

Static structure factor and collective diffusion of globular proteins in concentrated aqueous solution

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We report our measurement of the time average and the temporal autocorrelation function of the intensity of light scattered by the highly monomeric globular protein, bovine γ_{II} -crystallin, in aqueous solution as a function of wave number q , protein volume fraction ϕ , and temperature T . The time average intensity data is used to obtain the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$, as a function of ϕ and T . We show that $S(\phi, T)$ may be well characterized by modeling the proteins as interacting through the Baxter adhesive hard sphere pair interaction potential. The temporal autocorrelation function data is used to determine the collective diffusion coefficient $\tilde{D}(\phi, T)$ of the proteins as a function of ϕ and T . We then obtain the experimental hydrodynamic factor $\tilde{H}(\phi, T) \equiv S(\phi, T)[\tilde{D}(\phi, T)/D_0(T)]$, where $D_0(T)$ is the diffusion coefficient of the individual proteins in the $\phi \rightarrow 0$ limit. We find that \tilde{H} exhibits a different ϕ -dependence at low ($\phi \leq 0.016$) and high ($\phi \geq 0.02$) protein volume fractions. In the low ϕ domain our data for \tilde{H} are consistent with the theoretical result for the collective diffusion in the $q \rightarrow 0, t \rightarrow 0$ limit. However, for $\phi \geq 0.02$ we find a deviation from single exponential decay in the autocorrelation functions, and an unexpected, large change in the slope of the \tilde{H} vs ϕ relation. This crossover at such low ϕ suggests the existence of a heretofore unappreciated length scale in the dynamics of colloid solutions. Clearly, further theoretical insights are required to understand the origin of this crossover behavior. © 1996 American Institute of Physics. [S0021-9606(96)50401-X]

I. INTRODUCTION

In the past several years, there has been considerable activity directed at understanding both the equilibrium thermodynamic properties and the transport properties of dispersions of interacting colloidal particles (for a review see Ref. 1). As a result of this activity, significant progress has been made in the description of both the self-diffusion and the collective diffusion of colloidal particles. For self-diffusion, it has been shown that direct interactions are relatively unimportant compared to hydrodynamic interactions.² In contrast, for collective diffusion, the direct interactions play an important role. The present state of the theory for the collective diffusion takes into account the contributions due to both direct and hydrodynamic interactions.

Recently, there has also been a growing recognition that many physiological processes are significantly affected by the high macromolecular concentration that exists in many biological solutions. For example, it is becoming clear that in living cells this "macromolecular crowding" may influence many biochemical reactions³ and may also have a significant effect on the diffusion of macromolecules.⁴⁻⁶ Furthermore, the high concentration of macromolecules in many living cells has been shown to play an important role in a number of specific diseases. In particular, there is a group of diseases, referred to as protein condensation diseases,⁷ in which an important pathological event is the separation of a biological solution into coexisting protein-rich and protein-poor phases. These diseases include sickle cell anemia, cryoimmunoglobulinemia, amyloidosis, and cataract disease.

Clearly, many macromolecular solutions of biological

importance may be viewed as colloidal dispersions. It is therefore natural to investigate whether theories that have been successful in describing the equilibrium thermodynamic properties and the transport properties of colloidal dispersions may be used to explain those properties in solutions of biological macromolecules.

In this paper, we describe our use of light scattering techniques to determine the static structure factor and collective diffusion of a highly monomeric globular protein, bovine γ_{II} -crystallin, in concentrated aqueous solution. We use the static structure factor data to determine a reasonable pair interaction potential to use to model the interactions among the protein molecules. We use the collective diffusion data, together with the static structure factor data, to test whether the theory for collective diffusion in moderately concentrated colloidal dispersions is applicable to solutions of this biological macromolecule. This work may be viewed both as an experimental test of the currently accepted theory of the collective diffusion of colloidal dispersions and also as a contribution to the understanding of the effect of macromolecular crowding in biological systems.

Bovine γ_{II} -crystallin is a member of the γ -crystallin family of highly homologous eye-lens proteins. There are well-established methods for its purification⁸ and its structure has been well characterized. It has a molecular weight of 2.1×10^4 g/mol.⁹ X-ray crystallographic studies^{9,10} have shown that γ_{II} -crystallin is a globular protein in the shape of a prolate ellipsoid with axes 35×55 Å and have determined its structure to a resolution of 1.47 Å.

Aqueous solutions of this protein exhibit a liquid-liquid

phase transition,^{8,11} in which, upon lowering the temperature, the solution separates into two coexisting solutions with differing protein volume fractions ϕ . This phase transition has a critical point with a critical volume fraction $\phi_c = 0.19 \pm 0.01$.¹¹ Previous light scattering studies on aqueous solutions of this protein have addressed the equilibrium thermodynamic and the transport properties of these solutions in the vicinity of the critical point for this phase transition.^{12,13} In those studies, careful consideration was given to the effects of multiple light scattering. In the present paper, we describe measurements of the static structure factor and the collective diffusion of protein molecules as a function of the protein volume fraction ϕ at various temperatures T well above the critical temperature of these solutions. These measurements were made sufficiently far from the critical point that multiple light scattering effects are not significant.

We determined the static structure factor $S(q, \phi, T)$ for γ_{II} -crystallin in aqueous solution by measuring the time average of the intensity of light scattered by these solutions at up to twelve different scattering angles θ . The wave number q is related to the scattering angle θ by the relation $q = (4\pi m/\lambda)\sin(\theta/2)$, where m is the refractive index of the sample. Experimental determination of $S(q, \phi, T)$ provides information on the equilibrium properties of the system. In particular, in the limit $q \rightarrow 0$, $S(q, \phi, T)$ is related to the osmotic compressibility $(\partial\phi/\partial\Pi)_T$ by the relation

$$\lim_{q \rightarrow 0} S(q, \phi, T) \equiv S(\phi, T) = \frac{k_B T}{\Omega_p} \left(\frac{\partial\phi}{\partial\Pi} \right)_T, \quad (1)$$

where Π is the osmotic pressure, k_B is the Boltzmann constant, and Ω_p is the volume of a single protein molecule. The static structure factor is also formally related to the pair interaction potential $u(r)$ (see, for example, Ref. 14). However, in practice, it is not possible to use an experimental determination of $S(q, \phi, T)$ to unambiguously deduce $u(r)$. Instead, one must first hypothesize a form for $u(r)$, which may then be used to calculate $S(q, \phi, T)$. This calculated $S(q, \phi, T)$ may then be compared with experimental data to determine whether the hypothesized $u(r)$ is a reasonable model for the system of interest.

For γ_{II} -crystallin, we show that we may use the Baxter adhesive hard sphere pair interaction potential¹⁵ to model the interactions between the protein molecules in solution. This pair interaction potential consists of a hard core repulsion together with a very short-ranged attractive well. The Baxter potential has the desirable feature that it may be used to obtain an analytic expression for $S(\phi, T)$ by employing the Percus–Yevick approximation.¹⁵ We find that this expression for $S(\phi, T)$ agrees very well with our experimental data over the full range of ϕ that was studied ($0.012 \leq \phi \leq 0.21$). Thus, this paper demonstrates, in so far as the equilibrium properties are concerned, that the interactions among protein molecules may be successfully modeled as a pair interaction potential consisting of a hard core repulsion and a short-ranged attractive well.

