sensitivity of the spectrometer. Forced mixing of the solvent from the bottom compartment with that from the top introduced the erratic spectral contributions previously observed with filtered solvent which had not been centrifuged. Centrifuged solvent was found to introduce no spurious spectral components for a period of at least 20 hr after centrifugation.

Two series of experiments were then run using centrifuged and uncentrifuged polystyrene solutions to determine directly the effect of dust on line-width measurements of the diffusion constant. In the first series, solutions of NBS-705 polystyrene in 2-butanone ranging in concentration from 6.6×10^{-4} to 6.7×10^{-3} g cm^{-3} were cleaned by filtration through a 450-nm Flotronics filter, and the spectrum of each solution at 25° was determined at several angles. An average value over angle of $\Gamma_{1/2}/\sin^2(\theta/2)$ was calculated for each solution and used to calculate the diffusion constant D according to the relation

$$D = \frac{\Gamma_{1/2}(\lambda_0/n)^2}{16\pi \sin^2(\theta/2)} \tag{2}$$

where $\Gamma_{1/2}$ is the half-width at half maximum amplitude of the spectrum of the photomultiplier current, determined by least-squares fitting to a single Lorentizian, θ is the scattering angle, λ_0 is the wavelength of the laser light *in vacuo*, and n is the refractive index of the solution. A second series of experiments was then run which was similar to the first except that all solutions were centrifuged in a two-compartment cell for 2 hr at 1000*g* before spectra were measured.

Figure 1 shows a comparison of spectra determined for the same filtered sample of NBS-705 before and after centrifugation. The

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

[5] T. F. Reed and J. E. Frederick, *ibid.*, **4**, 72 (1971).

spectrum of the uncentrifuged sample does not fit a Lorentzian functional form, whereas the spectrum of the centrifuged sample is Lorentzian within experimental error. In addition, the centrifuged sample has a considerably broader spectrum. A more striking demonstration of the effects of dust is given in Figure 2, which compares the results obtained over a concentration range for centrifuged and uncentrifuged solutions. Use of data from the uncentrifuged solutions would obviously have led to completely erroneous conclusions about the concentration dependence of the diffusion constant or its extrapolated value D_0 at zero concentration. These experiments thus indicate that considerable caution is required to ensure that samples used for Rayleigh line-width measurements are as free of dust as possible. Accordingly, each solution for which results are presented was filtered and centrifuged for 2 hr at 1000*g* immediately before spectral determinations were made.

Results

Measurements were made at 25.0° over a range of concentration for each of three samples of polystyrene in 2-butanone. Two of these samples, NBS-705 and W-61970, have relatively narrow distributions of molecular weight. The third, NBS-706, has the fairly broad most probable distribution of molecular weights. Polystyrene NBS-705 was supplied by the National Bureau of Standards; the weight-average molecular weight obtained from light scattering was given as 179,300, and the ratio M_w/M_n of weight- to number-average molecular weights determined by fractionation was 1.07. Sample W-61970 was obtained from Waters Associates, who give M_w as 2,145,000 and M_w/M_n as 1.21. Polystyrene NBS-706 was supplied by the National Bureau of Standards, with M_n determined from osmotic pressure measurements to be 136,500. The weight-average value M_w was 257,800 as determined by light scattering and 288,100 as determined by sedimentation equilibrium.

For each solution the power spectral density of the photomultiplier current was determined at several scattering angles, usually 30, 40, and 50°. A replicate run was made at each angle in most cases. It was determined by a separate series of experiments, some of which have been reported elsewhere,[4,5] that for all three polymers the experimentally determined half-width at half-maximum, $\Gamma_{1/2}$, of the photocurrent was proportional within experimental error to the square of the scattering vector κ^2 given by

$$\kappa = (4\pi n/\lambda_0)\sin(\theta/2) \tag{3}$$

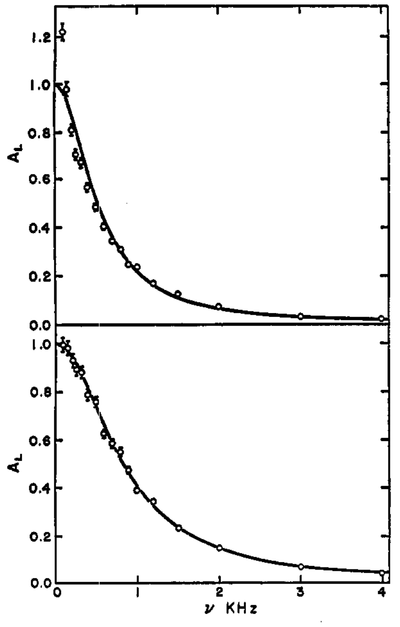

Figure 1. Photocurrent spectra due to light from NBS-705 polystyrene solution in 2-butanone with $\theta = 30°$, solute concentration 18×10^{-4} g cm^{-3}, and temperature 25.0°. The upper curve from the filtered solution has $\Gamma_{1/2} = 529$ Hz; the lower curve from the same solution after centrifugation has $\Gamma_{1/2} = 829$ Hz: ○, experimental; ——, least-squares fits to single Lorentzians.

