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Dynamic Light Scattering Application to Study Protein Interactions in Electrolyte Solutions

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Abstract. The concentration dependence of the diffusion coefficient of particles suspended in solution depends primarily on the occupied volume fraction and on repulsive and attractive forces. This dependency is expressed by the interaction parameter, which can be assessed experimentally by light scattering measurements and have been determined for the diffusion coefficient of BSA under different salt concentration conditions in the present work. The result shows that the diffusion coefficient of protein grows up with increasing protein concentration, and when the ionic strength turns up gradually the diffusion coefficient decreases with protein concentration's increasing. The concentration dependence of BSA diffusion coefficients is interpreted in the context of a two-body potential of mean force, which includes repulsive hard-sphere and Coulombic interactions and attractive dispersion. With the increase of ionic strength, Debye screening decreases, protein interaction changes from repulsion to attraction, and protein begins to aggregate. By means of the concentration dependence of BSA diffusion coefficients, one can obtain the parameters of protein interactions and can find that protein bears a net effective charge of -9.0 e and has a Hamaker constant of $2.8k_{\mathrm{B}}T$. This work demonstrates that DLS is an effective technique of studying protein interactions.

Key words: protein interactions, diffusion coefficient, dynamic light scattering

1. Introduction

Protein precipitation and crystallization are key steps in the recovery and characterization of virtually all proteins [1–6]. In industry, salt-induced protein precipitation is frequently used as a first-pass purification step, and in research, large, high-quality crystals are required for structure determination and studies of structure function relationships. For these applications, predictive models based on fundamental protein properties would be of considerable benefit. Because precipitation and crystallization are both aggregation processes driven by intermolecular interactions, it is crucial to understand how equilibrium interactions depend on experiment variables. Recent work in this area has focused on correlating precipitation and crystallization data with molecular quantities such as the potential of mean force between protein monomers and the osmotic second virial coefficient [1–3, 7]. In this paper we report the results of dynamic light scattering measurements of the diffusion coefficient and the extent of aggregation in solutions of a common protein,

BSA, with the salt concentration varying between dilute and near-salting-out conditions.

Dynamic light scattering (DLS) is a technique based on measuring the fluctuations in the intensity of light scattered from particles in a solution without perturbing the system [8]. It has been used to determine the diffusion coefficients and the sizes of particles in solution rapidly and exactly. The research results have been published in all kinds of journals [9–14].

2. Theoretical Considerations

2.1. Dynamic Light Scattering

In DLS experiments, the normalized intensity autocorrelation functions of the scattered light, $G^{(2)}(\tau) = \langle I_s(t)I_s(t+\tau)\rangle / I_s^2(t)$, is measured and is used to calculate the normalized time correlation function of the scattered electric field [9], $g^{(1)}(\tau) = \langle E_s(t)E_s^*(t+\tau)\rangle / I_s(t)$. For a Gaussian signal, the Seigert relation, $G^{(2)}(\tau) = \mathrm{A}\,(1+\beta|g^{(1)}(\tau)|^2)$ holds, where the coherence factor β measures the degree of coherence of the scattered light. For a monodisperse dilute solution, $g^{(1)}(\tau)$ is represented by a single exponential as follows

$$g^1(\tau) = \exp(-\Gamma\tau), \tag{1}$$

where $\Gamma = D_0 q^2$, the length of the scattering vector $q = |\vec{q}| = \frac{4\pi n}{\lambda_0}\sin(\theta/2)$. D_0 is the translational diffusion coefficient in infinitely-dilute solutions, which is related to the hydrodynamic radius R_s by the following Stokes-Einstein relationship [8]:

$$D_0 = K_B T/(6\pi\eta R_s), \tag{2}$$

where K_B is the Boltzman constant, and η is the solvent viscosity.

2.2. First Order Interaction Parameter

For concentrated systems the various types of interactions between particles are to be taken into account. The problem of interactions, as determined by DLS, has been under investigation for over thirties years, the most viable theories are the ones developed by Batchelor, Felderhof and Phillies [15–18].