In order to investigate the transport properties of γ_{II} -crystallin in aqueous solution, we have used the technique of quasielastic light scattering (QLS). This technique is ideal for studying the diffusion of particles in a wide variety of colloidal dispersions, including protein solutions. In QLS, one obtains the time autocorrelation function of the photo-counts, $\langle n(q, t)n(q, 0) \rangle$, due to light scattered by the sample of interest. Here, $n(q, t)$ is the number of photons counted by a detector that is placed at a scattering angle θ in a time interval between t and $t + \Delta t$, where Δt is a sample time. The measured quantity $\langle n(q, t)n(q, 0) \rangle$ may be related to the temporal autocorrelation function $f(q, t)$ of spontaneous fluctuations in the protein volume fraction by¹⁶

$$\frac{\langle n(q, t)n(q, 0) \rangle}{\langle n(q) \rangle^2} = 1 + b[f(q, t)]^2. \quad (2)$$

In this equation, b is a constant between 0 and 1 that depends on the number of coherence areas subtended by the detector and $f(q, t)$ is the intermediate scattering function,

$$f(q, t) = \frac{\langle \hat{\phi}(q, t)\hat{\phi}(-q, 0) \rangle}{\langle |\hat{\phi}(q)|^2 \rangle}, \quad (3)$$

where $\hat{\phi}(q, t)$ is the spatial Fourier component with wave number q of the fluctuations in ϕ about its mean value. Equation (2) is valid when the scattering volume is much larger than the volume over which the particles are correlated. This was always the case for the experiments presented here.

The intermediate scattering function $f(q, t)$ decays from a value of 1 at $t = 0$ to a value of 0 as $t \rightarrow \infty$. In the limit of very low protein concentration, this decay is exponential in time, with a decay rate $-q^2 D_0(T)$, i.e., $\ln[f(q, t)] = -q^2 D_0(T)t$, where $D_0(T)$ is the particle diffusion coefficient at infinite dilution. At moderate concentrations, $f(q, t)$ exhibits a deviation from exponential decay with time.¹ It is then convenient to characterize the time decay of $f(q, t)$ in terms of cumulants,¹⁷

$$\ln[f(q, t)] = -K_1 t + \frac{1}{2} K_2 t^2 + O(t^3), \quad (4)$$

where K_1 and K_2 are the first and second cumulants of $f(q, t)$, respectively. As can be clearly seen in the above equation, K_1 characterizes the time decay of $f(q, t)$ in the limit $t \rightarrow 0$.

The current status of the theory for the collective diffusion of colloidal particles has been reviewed extensively (see, for example, Ref. 1). The theory provides a prediction for the collective diffusion coefficient $D(\phi, T)$ in a colloidal dispersion on the basis of its microscopic properties. The collective diffusion coefficient $D(\phi, T)$ is related to the long wavelength limit of the first cumulant by the relationship^{1,18}

$$D(\phi, T) \equiv \lim_{q \rightarrow 0} \frac{K_1}{q^2} = D_0(T) \frac{H(\phi, T)}{S(\phi, T)}, \quad (5)$$

where $D_0(T)$ is, as defined previously, the diffusion coefficient at infinite dilution, $S(\phi, T)$ is the $q \rightarrow 0$ limit of the static structure factor, and $H(\phi, T)$ is a factor that accounts

for hydrodynamic interactions. The quantities $D(\phi, T)$ and $H(\phi, T)$ both characterize the system in the limit $t \rightarrow 0$ and $q \rightarrow 0$. A theoretical expression for $H(\phi, T)$ that is valid to first order in ϕ has been found¹⁹ in terms of the pair interaction potential $u(r)$. We shall discuss this expression in the next section. For hard spheres without attraction, numerical results have been obtained for $H(\phi, T)$ to higher order in ϕ .²⁰ It would be desirable if, in the future, such a calculation were extended to the case of the Baxter potential.

The measurements described in this paper allow us to make a comparison between our experimental data and the current theory for the collective diffusion in colloidal dispersions. We use the QLS data to determine the collective diffusion coefficient $\tilde{D}(\phi, T)$ as a function of ϕ at several different temperatures. Using the experimentally determined values for $\tilde{D}(\phi, T)$, along with a knowledge of $S(\phi, T)$ and $D_0(T)$, we then determine $\tilde{H}(\phi, T)$ as a function of ϕ . The symbols \tilde{D} and \tilde{H} represent the quantities derived from the experimental data. We use this notation to emphasize that we do not expect that, under all experimental conditions, the experimentally determined quantities $\tilde{D}(\phi, T)$ and $\tilde{H}(\phi, T)$ will always be identical to the theoretical quantities $D(\phi, T)$ and $H(\phi, T)$, which are defined in the limit $t \rightarrow 0$ and $q \rightarrow 0$. In contrast, we do expect that the experimentally determined values for the $q \rightarrow 0$ limit of the static structure factor will correspond to the theoretical expression for this quantity. Therefore we represent both the experimental and the theoretical $q \rightarrow 0$ limit of the static structure factor by the same symbol, $S(\phi, T)$. We compare the experimentally determined $\tilde{H}(\phi, T)$ with the currently accepted theoretical expression for $H(\phi, T)$ which is valid to first order in ϕ , by modeling the proteins as interacting through the Baxter pair interaction potential. We find that for $\phi \leq 0.016$, our $\tilde{H}(\phi, T)$ data are consistent with the theoretical predictions for $H(\phi, T)$. For $\phi \geq 0.02$, we find unequivocal disagreement between $\tilde{H}(\phi, T)$ and $H(\phi, T)$. This disagreement is consistent with the view that when memory effects become important, results obtained at finite t and q will differ significantly from those predicted in the limit $t \rightarrow 0$, $q \rightarrow 0$.²¹

The collective diffusion of proteins at low volume fractions has been compared with the above theory by Neal and co-workers.²² These investigators demonstrated a significant disagreement between the same theoretical expression used in this paper and experimental results for the collective diffusion of bovine serum albumin (BSA). In contrast, the collective diffusion of relatively large, sterically-stabilized silica particles has been shown to be in good agreement with the above theory.^{23–25} In the present work we determine the collective diffusion of γ_{II} -crystallin over a much wider range of ϕ than was investigated by Neal and co-workers. This enabled us to observe what appears to be a transition between a low ϕ regime in which the linear in ϕ theory applies and a higher ϕ regime where the present theory requires significant modification. Thus, the present work, we feel, provides an important new contribution to the study of the collective diffusion of colloidal dispersions and also to the study of the equilibrium and transport properties of protein solutions.