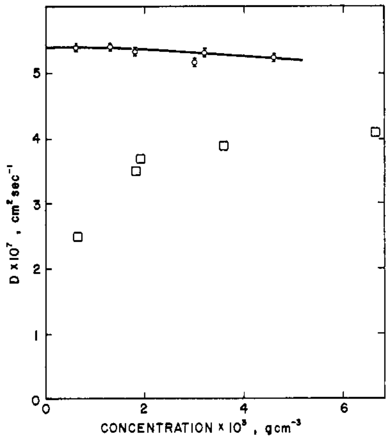

Figure 2. Concentration dependence of the diffusion constant of polystyrene NBS-705 in 2-butanone at 25.0°: ○, centrifuged solutions; □, filtered solutions containing residual dust.

Accordingly, the value of $\Gamma_{1/2}/\sin^2(\theta/2)$ could be averaged over angle and substituted in eq 2 to yield a value of the diffusion constant. For all calculations the refractive index of the solution, n, was taken as 1.374, the refractive index of the solvent. Calculated changes in n due to addition of solute were less than 0.2% at the highest polymer concentration studied and could thus be ignored. The wavelength λ_0 of the laser source *in vacuo* was 632.8 nm.

A representative spectral determination for a solution of NBS-705 is given in the lower section of Figure 1, and the concentration dependence of D is shown as the set of circular points in Figure 2. An estimate of the precision of the values of D obtained was made in the following way. The variance of the six values of D calculated from data obtained at three scattering angles was calculated for each solution. Because the measurements on each solution were taken under similar experimental conditions, the variance was nearly the same for all solutions in the concentration range studied. The average variance was accordingly taken as representative of each solution. The standard deviation (square root of the average variance) for all solutions in the concentration range is about 0.9%; error bars of magnitude plus or minus one standard deviation have been drawn in Figure 2. Extrapolation of these data to zero concentration yields for D_0 a value of 5.40×10^{-7} cm^2 sec^{-1} for NBS-705 polystyrene in 2-butanone at 25.0°.

Similar measurements were made on several solutions of polystyrene W-61970, for which excellent spectra were obtained; an example is shown in Figure 3. The lower set of points (circles) in Figure 4 represents the concentration dependence of the diffusion constant for this polymer. The estimated standard deviation of D in this range of concentrations, calculated as described above for NBS-705 solutions, is also 0.9%, a value too small to be shown conveniently in Figure 4. The value of D_0 for W-61970 estimated by extrapolation of these data to zero concentration is 1.62×10^{-7} cm^2 sec^{-1}.

Rayleigh spectral determinations were made for a series of solutions of the polydisperse sample NBS-706 in 2-butanone.

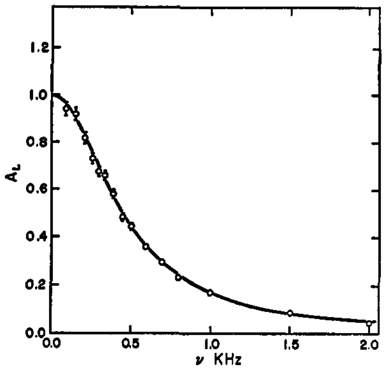

Figure 3. Photocurrent spectrum due to light scattered from a solution of W-61970 polystyrene in 2-butanone with $\theta = 40°$, solute concentration 4.1×10^{-4} g cm^{-3}, and temperature 25.0°: ○, experimental; ——, single Lorentzian of half-width 448 Hz obtained by least-squares fit.

It was previously reported[4] that for solutions of this polymer in cyclohexane the spectra observed by homodyning spectrometry were Lorentzian with half-width proportional to κ^2 This was found to be true also for the 2-butanone solutions over the concentration range of this study. Spectral data for NBS-706 were accordingly analyzed in the same way as for the two monodisperse samples. The closeness of fit between homodyning spectral data and a least-squares Lorentzian curve is illustrated in the lower section of Figure 5. For comparison, the upper section of Figure 5 shows a light spectrum calculated from theory[4] using $x = 0.1$, $z = 1.0$, and $\alpha = 0.5$. (The definitions and choices of values for these parameters have been given in detail previously.[4] Briefly, x is the product $\kappa^2\langle s^2\rangle$, where $\langle s^2\rangle$ is the mean-square radius of gyration; z is the parameter characterizing the breadth of the Schulz distribution function; and α is the exponent in the diffusion–molecular weight relation, given by eq 4.) The solid line is the result of least-squares fitting of the theoretically computed curve to a single Lorentizian using equal weighting of all points. A plot of D as a function of concentration is shown as the upper set of points (hexagons) in Figure 4. The estimated standard deviation in D is again about 0.9% of the measured value at each concentration. The zero-concentration value D_c obtained by extrapolating the apparent values of D obtained over the concentration range shown is 3.88×10^{-7} cm^2 sec^{-1}.

