Optical Probe Study of a Nonentangling Macromolecule Solution—Bovine Serum Albumin:Water*,[1]

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The diffusion coefficient D of polystyrene latex spheres in bovine serum albumin:water was studied as a function of protein concentration c for $0 < c < 200$ g/liter. The Stokes–Einstein equation for D fails by as much as 25 to 50%, D being larger than predicted from the sphere radius R and the solution viscosity. Probe particles with R as large as 0.62 μm were used. D fits well to the form $D = D_0\exp(-\alpha c^{\nu})$ for $\alpha = 0.004$ to 0.008 and $\nu = 0.96$ to 0.99. Serum albumin is a globular protein, so chain entanglement cannot cause these non-Stokes–Einsteinian effects, which are presumably due to sphere:albumin interactions. Polystyrene spheres in semidilute polyethylene oxide:water (G. S. Ullmann, K. Ullmann, R. M. Lindner, and G. D. J. Phillies, *J. Phys. Chem.* **89**, 692 (1985)) behave similarly to spheres in serum albumin:water, suggesting that chain entanglement may also not be important in probe diffusion through semidilute polymer solutions.

INTRODUCTION

Light-scattering spectra of multicomponent systems are in general expected to be quite complex. However, great simplifications arise if all solute components but one are isorefractive with the solvent, and if the nonisorefractive ("scattering") component is dilute (1, 2). In this special case, the isorefractive components scatter no light, and hence make no direct contribution to the light-scattering spectrum. The spectrum is due solely to the unique scattering component. As discussed in Appendix A, in the limit that this component is dilute, light-scattering spectroscopy *effectively* obtains the single-particle ("tracer" or "self") diffusion coefficient D of the scattering component as it moves through a multicomponent solution (2). D reflects the dynamics of all of the components of the system, including those components which scatter no light. By analogy with ESR and neutron scattering, in which dilute free-radical or isotopically labeled probes are used to study solution dynamics, we shall refer to the dilute, scattering component as an "optical probe," and to D as the "probe diffusion coefficient."

The results reported here are an extension of previous studies from this laboratory on the diffusion of optical probes in viscous liquids (3), colloid suspensions (2, 4), and neutral (5–7) and charged (8–10) synthetic polymers. Related work has been performed by Kops-Werkhofen *et al.* (11) on concentrated silica sphere suspensions and by Lodge (12) on mixtures of synthetic polymers. Besides the information which probe diffusion yields on fundamental properties of complex solutions, particle diffusion in macromolecule solutions arises naturally in several contexts, such as biopolymer transport *in vivo*.

Our original intent was to examine the range of validity of the Stokes–Einstein equation

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$$D = \frac{k_B T}{6\pi\eta R} \qquad [1]$$

where k_B is Boltzmann's constant, T is the absolute temperature, R is the radius of the (presumed to be spherical) probe particle, and η is the macroscopic shear viscosity of the solvent, as measured with a conventional capillary viscometer. By "solvent" we mean the fluid in which the probes are suspended, including both its small-molecule and its polymer components. Equation [1] provides an accurate description of the diffusion of dilute large particles through highly viscous, small-molecule solvent systems, such as water: glycerol (3). In solutions of synthetic polymers, including the water:polyacrylic acid mixtures studied by Lin and Phillies (8–10) and the water:polyethylene oxide systems studied by Ullmann *et al.* (5–7), the Stokes–Einstein equation does not always work. For probe particles in a system of fixed composition, Eq. [1] does predict the temperature dependence of D (8). However, at fixed temperature, Eq. [1] does not predict how D depends on η. (η of a water:polymer mixture may be varied at fixed T by changing the polymer concentration c.) When the polymer molecular weight M is large enough, we find that D is larger (7, 9, 10) than predicted by Eq. [1]. This non-Stokes–Einsteinian behavior depends on the probe diameter. The deviation from Eq. [1] increases with increasing c and M.

Modern theories of polymer dynamics (13, 14) divide polymer solutions into three concentration ranges: the dilute, the semidilute, and the concentrated. In the dilute range, the distance between adjoining polymer molecules is typically much larger than the polymer radius of gyration R_G. In the semidilute concentration regime $c > M/R_G^3$, neighboring polymer molecules are separated by typical distances less than R_G. Neighboring chain molecules therefore overlap, intertwine, and become entangled. The modern theories claim that the properties of semidilute polymer solutions, such as those studied in Refs. (5–10), are dominated by topological entanglements between adjoining polymer chains. [This use of "entanglement" is based on concentration and molecular weight concentrations, not on rheological data, and therefore is not necessarily equivalent to classical references to "entanglement effects" in the concentration and molecular weight dependence of the viscosity.]