II. THEORETICAL BACKGROUND

To model the interactions between γ_{II} -crystallin molecules in solution, we considered each molecule to be a hard sphere of radius a with an attractive rectangular well of width δ . Their pair interaction potential $u(r)$ may then be written as

$$u(r) = \begin{cases} \infty & 0 < r < 2a, \\ -v_0 & 2a < r < 2a(1 + \delta), \\ 0 & 2a(1 + \delta) < r, \end{cases} \quad (6)$$

where r is the center-to-center distance between two protein molecules.

In general, for a potential of this form with arbitrary well dimensions, there is no known analytic expression for $S(q, \phi, T)$. However, Baxter has shown that¹⁵ the Percus–Yevick equation may be solved to obtain an approximate analytic expression for $S(q, \phi, T)$ for this potential in a particular limit in which the width of the well goes to zero, while its depth becomes infinite in such a way that the second virial coefficient remains finite. Specifically, an approximate analytic expression for $S(q, \phi, T)$ may be obtained using the Percus–Yevick approximation for a potential of the form given in Eq. (6) in the limit that δ goes to zero and v_0 goes to infinity such that¹⁵

$$\lim_{\delta \rightarrow 0, v_0 \rightarrow \infty} \delta e^{\beta v_0} = \frac{1}{12\tau}, \quad (7)$$

where $\beta = (k_B T)^{-1}$. The parameter τ is a dimensionless quantity that fully characterizes the interaction potential and hence the equilibrium properties of the system at any given temperature. The case of pure hard spheres is recovered in the limit $\tau \rightarrow \infty$. The potential $u(r)$ given in Eq. (6) in the limit given by Eq. (7) is commonly referred to as the Baxter potential. The expression for $S(q, \phi, T)$ obtained in the Percus–Yevick approximation for the Baxter potential has been shown to be in excellent agreement with $S(q, \phi, T)$ obtained by Monte Carlo simulations for this potential.²⁶

By taking the $q \rightarrow 0$ limit of this expression for $S(q, \phi, T)$ for the Baxter potential, it may be shown that,^{15,27}

$$S(\phi, T)^{-1} = \left[\frac{1 + 2\phi - \Lambda\phi(1 - \phi)}{(1 - \phi)^2} \right]^2, \quad (8)$$

where

$$\Lambda = 6 \left(\frac{\tau}{\phi} + \frac{1}{1 - \phi} \right) - \sqrt{\left[6 \left(\frac{\tau}{\phi} + \frac{1}{1 - \phi} \right) \right]^2 - \frac{12(1 + \phi/2)}{\phi(1 - \phi)^2}}. \quad (9)$$

We may compare the ϕ dependence of $S(\phi, T)$ predicted by these expressions with that found experimentally and determine the corresponding value of τ at each temperature. For $\tau < (2 - \sqrt{2})/6$, there is a range of ϕ for which Λ does not have a real value. This indicates that for $\tau < (2 - \sqrt{2})/6$, the system undergoes a discontinuous transition between states of different ϕ . Thus, for the Baxter potential in the Percus–Yevick

approximation, a first order phase transition is exhibited with a critical value of $\tau = \tau_c = (2 - \sqrt{2})/6 \approx 0.0976$ and a corresponding critical volume fraction $\phi_c = (3\sqrt{2} - 4)/2 \approx 0.1213$.¹⁵ However, an analysis on the basis of the energy equation for the Baxter potential in the Percus–Yevick approximation shows that $\tau_c \approx 0.1185$ and $\phi_c \approx 0.32$.²⁸

We described above [Eq. (5)] that the collective diffusion coefficient $D(\phi, T)$ depends on $S(\phi, T)$, the free particle diffusion coefficient $D_0(T)$, and the hydrodynamic factor $H(\phi, T)$. Felderhof has obtained¹⁹ an expression for $H(\phi, T)$, that is valid to first order in ϕ , in terms of the pair interaction potential $u(r)$. Using a Taylor expansion in inverse powers of center-to-center distance for the hydrodynamic interactions between two spherical particles and keeping only the first several nonvanishing terms, Felderhof obtained that¹⁹

$$H(\phi, T) = 1 + \lambda(T)\phi + O(\phi^2), \quad (10)$$

where

$$\lambda(T) = \lambda_O + \lambda_D + \lambda_S + \lambda_A. \quad (11)$$

In the above equation, $\lambda(T)$ is represented as the sum of Oseen λ_O , hydrodynamic dipole λ_D , hydrodynamic short range λ_S , and hydrodynamic self λ_A contributions, where

$$\begin{aligned} \lambda_O &= \frac{3}{a^2} (1 - \zeta) \int_0^\infty dr r [e^{-\beta u(r)} - 1], \\ \lambda_D &= 1 - 3\zeta, \\ \lambda_S &= \frac{75}{4} \frac{(1 - \zeta)^3}{(1 + 2\zeta)^2} a^4 \int_{2a}^\infty dr \frac{e^{-\beta u(r)}}{r^5}, \\ \lambda_A &= \frac{3}{a^3} (1 - \zeta) \int_{2a}^\infty dr r^2 e^{-\beta u(r)} \left[-\frac{5}{4} \frac{1 - \zeta}{1 + 2\zeta} \frac{a^4}{r^4} \right. \\ &\quad \left. + \left(4 \frac{1 - 3\zeta}{1 + 2\zeta} - \frac{91}{40} \frac{1 - \zeta}{1 + 4\zeta} - \frac{1}{2} \frac{1 - 4\zeta}{1 + \zeta} \right. \right. \\ &\quad \left. \left. - \frac{1}{10} \frac{1 - 6\zeta}{1 - \zeta} \right) \frac{a^6}{r^6} \right], \end{aligned}$$

where the parameter ζ is included to account for the nature of the fluid flow at the particle surface. The parameter ζ can take a value between 0 and 1/3, where $\zeta = 0$ for stick boundary conditions and $\zeta = 1/3$ for slip boundary conditions. For stick boundary conditions, there is no fluid flow at the surface of the particles. For slip boundary conditions, fluid flows freely over the particle surface. The above expressions, together with a knowledge of $u(r)$, may be used to obtain a theoretical prediction for $H(\phi, T)$ to first order in ϕ .

For the Baxter potential, all of the contributions to $\lambda(T)$ in Eq. (11) involving the interaction potential $u(r)$ may be expressed in terms of the single parameter τ . We find that for stick boundary conditions ($\zeta = 0$)

$$\lambda_{\text{stick}}^{\text{approx}}(T) \equiv \lambda(T) = -\frac{1649}{256} + \frac{1}{\tau} \frac{250}{256} \approx -6.44 + \frac{0.977}{\tau}. \quad (12)$$

While for slip boundary conditions ($\zeta = 1/3$), we find

$$\lambda_{\text{slip}}^{\text{approx}}(T) \equiv \lambda(T) = -\frac{9}{2} + \frac{1}{\tau} \frac{241}{384} \approx -4.50 + \frac{0.628}{\tau}. \quad (13)$$

We introduce the superscript “approx” to emphasize that these expressions for λ are approximate since they are derived using only the first several terms of a Taylor expansion in inverse powers of the center-to-center distance of the hydrodynamic interactions.