For concentrated macromolecular solutions in the hydrodynamic regime, being modified to account for finite concentration effects and having been corrected to the first order in concentration, Eq. (2) can be written as follows

$$D_m = D_0[1 + \lambda\phi + O(\phi^2) + \cdots]. \tag{3}$$

For systems of monodisperse interacting spheres, interaction parameter λ may be expressed generally as follows [18]

$$\lambda = \lambda_{HS} + \int_{2a}^{\infty} f(r, 2a) \cdot \left[1 - \exp\left(-\frac{W_{total}(r)}{k_B T}\right)\right] dr, \tag{4}$$

where λ_{HS} is a hard-sphere term, a is particle radius, and $f(r, 2a)$ is a function that takes into account both direct interparticle forces and hydrodynamic interactions,

$$f(r, 2a) = \frac{24r^2}{(2a)^3} - \frac{12r}{(2a)^2} + \frac{90a}{8r^2} - \frac{93(2a)^3}{32r^4} - \frac{225(2a)^4}{32r^5}, \tag{5}$$

$W_{total}(r)$ is the total potential of mean force.

2.3. Energy of Interaction

For calculating the interaction parameter λ, the effective interaction potential should be known. As a first approximation this potential can be considered, according to the DLVO theory, as the sum of three contributions [15]

$$W_{total}(r) = W_{hs}(r) + W_{disp}(r) + W_{elec}(r), \tag{6}$$

The hard-sphere potential $W_{hs}(r)$ is

$$W_{hs}(r) = \begin{matrix} \infty, r \le 2a \\ 0, r > 2a \end{matrix}. \tag{7}$$

The attractive forces acting between two molecules in solution are caused by the Van der Waals induced dipole interaction:

$$W_{disp}(r) = -\frac{H_A}{12}\left[\frac{(2a)^2}{r^2 - (2a)^2} + \frac{(2a)^2}{r^2} + 2\ln\left(\frac{r^2 - (2a)^2}{r^2}\right)\right] \quad r > 2a, \tag{8}$$

where H_A denotes the Hamaker constant. Eq. (8) is divergent for $r = 2a$ and the numerical implementation of this expression requires a lower cut-off value that may considerably modify the magnitude of the attractive energy $W_{disp}(r)$. We have increased the lower integration bound by a value that can approximately qualify for the thickness of one Stern layer.

At low particle and ionic concentrations, the potential of mean force can be approximated by the well-known mean field expression

$$W_{elec}(r) = \frac{Z_p^2 e^2}{4\pi\varepsilon r} \frac{e^{-\kappa(r-2a)}}{(1+\kappa a)^2}, \tag{9}$$

where κ is the inverse Debye-Huckel length,

$$\kappa = \left(\frac{e^2 N_A}{\varepsilon k_B T} \sum_j Z_j^2 n_j \right)^{1/2}, \tag{10}$$

and e denotes the electronic charge, N_A Avogadro's number, ε the permittivity of the solvent, Z_j the ionic valence and n_j is the number concentration of the jth ion.

3. Materials and Methods

The sample preparation is a critical component for measurements of light scattering from protein solutions. All chemicals used were reagent grade. Deionized water was used as solvent. The bovine serum albumin purchased from SIGMA Company. The buffers used were Na-acetate at 50 mM, adjusted to 0.05, 0.1, 0.2, and 0.5M by adding sodium chloride alone. The samples were shook, and filtered through a 0.2-μm nitrocellulose membrane filter. The investigated protein volume fractions were 0.01, 0.02, 0.03 and 0.04 with a fixed pH of 5.4. All samples were centrifuged at 15000 rpm for 40 minutes prior to the light-scattering experiment. For each sample, the measure time was 2 minutes at a temperature of 27 °C. Each measurement was repeated six times. The last results were average value of six times measurements.