The failure of Eq. [1] for probes in polymer solutions might be due to entanglement. However, a globular protein cannot form topological entanglements. Allis and Ferry (15) have found that nondenatured solutions of serum albumin in water show no viscoelastic effects, only Newtonian viscosity, over the broad frequency range 0.04–400 Hz. If a protein neither entangles nor shows viscoelastic effects, it might be supposed that effects leading to non-Stokes–Einsteinian behavior are also absent, so that the Stokes–Einstein equation would be valid for the diffusion of a large probe particle through a concentrated protein solution.

We here report an experimental study on the diffusion of polystyrene latex spheres through water:serum albumin:0.15 M NaCl. The Stokes–Einstein equation fails, the probe particles being found to diffuse faster than predicted from Eq. [1]. From the standpoint of biophysical chemistry, this finding is disappointing. If Eq. [1] were valid in protein solutions, probe diffusion measurements would provide a convenient microscale method for determining the viscosity of biopolymer solutions, thereby allowing hydrodynamic studies of particle shape while using extremely small amounts of material. This paper is concerned with physicochemical issues, including the dependence of D on protein concentration and probe radius, and the correlations between this study and optical probe studies on synthetic polymer solutions.

EXPERIMENTAL METHODS

Bovine serum albumin (BSA; Sigma, crystallized and lyophilized, essentially fatty acid

free) and carboxylate-modified polystyrene latex spheres of diameters 0.12 μm (nominal surface charge 0.12 meq/g polymer), 0.70 μm (nominal surface charge 0.46 meq/g polymer), and 1.28 μm (nominal surface charge 0.12 meq/g polymer) were obtained commercially. In pure water, the 0.12-, 0.7-, and 1.28-μm spheres have diffusion coefficients of 4.64×10^{-8}, 7.44×10^{-9}, and 3.66×10^{-9} cm^2/s, which imply effective hydrodynamic radii of 517 Å, 0.322 μm, and 0.655 μm, respectively.

Protein solutions were made by dissolving weighed amounts of BSA in 0.15 *M* NaCl. The solution pH was adjusted to 7.0 with small amounts of NaOH. Each protein solution was filtered into a scattering cell through a 0.22- or 0.45-μm Nucleopore filter. The polystyrene latex spheres were then micropipetted into the filtered protein solutions without themselves being filtered.

Viscosities were obtained with a calibrated Cannon–Fenske (capillary) viscometer. Diffusion coefficients were measured with a quasi-elastic light-scattering spectrometer, using a 20-mW He–Ne laser and 90° scattering angle. The working temperature was 24.8 $\pm$ 0.2°C. The detector was an RCA 7265 photomultiplier tube. A pair of irises placed in the path of the scattered light restricted the detector to viewing a few coherence areas. Correlation studies were made with 64- and 144-channel Langley–Ford digital correlators. The correlators were adjusted so that the half-maximum of the spectrum appeared between the eighth and the fifteenth correlator channel. The first three data points of the spectrum, which contained possible artifacts due to weak scattering by the serum albumin, were not used in the spectral analysis.

Spectra were analyzed with Koppel's method of cumulants (16). The optimal number of cumulants was chosen with statistical tests. The best fit was assumed to give a positive or zero value for the second cumulant (since no known physical effect other than noise leads to negative values of this parameter), to tend to minimize the root-mean-square difference between the measured and the calculated spectrum, and to tend to minimize the magnitude of the quality parameter

$$Q = \sum_{i=1}^{N-1} (C_i - S_i)(C_{i+1} - S_{i+1}). \quad [2]$$

Here C_i is the calculated spectrum in the ith channel, S_i is the measured spectrum in the ith channel, and the sum on i is over all but the final signal channel in the correlator.

D is related to the first cumulant K_1 by

$$D = K_1/k^2 \quad [3]$$

where k is the magnitude of the scattering vector. To determine k, the index of refraction of each solution was measured with a Bausch & Lomb Abbe-56 refractometer.

RESULTS

D of each probe species was determined in BSA solutions with protein concentrations covering the range 0–200 g/liter. The viscosity of each solution was also determined. Multiple measurements on a single sample found that D remains constant over periods of 3–6 h; the polystyrene spheres do not aggregate slowly on a multihour time scale.

Figures 1a–c show measurements of D (open points) for all three sphere sizes, as a function of protein concentration. Each point is an average of several (generally three) measurements made on a single sample; the error bars indicate the range of scatter in D. Each set of measurements is normalized by D_0, the diffusion coefficient of the probe particles in the limit of low protein concentration. At low protein concentration the diffusion coefficient of the probes is quite close to D in pure water. As c is increased, D falls, the decline being slower than linear in c.