A different expression for $\lambda(T)$ has been obtained by Cichocki and Felderhof²⁹ for the Baxter potential by making use of an asymptotic expression for the two-particle hydrodynamics that is valid when the spheres are close to touching. They found that for stick boundary conditions,²⁹

$$\lambda_{\text{stick}}^{\text{asympt}}(T) \equiv \lambda(T) = -6.546 + \frac{0.875}{\tau}. \quad (14)$$

Comparison of this result with Eq. (12) shows that the ϕ -dependence of $H(\phi, T)$ is not very sensitive to the particular approximation chosen for the hydrodynamic interactions.

For aqueous γ_{II} -crystallin solutions, we have determined experimentally the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$, the collective diffusion coefficient $\hat{D}(\phi, T)$, and the free particle diffusion coefficient $D_0(T)$. Hence, we have obtained experimental data for $\hat{H}(\phi, T)$ [using Eq. (5)] that may be compared with the theoretical predictions for $H(\phi, T)$ given by Eq. (10) with $\lambda(T)$ given by either $\lambda_{\text{stick}}^{\text{approx}}(T)$ [Eq. (12)], $\lambda_{\text{slip}}^{\text{approx}}(T)$ [Eq. (13)], or $\lambda_{\text{stick}}^{\text{asympt}}(T)$ [Eq. (14)].

III. MATERIALS AND METHODS

Two different bovine γ_{II} -crystallin samples were prepared. One was used for QLS measurements at very low protein volume fractions ($0.0036 \leq \phi \leq 0.016$). The other was used for both QLS and time average scattered intensity measurements at a wide range of higher protein volume fractions ($0.012 \leq \phi \leq 0.21$).

Bovine γ_{II} -crystallin was isolated from calf lenses by column chromatography using a method described elsewhere.^{8,30} After purification, the protein samples were exchanged into a 100 mM sodium phosphate buffer solution (ionic strength 240 mM, pH 7.1) containing 3 mM sodium azide. For the higher protein volume fraction measurements, the protein sample was passed through a 0.22 μm filter (Millex-GV, Millipore, Bedford, MA) to remove any large protein aggregates, prior to being exchanged into the phosphate buffer. For the very low protein volume fraction measurements, 100 mM dithiothreitol (DTT) was added to the phosphate buffer, prior to introducing the protein sample into it. DTT has been shown to prevent oxidation of thiol groups in the protein and thus to prevent protein aggregation.³¹ The samples were then either concentrated by ultrafiltration (Centricon-10 and Centriprep-10, Amicon, Beverly, MA) or diluted with phosphate buffer to the desired protein volume fraction, ϕ .

The protein concentration of the samples were determined by UV absorption as described elsewhere.¹¹ The samples were then transferred into cylindrical glass scattering cells and centrifuged at $4300 \times g$ for at least 30 min to remove dust from the scattering volume.

For aqueous γ_{II} -crystallin solutions, the critical volume fraction ϕ_c is known to be 0.19 ± 0.01 .¹¹ The critical temperature T_c for liquid–liquid phase separation of these protein solutions was determined by cloud point measurements as described elsewhere.¹¹ For the solution described in this paper, in the absence of DTT, $T_c = 3.9 \pm 0.1$ °C. In the presence of DTT, the T_c of aqueous γ_{II} -crystallin solutions is depressed by several degrees.³¹ This suppression of T_c is sufficiently small that it may be neglected in the analysis of the data presented in this paper.

The light scattering measurements were made using an apparatus which is described in detail elsewhere³² and is based on the design of Haller, Destor, and Cannell.³³ We used a vertically polarized argon ion laser operating at a wavelength λ of 4880 Å. The light scattered by the sample was detected at up to twelve fixed angles ($11.50^\circ \leq \theta \leq 162.60^\circ$). Thus, the range of wave numbers q sampled was $3.4 \times 10^4 \leq q \leq 3.4 \times 10^5 \text{ cm}^{-1}$.

We used measurements of the time average of the intensity of light scattered by these solutions to determine the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$. Specifically, we obtained the time average of the photocounts due to light scattered from a solution as a function of angle by employing the following protocol. The light was collected at each angle for less than two seconds. The transmitted photocounts $n(q=0)$ and the scattered photocounts at each angle $n(q)$ were measured sequentially and the ratio $n(q)/n(0)$ was obtained. These measurements were repeated at least thirty times and the average value $\langle n(q)/n(0) \rangle$ was determined with a dust discrimination algorithm. This procedure ensured that the results were largely insensitive to drifts in laser power or photomultiplier response, and corrected $n(q)$ for sample turbidity. The $\langle n(q)/n(0) \rangle$ data were then used to determine $S(\phi, T)$ by using a protocol that is described in detail elsewhere.^{12,30} This protocol involved comparing the $\langle n(q)/n(0) \rangle$ data for the sample of interest with $\langle n(q)/n(0) \rangle$ data for the known scattering standard, spectrographic grade toluene.

We obtained the temporal autocorrelation function of the photocounts $\langle n(q, t)n(q, 0) \rangle$, using a Langley–Ford (Amherst, MA) Model 1096 correlator with 144 channels. The last 16 channels of the correlator were delayed by 1024 sample times. The sample time was chosen so that the first 128 channels would span two average decay times of the correlation function.

For each correlation function, we determined the value of $\langle n(q) \rangle^2$ in two ways. First, we obtained $\langle n(q) \rangle^2$ by taking an average of the number of counts in the delayed last sixteen channels of the correlator. Second, we calculated $\langle n(q) \rangle^2$ on the basis of the total number of photons detected during the acquisition of the correlation function. The two methods always provided values for $\langle n(q) \rangle^2$ that agreed within experimental uncertainty. In the analysis of the corre-

lation functions, the second method of determining $\langle n(q) \rangle^2$ was always used.

We characterized the autocorrelation functions in terms of a distribution $G(\Gamma_i)$ of decay rates Γ_i , so that $f(q, t) = \sum_i G(\Gamma_i) \exp(-\Gamma_i t)$, where $\sum_i G(\Gamma_i) = 1$. In order to obtain an estimate for $G(\Gamma_i)$ for each correlation function, we used a constrained regularization algorithm,³⁴ which is described in more detail elsewhere.³⁰

For the very low protein volume fraction measurements ($0.0036 \leq \phi \leq 0.016$), we found that a small dust contribution to the temporal autocorrelation functions was unavoidable. In the analysis of these measurements, we used the constrained regularization algorithm to resolve the dust contribution from the protein contribution to the autocorrelation functions. We were then able to determine the rate of decay Γ of the protein contribution. For these samples, we used Γ , thus determined, to calculate $\tilde{D}(\phi, T)$ using the relationship $\Gamma = q^2 \tilde{D}(\phi, T)$.

For the autocorrelation functions obtained at higher protein volume fractions ($0.012 \leq \phi \leq 0.21$), the dust contribution was not significant. For each of these autocorrelation functions, we made a least-squares fit of the second order cumulant expansion [Eq. (4)] to obtain a value for the first cumulant K_1 . We then used K_1 to determine $\tilde{D}(\phi, T)$ using Eq. (5).