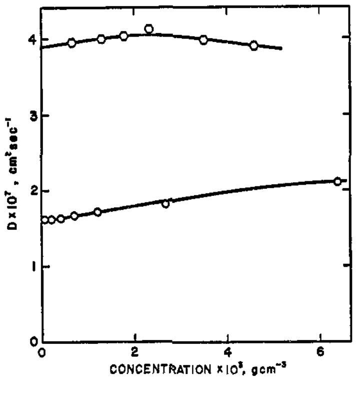

Figure 4. Concentration dependence of the diffusion constant for two polystyrenes in 2-butanone at 25.0°: ○, W-61970; ⬡, NBS-706.

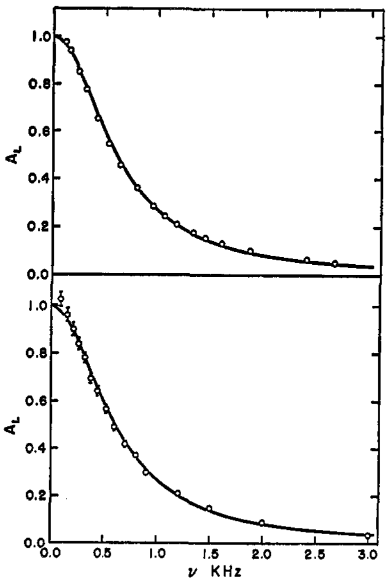

Figure 5. Top: ○, calculated light spectrum for a polydisperse sample with $z = 1$, $\alpha = 0.50$, and $x = 0.1$; frequency units are arbitrary; ——, least-squares Lorentzian. Bottom: photocurrent spectrum due to light scattered from NBS-706 polystyrene in 2-butanone with $\theta = 30°$, concentration 6.6×10^{-4} g cm^{-3}, and temperature 25.0°; ○, experimental; ——, Lorentzian with $\Gamma_{1/2} = 602$ Hz obtained by a least-squares fit.

Discussion

It has been shown previously[4] that the theory of Tagami and Pecora[2,3] for the spectrum of light scattered from solutions of polydisperse random coils can be used to calculate the ratio of D_c/D_w. The value of D_c has been determined directly by the experiments just described, and the value of D_w can be determined from the experimental relationship between the diffusion constant D_0 and molecular weight for monodisperse samples. The measurements reported above for NBS-705 and W-61970 serve to establish this relationship adequately over the limited range required, since these samples have molecular weights below and above, respectively, the known value of M_w for NBS-706. D_w can then be readily obtained by interpolation.

It is well established that for random-coil polymers in dilute solution the diffusion constant and molecular weight are related by an expression of the form

$$D_0 = CM^{-\alpha} \tag{4}$$

where C and α are constants characteristic of the polymer–solvent system at a given temperature. The appropriate plot for presentation of data is accordingly a doubly logarithmic plot of D_0 against M. This plot is shown in Figure 6, where circles are used to plot the diffusion constants of NBS-705 and W-61970. The values of the parameters in eq 4 obtained from Figure 6 are $C = 1.96 \times 10^{-4}$ and $\alpha = 0.49$. This value of α is slightly lower than would be indicated by either theory or experiment.[1] The small discrepancy could easily be due to a small error in the stated molecular weight of W-61970 which would result in an uncertainty in the horizontal position of this point. However, the value of molecular weight at which interpolation is to be performed lies much closer to the molecular weight of NBS-705, and a small error in the position of the point for W-61970 is accordingly tolerable. An error in the horizontal position of NBS-705 is less likely, since this is one of the most carefully characterized polymers available.

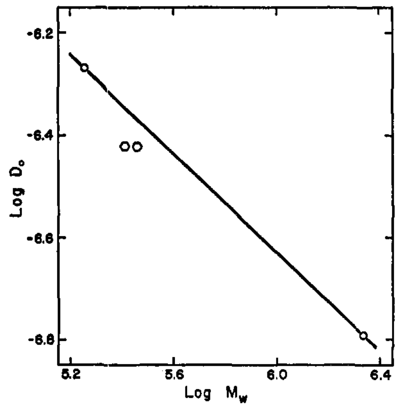

Figure 6. Dependence of D_0 on M_w for three polystyrenes in 2-butanone at 25.0°: ○, narrow-distribution polymers; ○, single measurement for NBS-706, plotted at two values of M_w obtained by light scattering and sedimentation equilibrium.