The filled points and the dashed line in Figs. 1a–c indicate the fluidity η^{-1} of the solvent, as normalized by the fluidity η_0^{-1} of pure water. At concentrations above 100 g/liter, the concentration dependence of D for

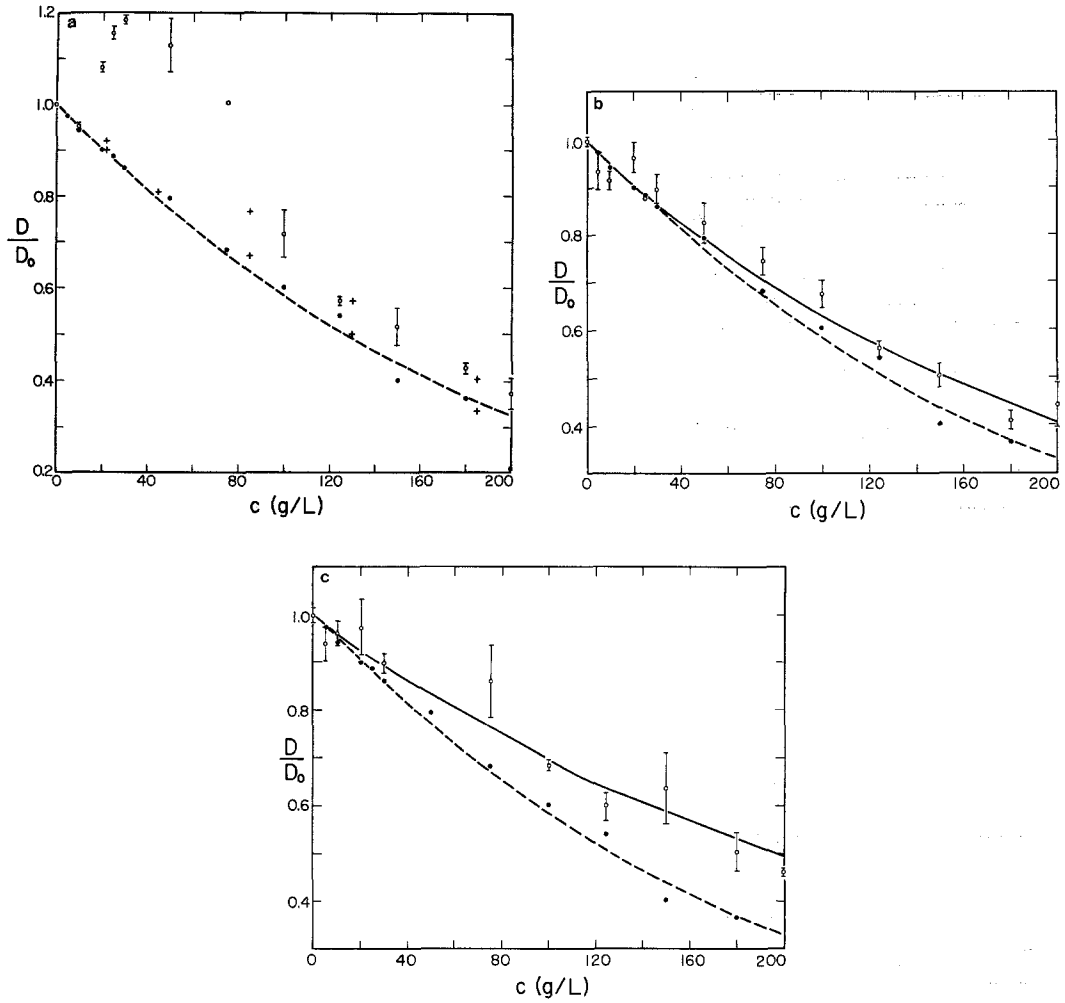

FIG. 1. Concentration dependence of the diffusion coefficient D of (a) 517-Å-, (b) 0.322-μm-, and (c) 0.655-μm-radius carboxylate modified polystyrene spheres in bovine serum albumin:water solutions. Values of D (open points) are normalized by D_0, the diffusion coefficient of the spheres in the limit of low protein concentration. The solid curves are fits of the data to Eq. [4], using parameters of Table I. Crosses are the self-diffusion coefficient D_s/D_{s0} of BSA, as taken from Refs. (19, 20). Filled points and dashed line are the normalized fluidity η^{-1}/η_0^{-1} of the BSA solutions.

the 0.12-μm spheres parallels the concentration dependence of η^{-1}/η_0^{-1}; at lower protein concentrations, the 0.12-μm spheres show a more complex concentration dependence. At protein concentrations above 25 g/liter, D of the 0.7- and 1.28-μm spheres rises above the fluidity curve, that is, for $c > 25$ g/liter the larger spheres diffuse more rapidly than expected from the macroscopic viscosity of the solution.