IV. EXPERIMENTAL RESULTS AND COMPARISON WITH THEORY

We have made (i) time average scattered intensity measurements at a wide range of relatively high protein volume fractions ($0.012 \leq \phi \leq 0.21$) and (ii) QLS measurements at both very low protein volume fractions ($0.0036 \leq \phi \leq 0.016$) and at a wide range of higher protein volume fractions ($0.012 \leq \phi \leq 0.21$). All of these measurements were made at a series of different temperatures.

From the measurements of the time average scattered intensity $\langle n(q)/n(0) \rangle$, we determined the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$ as a function of ϕ at four temperatures (25.0, 20.0, 15.0, and 10.0 °C). In order to do this, we first extrapolated the $\langle n(q)/n(0) \rangle$ data to $\phi=0$ for each temperature and determined the molecular weight of the protein as described in Ref. 30. The values for the molecular weight thus obtained were consistent with the accepted value⁹ of 2.1×10^4 g/mol. Using the accepted value of the molecular weight and the $\langle n(q)/n(0) \rangle$ data, we then calculated $S(\phi, T)$ using the protocol described previously.^{12,30} Figure 1 contains plots of $S(\phi, T)^{-1}$ vs ϕ for the four temperatures studied. This figure shows that for $\phi \leq 0.1$, $S(\phi, T)^{-1}$ decreases dramatically as ϕ increases. For ϕ between 0.1 and 0.21 it appears that $S(\phi, T)^{-1}$ remains roughly constant.

We compared the experimental values for $S(\phi, T)$ with the prediction for this quantity on the basis of the Baxter potential. We found that Eq. (8), with an appropriate choice of τ for each temperature, was in very good agreement with the experimental $S(\phi, T)$ data [Figs. 1(a)–1(d)]. The values of τ that were chosen for the four temperatures studied are

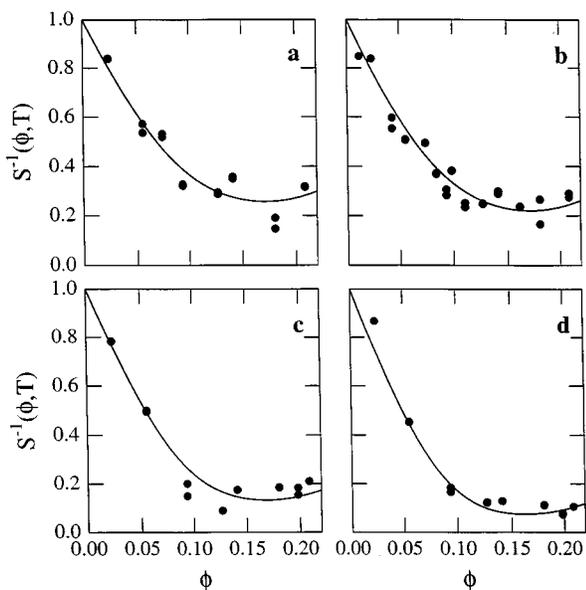


FIG. 1. The inverse $q \rightarrow 0$ limit of the static structure factor in the long wavelength limit $S(\phi, T)^{-1}$ vs ϕ at the various temperatures studied. The data is for γ_{II} -crystallin in 100 mM phosphate buffer. (a) 25.0 °C, (b) 20.0 °C, (c) 15.0 °C, and (d) 10.0 °C. The solid curves are plots of $S(\phi, T)^{-1}$ for the Baxter adhesive hard sphere potential with the value of τ at each temperature given in Table I.

listed in Table I. It is possible to interpret the magnitude and the temperature dependence of τ in terms of the parameters δ and v_0 which characterize the interaction potential of the protein molecules. To do so, we consider Eq. (7) not as a rigorous limit but instead as an approximate expression applicable to small but finite ranges of interaction δ . Using this approximation we have found that $\delta \approx 0.06$ and $v_0 \approx 2.7k_B T_c$. By extrapolating the temperature dependence of τ contained in Table I to the critical temperature $T_c = 3.9 \pm 0.1$ °C, we find that $\tau = 0.098 \pm 0.002$ at T_c . This value of τ is in very good agreement with the value $\tau = (2 - \sqrt{2})/6 \approx 0.0976$ that is expected at the critical temperature in the Percus–Yevick approximation.¹⁵

We now examine the transport properties of γ_{II} -crystallin in aqueous solution using QLS measurements.

The QLS measurements at very low protein volume fractions ($0.0036 \leq \phi \leq 0.016$) were used to determine the collec-

TABLE I. The values of the parameter τ , the free particle diffusion coefficient $D_0(\phi, T)$, and the theoretical values of $\lambda(T)$, for the five temperatures at which the protein solutions were studied.

T (°C)	τ	$D_0(T)$ (10^{-6} cm ² /s)	$\lambda_{\text{stick}}^{\text{approx}}(T)$	$\lambda_{\text{slip}}^{\text{approx}}(T)$	$\lambda_{\text{stick}}^{\text{asympt}}(T)$
25.0	0.119 ± 0.001	1.03 ± 0.01	1.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
20.0	0.116 ± 0.001	0.90 ± 0.01^b	2.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
15.0	0.109 ± 0.001	0.77 ± 0.01^b	2.6 ± 0.1	1.3 ± 0.1	1.5 ± 0.1
13.0	0.107 ± 0.002^a	0.70 ± 0.01	2.7 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
10.0	0.104 ± 0.001	0.66 ± 0.01^b	3.0 ± 0.1	1.6 ± 0.1	1.9 ± 0.1

^aDetermined by interpolation of the experimental values of τ .

^bCalculated using Eq. (15) and $D_0(T=25.0$ °C).

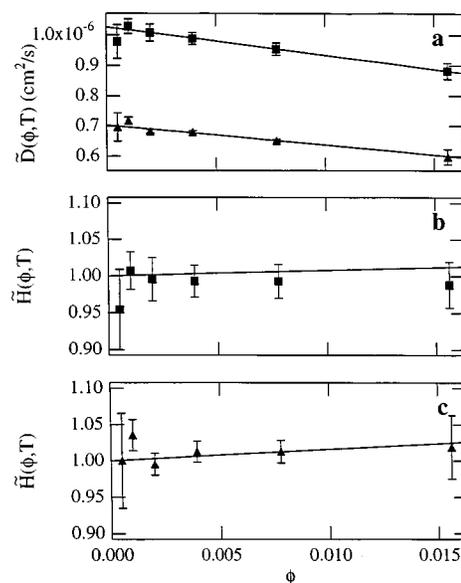


FIG. 2. Results of QLS measurements at low protein volume fraction. (a) The collective diffusion coefficient $\tilde{D}(\phi, T)$ at $T=25.0$ °C (squares) and $T=13.0$ °C (triangles). The solid lines are least squares fits of straight lines to the data. (b) The hydrodynamic factor $\tilde{H}(\phi, T)$ at $T=25.0$ °C. (c) The hydrodynamic factor $\tilde{H}(\phi, T)$ at $T=13.0$ °C. In (b) and (c), the straight lines are the theoretical predictions for $H(\phi, T)$ given by Eq. (10) with $\lambda(T) = \lambda_{\text{stick}}^{\text{asympt}}(T)$.