Because small changes in the value of α cause large changes in the value of C, this parameter is not well determined by the data given here.

Also shown in Figure 6 is the zero-concentration value D_c obtained for the polydisperse NBS-706. The value of log D_c is plotted at two values of log M, one corresponding to the value of M_w obtained by light scattering (257,800 ± 930), the other plotted at the value of M_w determined by sedimentation equilibrium (288,100 ± 9600). D_w may then be read from the graph as either 4.52 or 4.30 $\times$ 10^{-7}, depending on the value of M_w chosen, and the ratio D_c/D_w is then either 0.86 or 0.90. We believe the value of 0.86 resulting from the use of the more precise light-scattering value of M_w to be the better choice.

The theoretical prediction of D_c/D_w is given in Table I of ref 4 as 0.82, where $z = 1.0$, $x = 0.10$, and $\alpha = 0.5$. This is in reasonable agreement with the experimental value of 0.86. However, theoretical and experimental results have not been treated equivalently. Values of D_c/D_w given in ref 4 were calculated using the true half-width of calculated spectra, whereas experimental spectral half-widths were determined by least-squares fitting. If the theoretical curve shown in Figure 5 is fitted by least squares to a single Lorentzian, the resulting new theoretical value of D_c/D_w is 0.85, in excellent agreement with the probable best experimental value of 0.86. This agreement is probably fortuitously good, since the value of D_w can be no more accurate than the molecular weight determinations of the samples used, and the value of D_c depends somewhat on the manner in which it is obtained from the theoretical curve.

It should also be noted that this comparison of the theoretical and experimental values of the ratio D_c/D_w contains an implicit approximation. The theoretical prediction of D_c/D_w is made[4] by dividing the true half-width of the slightly non-Lorentzian calculated light spectrum by the half-width of the Lorentzian light spectrum which would be observed for a species with diffusion constant D_w. The experimental value of D_c/D_w is obtained from photocurrent spectral half-widths which result from homodyning of the light spectra. Homodyning of a Lorentzian light spectrum from a monodisperse polymer results in a Lorentzian photocurrent spectrum having exactly twice the width of the light spectrum. However, homodyning of the slightly non-Lorentzian light spectrum predicted for polydisperse materials like NBS-706 results in a slightly non-Lorentzian photocurrent spectrum with a half-width which is no longer exactly twice the light spectral half-width. Such measurements on a polydisperse sample accordingly cannot determine precisely the half-width of the light spectrum, and derived values of D_c are not exact. However, when the deviation of the light spectrum from a Lorentzian form is small, the ratio D_c/D_w calculated from photocurrent spectral half-widths should be only slightly in error. The extent of the error could be established by convolution of calculated non-Lorentzian light spectra; however, this involves lengthy numerical computation and has accordingly not been attempted.

Detailed discussion of the concentration dependence of the diffusion coefficient for NBS-705 and W-61970 has been deferred until data for a wide range of molecular weights can be obtained by Rayleigh line-width measurements. The present results are qualitatively consistent with the results of Tsvetkov and Klenin,[6] but quantitative comparison with the older literature is restricted by the possible lower accuracy of characterization of the molecular weight or polydispersity of the samples used. More importantly, conventional techniques of measuring the diffusion constant weight the effects of sample polydispersity differently from line-width measurements. It accordingly seems that quantitative analyses are best performed using data obtained by a single technique until comparisons are available of frictional properties as obtained by both Rayleigh line-width and conventional measurements on the same system.

In summary, the experimental results presented indicate that the theory of Pecora for the spectrum of light scattered from dilute solutions of polydisperse random-coil macromolecules is quantitatively correct within the present accuracy of homodyning measurements of the Rayleigh line-width and macromolecular characterization methods. The theory may accordingly be used to relate the apparent diffusion constant of a polydisperse sample obtained from line-width measurements to the diffusion constant of the species of molecular weight M_w when an estimate of the degree of polydispersity is available. Care must be taken, however, to avoid the serious errors caused by even small quantities of dust in samples to be used for Rayleigh line-width studies.

Acknowledgments. Support of this work under National Science Foundation Grant No. GP-8328 is gratefully acknowledged.

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(6) V. N. Tsvetkov and S. I. Klenin, *J. Polym. Sci.*, 30, 187 (1958).