It is well known (17) that proteins can be adsorbed irreversibly by polystyrene spheres. Such adsorption increases the physical radius of the probe particles. Furthermore, if the spheres are only partially coated with protein molecules, the protein molecules can crosslink spheres, forming sphere dimers and oligomers, or even precipitating the spheres from solution. If the average hydrodynamic radius of the probe particles were increased, whether

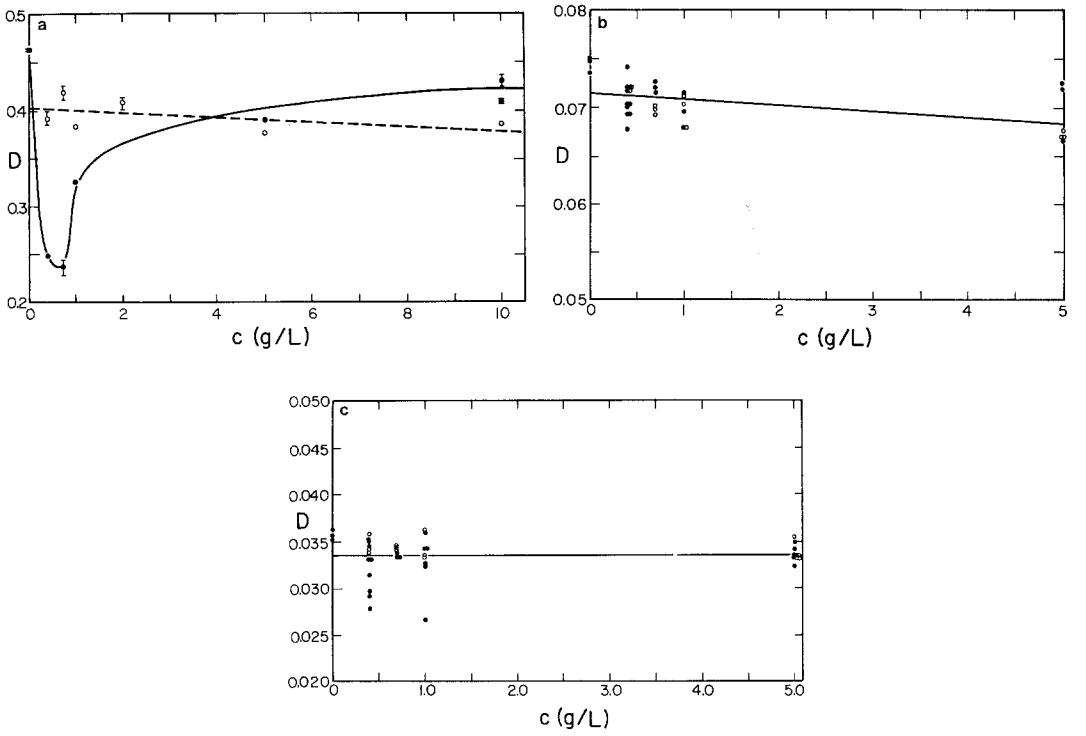

FIG. 2. Dependence of D of (a) 517-Å, (b) 0.322-μm, and (c) 0.655-μm spheres in BSA for (○) diluted spheres and (●) undiluted spheres, as described in the text. Straight lines are linear best fits of the data. The solid curve of Fig. 2a is drawn to guide the eye.

by protein adsorption or by oligomerization, D would be expected to be reduced, causing measurements of D/D_0 to fall below the fluidity curve. As seen in Fig. 1, D/D_0 actually deviates above the fluidity curve. These potential artifacts have the wrong sign to explain most of our data.

Figure 2a plots the diffusion coefficient of the 0.12-μm spheres in dilute protein solutions, demonstrating the consequences of oligomer formation on the apparent hydrodynamic radius. In Fig. 2, the filled circles refer to experiments in which the spheres were placed directly into serum albumin solutions of the indicated concentrations. If the serum albumin was dilute ($0.4 < c < 5$ g/liter), the spheres were only partially coated with protein, leading to crosslinking, oligomer formation, and a reduction of D. On the other hand (open circles), if the spheres were first placed in 10 g/liter serum albumin, and then diluted with distilled water to the indicated protein concentrations, the individual spheres remained completely coated with protein, and did not oligomerize. The dashed line indicates the concentration dependence of the fluidity of dilute protein solutions; D of the fully coated, nonoligomerizing spheres (open circles) simply follows η^{-1}. D in the limit $c \rightarrow \theta$ is less than D in pure water; the thickness of the adsorbed serum albumin layer is inferred to be 300 Å. This layer of protein need not be solid. Because of hydrodynamic screening, a limited amount of adsorbed protein jutting out for 300 Å, at a few points on the sphere surface, would have almost the same effect on D as a solid protein coat 300 Å thick.