tive diffusion coefficient $\tilde{D}(\phi, T)$. Figure 2(a) contains plots of $\tilde{D}(\phi, T)$ vs ϕ for the two temperatures studied (25.0 and 13.0 °C). This figure shows that $\tilde{D}(\phi, T)$ decreases with increasing ϕ and decreasing T . For this range of ϕ , the $\tilde{D}(\phi, T)$ data are consistent with a linear dependence on ϕ . We linearly extrapolated the $\tilde{D}(\phi, T)$ data as a function of ϕ to $\phi=0$ to determine the free particle diffusion coefficient $D_0(T)$. The values for $D_0(T)$ at $T=25.0$ °C and 13.0 °C, thus determined, are contained in Table I. The Stokes–Einstein relation,¹⁹

$$D_0(T) = \frac{k_B T}{6(1 - \zeta)\pi\eta a}, \quad (15)$$

provides a relation between $D_0(T)$ and the effective hydrodynamic radius a of the scattering particles. In this equation, η is the viscosity of the suspending fluid and the parameter ζ depends on the nature of fluid flow at the effective surface of the particle. For stick boundary conditions ($\zeta=0$), we find $a = 23.9 \pm 0.3$ Å at $T=25.0$ °C. This value is consistent with the known structure of γ_{II} -crystallin.⁹ On the basis of Eq. (15), the difference between $D_0(T=25.0$ °C) and $D_0(T=13.0$ °C), and therefore the difference in the value of $\tilde{D}(\phi, T)$ at $T=25.0$ °C and at $T=13.0$ °C, is due largely to the difference in η at these two temperatures.

The hydrodynamic factor $\tilde{H}(\phi, T)$ was calculated by using Eq. (5) and the experimentally determined values of $\tilde{D}(\phi, T)$ and $D_0(T)$. For these calculations, $S(\phi, T)$ was obtained by using Eq. (8) and the values for τ given in Table I. Figures 2(b) and 2(c) contain plots of $\tilde{H}(\phi, T)$ vs ϕ at $T=25.0$ °C and 13.0 °C, respectively. In these figures, the straight lines are the theoretical predictions for $H(\phi, T)$.

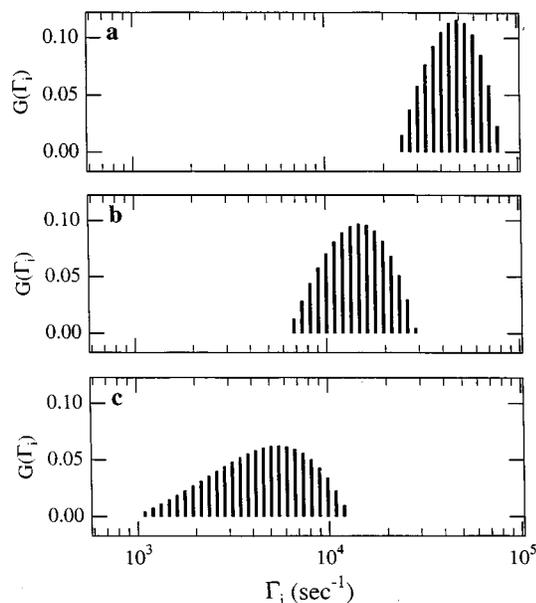


FIG. 3. The distribution of decay rates obtained from autocorrelation functions using the constrained regularization algorithm. Data is for γ_{11} -crystallin in 100 mM phosphate buffer at $T=20^\circ\text{C}$. The correlation functions were obtained at $q=2.43\times 10^5\text{ cm}^{-1}$. (a) $\phi=0.012\pm 0.001$, (b) $\phi=0.101\pm 0.001$, (c) $\phi=0.161\pm 0.001$.

These predictions are given by Eq. (10) with $\lambda(T)$ given by $\lambda_{\text{stick}}^{\text{asympt}}(T)$ [Eq. (14)]. These figures show that the predictions for $H(\phi, T)$ using $\lambda_{\text{stick}}^{\text{asympt}}(T)$ are consistent with the data over this range of ϕ . We also found that the predictions for $H(\phi, T)$ using either $\lambda_{\text{stick}}^{\text{approx}}(T)$ [Eq. (12)] or $\lambda_{\text{slip}}^{\text{approx}}(T)$ [Eq. (13)] were also consistent with the data in Figs. 2(b) and 2(c).

We now describe the results of the QLS measurements over a wide range of higher protein volume fractions ($0.012\leq\phi\leq 0.21$) at four different temperatures (25.0, 20.0, 15.0, and 10.0°C). As a first step we investigated the shape of the temporal autocorrelation functions. We found that at the lower protein volume fractions in this range of ϕ the autocorrelation functions appeared to be exponential. However, as the protein volume fraction was increased and the temperature decreased the correlation functions became non-exponential.

At nonzero q , a deviation from exponential decay is to be expected on the basis of the present theory for diffusion in colloidal dispersions (see, for example, Ref. 1). To characterize this nonexponential decay, Cichocki and Felderhof have suggested that the autocorrelation functions be analyzed in terms of a distribution of decay rates.³⁵ We determined such distributions from the experimentally obtained autocorrelation functions by using the constrained regularization algorithm described above. Representative results are shown in Fig. 3. The three distributions in this figure were determined from autocorrelation functions that were obtained at wave number $q=2.43\times 10^5\text{ cm}^{-1}$ and $T=20^\circ\text{C}$ at three different protein volume fractions ($\phi=0.012\pm 0.001$, 0.101 ± 0.001 , and 0.161 ± 0.001). This figure shows that as the protein vol-

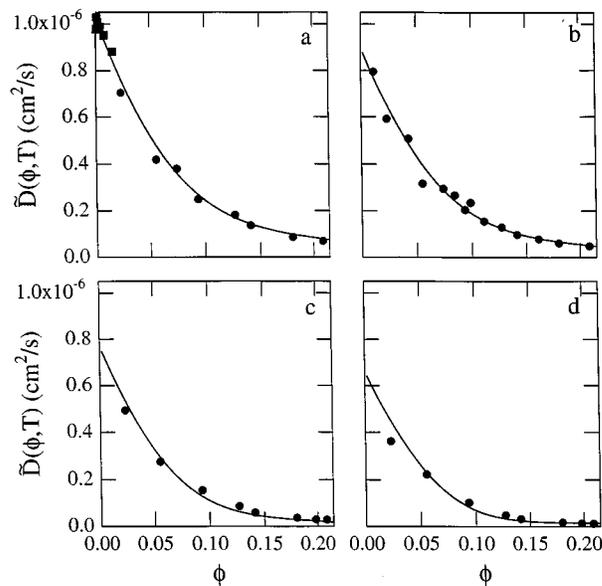


FIG. 4. The collective diffusion coefficient $\bar{D}(\phi, T)$ vs ϕ for the four temperatures studied. (a) 25.0°C , (b) 20.0°C , (c) 15.0°C , and (d) 10.0°C . The solid lines are eye guides. In (a), the data obtained at very low protein volume fraction (squares) is also included. These data are also presented in Fig. 2(a).

ume fraction is increased, the average of the distribution decreases and the distribution becomes increasingly broad. Unfortunately, to the best of our knowledge, the present theory does not yet provide a quantitative prediction for the distribution of decay rates for particles with both attractive and hydrodynamic interactions.