Figures 2b and c show similar dilution experiments on the 0.7- and 1.28-μm spheres. In these cases, the sphere concentrations were lower, substantially eliminating sphere oligo-

merization due to protein crosslinking. The concentration dependence of D is not changed by the method used to prepare the samples; the thickness of the adsorbed protein layer is less than the fractional error in the estimate of R.

With the 0.12-μm spheres, in addition to the oligimerization effect of Fig. 2a, we observed another systematic irregularity in D. As seen in Fig. 1a, in the concentration range $10 < c < 100$ g/liter, D increased with increasing protein concentration, so that $D > D_0$. A similar phenomenon was observed with the 1.28-μm spheres in 18500 MW polyethylene oxide (6). An obvious interpretation is that the protein/polymer component is causing sphere dimers, present in the initial stock solution of spheres, to separate. Monomerization of sphere assemblies would increase the average D of particles in solution, though the change in D is rather large for a simple dimer-to-monomer transition, unless the fraction of dimerized spheres was improbably large. Furthermore, if the increase in D were a dimer $\rightarrow$ monomer transition, one might have expected that polyethylene oxides of molecular weight 7500, 1×10^5, or 3×10^5 would have at least some tendency to promote the same transition. They do not; nor does 0.1 wt% Triton X-100. This anomaly in D was not studied further; the corresponding data on 0.12-μm spheres for $10 < c < 100$ g/liter were not included in the numerical analysis below. The larger spheres are considerably better behaved than the 0.12-μm spheres.

The self-diffusion coefficient D_s of serum albumin—the probe diffusion coefficient in serum albumin of an albumin-size probe—has been measured with good accuracy by Keller *et al.* (19) and Kitchen *et al.* (20). Their data for D_s/D_{s0} are indicated in Fig. 1a by the crosses. D_s matches the solution fluidity. The probe diffusion coefficient thus agrees with Eq. [1] if the probe particles are small, but comes into disagreement with Eq. [1] as the probe particles are enlarged. There is a common intuition that the Stokes–Einstein equation is a near-continuum approximation (very large probe, very small "solvent" molecules), which is more likely to fail if the probe and the surrounding molecules are similar in size. Equation [1] would therefore be expected to fail for small probe particles and not large ones; our experimental data are therefore somewhat counterintuitive.

DISCUSSION

In previous work on optical probe diffusion in water:polyacrylic acid (8–10) and water:polyethylene oxide (5–7), it was found that the concentration dependence of the diffusion coefficient is fit well by the form

$$\frac{D}{D_0} = \exp(-\alpha c^{\nu}), \qquad [4]$$

where α and ν are adjustable parameters which vary from system to system. A similar expression, with different values of α and ν,

$$\frac{\eta}{\eta_0} = \exp(-\alpha c^{\nu}) \qquad [5]$$

was found to describe the behavior of the viscosity. A nonlinear least-squares fitting procedure was used to obtain values for α and ν which give the best description of our data. Since the noise sources in our measurements all lead to errors in D and η which are a fixed fraction of the absolute values of these parameters, and errors in c are negligible, the correct quantity to minimize is

$$\sum_{i=1}^{T} \left[\ln \frac{D_i}{D_0} + \alpha c_i^{\nu} \right]^2. \qquad [6]$$

D_i and c_i are the diffusion coefficient and the concentration for the ith of the T data points.

The results of the procedure are indicated in Table I and by the lines in Figs. 1a–c. ν is in the range 0.92–0.97, which is larger than the $\nu \approx \frac{2}{3}$ observed for polystyrene spheres in water:polyacrylic acid or the $\nu \sim 0.6$ to 0.76 observed in water:polyethylene oxide using serum albumin as a probe. However, in water:polyethylene oxide, with polystyrene sphere probes, Ullmann *et al.* (5–7) observed

TABLE I

Nonlinear Least-Squares Optimizing Parameters for Representing D/D_0 of Polystyrene Spheres in Water: BSA by the Form $D/D_0 = \exp(-\alpha c^{\nu})$

Sphere	α	ν
0.120	8.0×10^{-3}	0.92
0.700	5.3×10^{-3}	0.97
1.28	4.4×10^{-3}	0.96

values for ν close to the 0.9–1.0 found here.

The α for spheres in serum albumin solutions is substantially smaller than α for spheres in solutions of synthetic polymers. As shown in Table II in previous studies α has almost always been within a factor of 2 of 0.1. Table II includes our previous probe diffusion studies on systems in which Eq. 1 fails. Not included are findings on solutions of low-molecular-weight polymers in which the Stokes–Einstein equation is obeyed.

Figure 3 is a log–log plot of α against molecular weight for the systems in Tables I and II. Careful examination shows that α depends substantially on the polymer molecular weight. In preparing Fig. 3, consideration was given to the effective molecular weight of a polymer in solution. Synthetic polymers typically have loose, extended structures; as a polymer molecule diffuses, the solvent which it encloses is carried along by hydrodynamic interactions. This solvent is not included in the nominal polymer molecular weight. A globular protein has a compact structure with only limited amounts of water of hydration, so that the molecular weight of a protein includes more or less all the material in the volume enclosed by the protein. A globular protein thus has a much larger molecular weight than a random coil polymer having the same size. To correct for this semantic difference, we plotted the synthetic polymers at their nominal molecular weights. Data for probes in serum albumin were plotted both at the true molecular weight

TABLE II

Fits of Experimental Measurements (5–10) of the Diffusion Coefficient to Eq. [4] for Various Polymers and Probe Species

Polymer	M	c	Probe	R	α	ν
PEO	1×10^5	0–3	PSL	208 Å	(0.013)	(1.95)
				517 Å	0.046	1.35
				0.32 μm	0.064	1.00
	3×10^5	0–3	PSL	208 Å	0.14	0.88
				517 Å	0.14	0.92
				0.32 μm	0.12	0.82
	1×10^5	0–5	BSA	37 Å	0.08	0.6
	3×10^5	0–3	BSA	37 Å	0.25	0.76
PAA	3×10^5	0–150	PSL	204 Å	0.236	0.65
				800 Å	0.208	0.66
				0.62 μm	0.184	0.67
				1.5 μm	0.151	0.67
	1×10^6	0–20	PSL	204 Å	0.227	0.63
				800 Å	0.262	0.54
				0.62 μm	0.696	0.74

Note. PEO = polyethylene oxide:water; PAA = polyacrylic acid:water; PSL = polystyrene latex; BSA = bovine serum albumin; ( ) = marginal results.

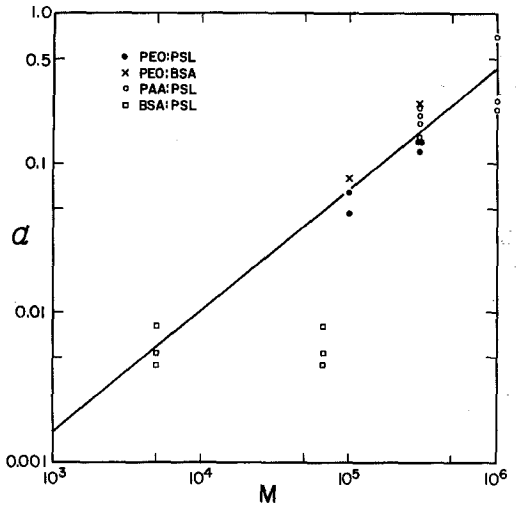

FIG. 3. The parameter α of Eq. [4] as a function of M, based on Tables I and II. Results for polystyrene latex probes in serum albumin are plotted at an effective M of 5×10^3 amu determined from the hydrodynamic radius of BSA (see text for details).

(67,000 amu) of serum albumin and at the molecular weight (5000 amu) of a polyethylene oxide species whose radius of gyration R_g equals the hydrodynamic radius (37 Å) of serum albumin. (R_g was obtained from the data of Destor *et al.* (18) on polyethylene oxide, and the scaling relation (12) $R_g \sim M^{0.6}$ for neutral polymers.)

In Fig. 3 the solid line indicates a linear least-squares fit to the points. Our data are consistent with $\alpha \sim M^{\alpha}$ with $\gamma = 0.8 \pm 0.2$. In semidilute solutions, we do not find that α is independent of M, as predicted from some scaling arguments. There is a substantial spread in α for the 10^6-amu polyacrylic acid samples.

The dynamic behavior of semidilute polymer solutions has often been interpreted in terms of topological entanglements between polymer chains, a topological entanglement being a knotted configuration of polymer chains, in which chains cannot readily move relative to each other in any direction. Serum albumin is a compact ellipsoidal particle; in the sense given here, serum albumin molecules cannot entangle. Entanglement effects therefore do not cause the concentration dependence of D in this system.