We used the experimentally determined values of the first cumulant K_1 for each autocorrelation function to determine the collective diffusion coefficient $\bar{D}(\phi, T)$. Specifically, as described in Sec. III, we determined K_1 by making a least-squares fit of Eq. (4) to each autocorrelation function. We found that Eq. (4) provided a very good characterization of the autocorrelation functions over the range of q , ϕ , and T studied. We then determined $\bar{D}(\phi, T)$, as defined in Eq. (5), at each temperature and protein volume fraction by extrapolating K_1/q^2 to $q=0$. Figure 4 contains plots of $\bar{D}(\phi, T)$ vs protein volume fraction for the four different temperatures studied.

We determined $\bar{H}(\phi, T)$ from the experimental data for $\bar{D}(\phi, T)$ using Eq. (5). In these calculations, we used Eq. (8) to provide values for $S(\phi, T)$. As we have shown above, this expression with appropriate choice of τ for each temperature (see Table I) accurately represents our experimental data. We calculated $D_0(T)$ for $T=20.0, 15.0$, and 10.0°C by using the experimentally determined value of $D_0(T=25.0^\circ\text{C})$ and by assuming that $D_0(T)$ is given by the Stokes–Einstein equation [Eq. (15)]. The values of $D_0(T)$ at the temperatures of interest are listed in Table I.

Figure 5 contains plots of the experimentally deduced values of $\bar{H}(\phi, T)$ vs ϕ for the four temperatures studied. This figure shows that for $0.012\leq\phi\leq 0.21$, $\bar{H}(\phi, T)$ de-

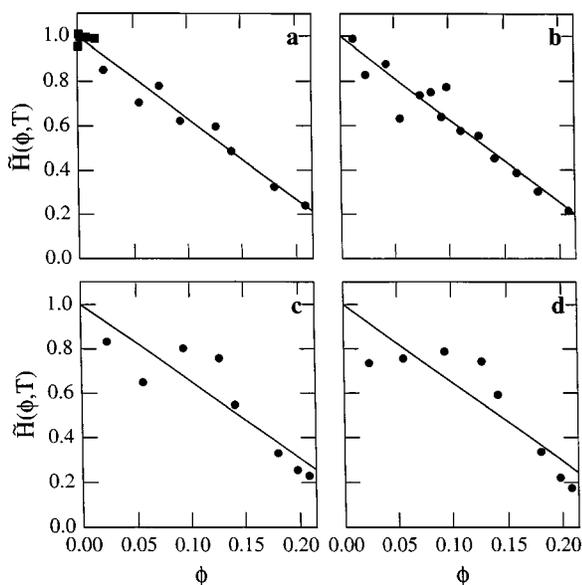


FIG. 5. The hydrodynamic factor $\hat{H}(\phi, T)$ vs ϕ for the four temperatures studied. (a) 25.0 °C, (b) 20.0 °C, (c) 15.0 °C, and (d) 10.0 °C. The solid lines are least-squares fits of the data to straight lines. In (a), the data obtained at very low protein volume fraction (squares) is also included. These data are also presented in Fig. 2(b).

creases dramatically with ϕ at all four temperatures. This is clearly in disagreement with the theoretical predictions for $H(\phi, T)$ to first order in ϕ . According to Table I, $\lambda_{\text{stick}}^{\text{approx}}(T)$, $\lambda_{\text{slip}}^{\text{approx}}(T)$, and $\lambda_{\text{stick}}^{\text{asympt}}(T)$ all have positive values for the temperatures of interest. Consequently, regardless of which of the three specific methods is used to calculate $\lambda(T)$, the theory predicts $H(\phi, T)$ to increase with ϕ at the temperatures studied. In contrast, the experimental data shows that at all temperatures studied $\hat{H}(\phi, T)$ decreases with the slope $(\partial\hat{H}/\partial\phi)_T \approx -4$. Thus, although we find that $H(\phi, T)$ is consistent with the data for $\hat{H}(\phi, T)$ at low protein volume fractions ($0.0036 \leq \phi \leq 0.016$), we find that clear disagreement between $H(\phi, T)$ and $\hat{H}(\phi, T)$ becomes evident for $\phi \geq 0.02$.

V. DISCUSSION AND CONCLUSION

We have studied the mean intensity and the temporal autocorrelation function of the intensity of light scattered by γ_{II} -crystallin in aqueous solution as a function of q , ϕ , and T . The mean intensity data were used to find the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$. This data provides information on the pair interaction potential, $u(r)$. However, it is generally not possible to unambiguously deduce $u(r)$ from experimental $S(\phi, T)$ data. Instead, one must hypothesize a form for $u(r)$, which may then be used to calculate $S(\phi, T)$. This calculated $S(\phi, T)$ may then be compared with experimental data to determine whether the hypothesized $u(r)$ is a reasonable model for the system of interest.

We chose the potential $u(r)$ to have the form of a spherically symmetric attractive square well with a repulsive hard core. This choice is consistent with the experimental observation that this protein solution exhibits liquid–liquid phase

separation. Furthermore, experimental measurements of the osmotic pressure and static structure factor of similar protein solutions also indicate the presence of an attractive interaction.^{36,37} We have regarded the range of this attractive interaction as small in comparison with the size of the protein. This assumption is consistent with the fact that the Debye screening length is quite small under our solution conditions. Further support for this presumption is provided by Monte Carlo simulations which show³⁸ that the observed critical volume fraction $\phi_c = 0.19$ is consistent with a small relative range parameter, viz., $\delta \leq 0.25$. Indeed, we carried this short range assumption to its logical limit and have represented the protein–protein interactions by the well known Baxter potential for adhesive spheres. This model has two distinct advantages. The first is that the entire effect of interprotein interactions on the equilibrium thermodynamic properties can be represented by a single parameter τ . The second is that the Baxter potential permits an analytical representation of the $q \rightarrow 0$ limit of the static structure factor $S(\phi, T)$ in the Percus–Yevick approximation. These two advantages provide a very convenient means for the comparison between theory and experiment.

By making such a comparison we have found, at each temperature T , a value of τ such that the theoretical expression for the structure factor $S(\phi, T)$ accurately represents the experimental data as shown in Fig. 1. If we plot the values of τ deduced from our data as a function of temperature and extrapolate to $T = T_c$ we find that the corresponding τ is equal to 0.098 ± 0.002 in excellent agreement with the theoretical prediction $\tau = 0.0976$. The observed temperature dependence of τ is in fact a consequence of the finite range and depth of the attractive part of the potential. If we make use of Eq. (7) without taking the limit $\delta \rightarrow 0$, we deduce from the magnitude and the temperature dependence of τ that the relative range δ is equal to 0.06 which is consistent with our underlying assumption that $\delta \ll 1$. In this connection it should be mentioned that the numerical solution of the Percus–Yevick equations for the potential with finite range and depth²⁷ shows that the Baxter analytical solution is quite accurate up to $\delta \approx 0.1$. We conclude that the Baxter model provides a self-consistent means for the accurate description of our data on the structure factor.