The behaviors of polystyrene latex spheres in concentrated BSA and in polyethylene oxide are rather similar. In each case, the diffusion coefficient follows Eq. [4], and is larger than expected from the fluidity of the solution. In BSA, this behavior cannot be due to polymer entanglement, suggesting that the like behavior seen in polyethylene oxide solutions is also not exclusively controlled by entanglement effects. As an alternative, one notes the possible role of collisions, similar to those responsible for the dynamic frictional contribution to the mutual diffusion coefficient [21]. Serum albumin molecules cannot pass through each other, so collisions hinder translation. Furthermore, serum albumin is ellipsoidal, so its rotational motions are hindered in concentrated solution. Collisions between adjoining molecules, restricting rigid-body translation and rotation, act equally in protein and synthetic polymer solutions, which would account for the similar behaviors of D/D_0 in the two systems.

It is presumably possible to interpret the concentration dependence of D in terms of the forces between the polystyrene spheres and the protein molecules. In monodisperse protein solutions (22, 23), the mutual diffusion coefficient D_m of the protein has been successfully interpreted in terms of hydrodynamic, electrostatic, and hard-sphere interactions, as determined from the self-diffusion coefficient and the osmotic compressibility. Repulsive protein–protein interactions enhance D_m relative to the predictions of the Stokes–Einstein equation. This enhancement of D_m is not physically relevant here. As shown in the Appendix, D is given by $\mu_{AA}k_BT$, μ_{AA} being the mobility of a sphere in the protein:water mixture. Electrostatic protein–protein and protein–probe repulsions do contribute to diffusion through the $c_i h_{ij}(k)$ terms of Eq. [A5]. However, from Eqs. [A2] and [A6], protein–probe and protein–protein repulsions drive the D^+ spectral mode, while the light-scattering intensity is almost entirely

concentrated in the D^- spectral mode. That is, the protein–probe repulsions do speed up probe motions, but not those particular motions to which our equipment is sensitive.

We recently reported (2, 4) an experimental study of probe diffusion in a bidisperse mixture of polystyrene spheres. D of the large, dilute spheres was studied as the concentration c of the smaller, more concentrated spheres was changed; comparison was made between systems of moderate and minimal ionic strength. Reference (4) reviews a variety of theories which predict dD/dc, the most robust test of a theory being its prediction of the correlation between dD/dc and the ionic strength of the solvent. Theories based on the N-particle Smoluchowski equation predict the wrong sign for this correlation, regardless of whether or not memory function effects are included; predictions based on the Kirkwood force fluctuation formula (24) or on the Langevin equation appear to get the sign right. None of these calculations deduces the solution viscosity under the same conditions, so a comparison with our experiments is not possible.

In conclusion, our main finding is that the Stokes–Einstein equation does not work for large (radii up to 0.62 μm) spherical probes in solutions of a globular protein. We find that D is not simply determined by the macroscopic shear viscosity of the solution. A simple scaling equation, using empirical constants, does predict D. Since serum albumin molecules cannot form topological entanglements, the chain entanglement effects often (13, 14) ascribed to semidilute polymer solutions cannot have significance here. D for polystyrene spheres in high-molecular-weight polyethylene oxide behaves much like D for spheres in BSA, suggesting that topological entanglements (as contrasted with simple collisions) may not be central to probe dynamics in polyethylene oxide solutions.

APPENDIX A

The quasi-elastic light-scattering spectrum of a system which contains two interacting Brownian components was first obtained by this author (25, 26) for the case that the two components obey the linear flow equations

$$\frac{d}{dt}a_A(k,t) = -D_{AA}k^2a_A(k,t) - D_{AB}k^2a_B(k,t)$$

$$\frac{d}{dt}a_B(k,t) = -D_{BA}k^2a_A(k,t) - D_{BB}k^2a_B(k,t), \quad [A1]$$

where $a_i(k,t)$ is the amplitude of the kth spatial Fourier component of species i's concentration, k is the magnitude of the scattering vector, and D_{ii} and D_{ij} are the mutual and cross diffusion coefficients. In general, the electric field correlation function $g^{(1)}(t)$ contains two decaying exponentials, so that

$$\begin{aligned} g^{(1)}(t) = I_0A^{-1}(D^+ - D^-)[e^{-D^+k^2t}\{(D_{AA} - D^-) \\ \times (\epsilon_a^2\alpha + \epsilon_a\epsilon_b\gamma) + (D^+ - D_{AA}) \\ \times (\epsilon_b^2\beta + \epsilon_a\epsilon_b\gamma) + D_{AB}(\epsilon_a^2\gamma + \epsilon_a\epsilon_b\beta) \\ + D_{BA}(\epsilon_a\epsilon_b\alpha + \epsilon_b^2\gamma)\} \\ + e^{-D^-k^2t}\{(D^+ - D_{AA})(\epsilon_a^2\alpha + \epsilon_a\epsilon_b\gamma) \\ + (D_{AA} - D^-)(\epsilon_a\epsilon_b + \epsilon_b^2\beta) \\ - D_{AB}(\epsilon_a^2\gamma + \epsilon_a\epsilon_b\beta) \\ - D_{BA}(\epsilon_a\epsilon_b\alpha + \epsilon_b^2\gamma)\}], \end{aligned} \quad [A2]$$