With this reasonable representation of the interaction potential, we next turn our attention to the transport properties of γ_{II} -crystallin in aqueous solution. We obtained the time autocorrelation function of the intensity of light scattered by the aqueous γ_{II} -crystallin solutions. We analyzed these autocorrelation functions with a constrained regularization algorithm to obtain corresponding distributions of decay rates. This analysis revealed a broad distribution of decay rates when the protein volume fraction is sufficiently high. A theoretical prediction for the distribution of decay rates has been obtained for a suspension of hard spheres without hydrodynamic interactions.^{35,21} However, such a prediction is not yet available for a system of particles with both attractive and hydrodynamic interactions, such as the system under study in this paper. We have presented representative plots of the distribution of decay rates in this paper to facilitate comparison

with theoretical results that may be obtained in the future.

We also used the QLS measurements to determine the collective diffusion coefficient $\tilde{D}(\phi, T)$ as a function of ϕ and T . We found that $\tilde{D}(\phi, T)$ decreases with both increasing ϕ and decreasing T . We used the experimental values for $\tilde{D}(\phi, T)$ to obtain the hydrodynamic factor $\tilde{H}(\phi, T)$ and compared the experimental data for $\tilde{H}(\phi, T)$ with the current theoretical expression for $H(\phi, T)$, the hydrodynamic factor in the limit $q \rightarrow 0$, $t \rightarrow 0$. Since the Baxter adhesive hard sphere potential provided an excellent characterization of the experimental data for $S(\phi, T)$, we used it in the theory for $H(\phi, T)$. We found that the theoretical prediction for $H(\phi, T)$, to first order in ϕ , is consistent with the $\tilde{H}(\phi, T)$ data for $\phi \leq 0.016$. However, at higher protein volume fractions, we found unequivocal disagreement between the $H(\phi, T)$ and the $\tilde{H}(\phi, T)$ data. The theory predicts, to first order in ϕ , that $H(\phi, T)$ will increase with increasing ϕ . In contrast, we found that for $\phi \geq 0.02$, $\tilde{H}(\phi, T)$ steeply decreases with ϕ , as shown in Fig. 5.

This disagreement between theory and experiment cannot be ascribed to our choice of the Baxter model for the protein-protein interactions. Indeed, if we choose a square well potential with width $\delta = 0.25$, which is an upperbound on the width of the potential suggested by Monte Carlo simulations,³⁸ we find that the hydrodynamic factor is essentially the same as in the limit $\delta \rightarrow 0$. Therefore, the Baxter model serves as a good approximation of a short range potential both for the calculation of $S(\phi, T)$ and $H(\phi, T)$.

Our experimental data suggest that $\tilde{H}(\phi, T)$ has a different ϕ -dependence at low ($\phi \leq 0.016$) and high ($\phi \geq 0.02$) protein volume fractions. This change in the ϕ -dependence of $\tilde{H}(\phi, T)$ cannot be regarded as a reflection of the phase transition which occurs in this system. As we have shown previously,¹³ critical phenomena affect the dynamical behavior only in the immediate vicinity of the critical point $\phi_c = 0.19$ and $T_c = 3.9$ and are insignificant in the ϕ - T domain studied here. We may speculate, therefore, that this difference in the ϕ -dependence occurs because the system makes a transition from one dynamic regime to another as the protein volume fraction increases beyond $\phi \approx 0.02$. In considering this transition, one must recall that the QLS measurements probe the dynamic behavior of the system over a length scale that is on the order of q^{-1} . For our protein solutions, q^{-1} is large compared to the size of the protein molecules, the range of their interaction and the average distance between them. There is, however, another important length in this problem related to the long range hydrodynamic interactions; viz, the hydrodynamic screening length l . According to Tokuyama and Oppenheim² this length scales with particle size a and volume fraction ϕ as $l \sim a/\sqrt{\phi}$. We may expect different dynamic behavior depending on whether our probe length q^{-1} is large or small compared to the screening length l . The transition between the two behaviors occurs when $ql \approx 1$. Clearly then, it is useful to introduce a transition volume fraction ϕ_T which scales with particle size and wave number as $\phi_T \sim (qa)^2$. At low protein volume fraction $\phi < \phi_T$ the length probed by a QLS measurement will be short compared to the screening

length and we will see individual particle motion. When $\phi > \phi_T$ the length probed by a QLS measurement will be large compared to the screening length and we will observe the effects of multiparticle interaction. Since ϕ_T is proportional to a^2 , we expect that large particles will obey the low ϕ behavior up to much higher volume fractions than will small particles. This expectation is consistent with reports that for relatively large sterically stabilized silica particles with radii between 23 and 47 nm the experimental data for $\tilde{H}(\phi, T)$ are in good agreement with theoretical predictions for $H(\phi, T)$ over a wide range of concentrations,²³⁻²⁵ whereas for the small protein BSA (with $a = 3$ nm), a significant discrepancy even at very low concentration has been reported.²² If our conjecture is correct, then the experimentally observed transition volume fraction ϕ_T should scale as q^{-2} . This prediction may be tested by measurements of the position of the maximum in $\tilde{H}(\phi)$ over the experimentally accessible range of q .

Our experimental data show that the theoretical prediction of $H(\phi, T)$ to first order in ϕ exhibits unequivocal disagreement with our $\tilde{H}(\phi, T)$ data for $\phi > \phi_T$, where ϕ_T is in fact quite small for our system ($\phi_T \approx 0.02$). This failure may be the consequence of the fact that, for $\phi \geq \phi_T$, the theoretical prediction based on the short time kinetics is no longer applicable in the time domain probed experimentally. The experimentally determined first cumulant typically reflects the diffusion of particles over the characteristic time scale $t \sim (Dq^2)^{-1}$. This value can be related to the $t \rightarrow 0$ result only if the autocorrelation function does not contain a wide distribution of relaxation times. Ackerson¹⁸ showed that (when all interactions, including hydrodynamic interactions, are pairwise additive) such single exponential behavior should be the case when q^{-1} is larger than all other characteristic lengths of the system. However, for $\phi \geq \phi_T$, as we demonstrated above, q^{-1} is actually comparable to the hydrodynamic screening length. Under these conditions we may expect that the autocorrelation function will exhibit a deviation from exponential decay, as we have found experimentally. Thus, we expect that the approach taken in the theory¹⁹ does not apply to the quantities measured experimentally when the concentration exceeds ϕ_T .

While this analysis is speculative, the experimental data imply the existence of some heretofore unappreciated length scale which depends on the particle concentration. This length manifests itself in the dynamic light scattering experiments in relatively dilute solution, $\phi \sim 0.02$, in the case of small particles such as proteins. Clearly the supposition presented here opens the door to promising opportunities for further theoretical and experimental investigation of the dynamic properties of the concentrated colloidal dispersions.

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