where

$$\begin{aligned} D^\pm &= \tfrac{1}{2}(D_{AA} + D_{BB}) \\ &\quad \pm \{[\tfrac{1}{2}(D_{AA} - D_{BB})]^2 + D_{AB}D_{BA}\}^{1/2} \\ A &= \epsilon_a^2\alpha + 2\epsilon_a\epsilon_b\gamma + \epsilon_b^2\beta \\ \alpha &= \langle|a_A(k,0)|^2\rangle \\ \beta &= \langle|a_B(k,0)|^2\rangle \\ \gamma &= \langle|a_A(-k,0)a_B(k,0)|\rangle \end{aligned} \quad [A3]$$

and where the scattering cross sections of the two species are ϵ_a^2 and ϵ_b^2, respectively, and where the brackets "$\langle\cdots\rangle$" denote an ensemble average.

In our special case, we identify A and B with the polystyrene spheres and the serum albumin, respectively. In this case D_{BB}

$> D_{AA}$, $\epsilon_a > \epsilon_b$, $N_A\epsilon_a^2 \gg N_B\epsilon_b^2$ (for our data analysis, the scattering by B is negligible, so $N_B\epsilon_B^2 \approx 0$), and ϕ_A, $C_A \approx 0$, where N_A, ϕ_A, and C_A are the number, volume fraction, and concentration of the spheres in the system.

In the experiments treated here, the volume fraction $\phi_B = C_B\bar{v}_B$ of serum albumin is not small, so reference frame corrections (27, 28) must be used to relate the D_{ij} of Eq. [A1], which apply in the experimental (volume-fixed) frame, to the D_{ij}^0 which apply in the theoretically relevant solvent-fixed frame. One has

$$D_{ij} = D_{ij}^0 - c_i \sum_{k=1}^{n} \bar{v}_k D_{kj}^0 \qquad [A4]$$

where $\bar{v}_k$ is the partial volume of species k, and where the sum goes over all n macromolecule species in the system.

By comparison with Ref. (2), Eq. [A4], and the associated discussion, the D_{ij} are related to the mobilities μ_{ij} and the Fourier transforms $h_{ij}(k)$ of the i–j radial distribution function by

$$D_{ij}^0 = \mu_{ij}(K_BT\delta_{ij} + c_i h_{ij}(k)), \qquad [A5]$$

δ_{ij} being the Kronecker delta. In the absence of hydrodynamic interactions, one would have $\mu_{ii} = (f_i)^{-1}$, f_i being the drag coefficient of i, and $\mu_{ij} = 0$, $i \neq j$.

If A is dilute, $c_A h_{AA}(k) \ll k_BT$ and $D_{AA}^0 = \mu_{AA}k_BT$, $D_{BB}^0 = \mu_{BB}(k_BT + c_B h_{BB}(k))$, $D_{AB}^0 = \mu_{AB}c_A h_{AB}(k) \cong 0$, and $D_{BA}^0 = \mu_{BA}c_B h_{AB}(k)$ so that $D_{AA} = \mu_{AA}k_BT$, $D_{BB} = \mu_{BB}(k_BT + c_B h_{BB}(k))$, and $D_{AB} = 0$. If D_{AB} vanishes and $D_B > D_A$, one has $D^+ = D_{BB}$ and $D^- = D_{AA}$. On neglecting the smaller terms, Eq. [A1] reduces to

$$g^{(1)}(t) = I_0 e^{-D_{AA}k^2t}. \qquad [A6]$$

That is, in the limit that the probe (scattering) species is dilute, the light-scattering spectrum contains as single exponential of characteristic decay time $1/D_{AA}k^2$. Formally, D_{AA} is the mutual diffusion coefficient of the spheres through the water:protein mixture. However, we are in the limit $c_A h_{AA}(k) \to 0$, so D_{AA} is numerically indistinguishable from the tracer diffusion coefficient $D_s = k_BT/f$ of the spheres.

We have now confirmed our assertion in the introduction that under our experimental conditions "light-scattering spectroscopy *effectively* obtains the single-particle ("tracer" or "self") diffusion coefficient of the scattering component as it moves through a multicomponent solution."

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