Dynamic light scattering system consisted of Verdi-10 laser (Coherent corp.) tuned to 532 nm, and operating at an output power 10 mw, BI-200SM goniometer and BI-9000AT digital autocorrelator. Sample cell were mounted at the center of a temperature-controlled, refractive index matched bath. All measurements were made at a scattering angle of 90 °.

4. Results and Discussion

The DLS measurements are performed in the long-time, or hydrodynamic, regime, where the time scale of the light scattering experiment τ is much greater than either the intrinsic time scale for Brownian motion or that for direct interparticle interactions. Table I shows diffusion coefficient of BSA in different ionic strength solutions at pH 5.4, temperature 27 °C.

4.1. Monomer Diffusion Coefficient D_0 and Interaction Parameter λ

Figure 1 shows a plot of normalized diffusion coefficient $D^{(c)}/D_0$ as a function of the protein volume fraction Φ at pH 5.4 and temperature 27 °C. The intercept corresponds to infinite dilution diffusion coefficient. The data do not collapse at

Table I. Diffusion coefficient of BSA in different ionic strength solutions with pH 5.4 and temperature 27 °C

I (M)	φ ($\times 10^3$)	D ($\times 10^7$ cm^2/s)	Error	I (M)	φ ($\times 10^3$)	D ($\times 10^7$ cm^2/s)	Error
	10	6.85	0.05		10	6.67	0.03
0.05	20	6.89	0.03	0.20	20	6.60	0.04
	30	7.01	0.05		30	6.54	0.04
	40	7.05	0.03		40	6.44	0.05
	10	6.72	0.04		10	6.62	0.03
0.10	20	6.7	0.03	0.50	20	6.51	0.05
	30	6.65	0.03		30	6.44	0.04
	40	6.63	0.05		40	6.29	0.03

Note. I means ionic strength and φ indicates protein volume fraction.

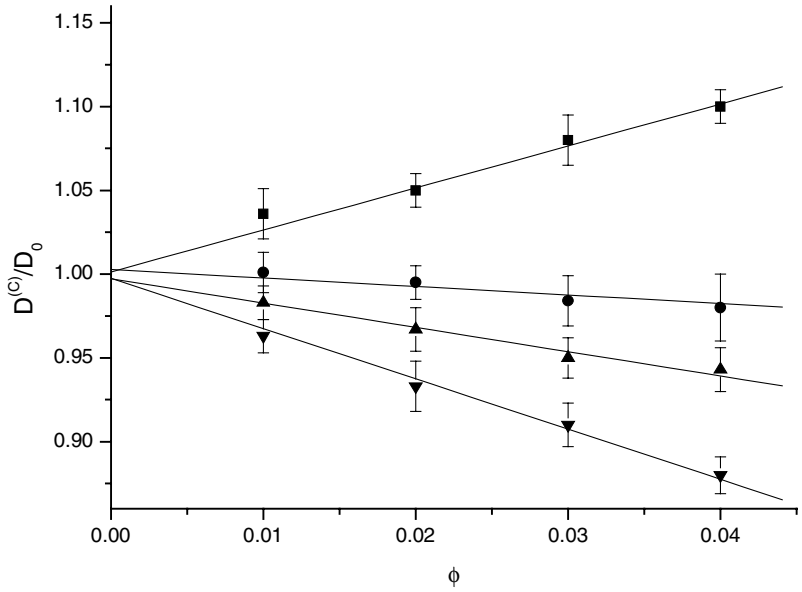

Figure 1. Normalized diffusion coefficient of BSA monomers as a function of the protein volume fraction Φ Each point is the average of six measurements. Error bars denote six standard deviations. The slopes of the curves yield the interaction parameter λ through Eq. (3); NaCl molarities are: 0.05 M (■); 0.1 M (●); 0.2 M (▲); 0.5 M (▼).

a single point on the ordinate axis as expected. At low salt concentrations, the diffusivity increases with protein concentration increasing. With increasing salt concentration this trend is eventually reversed. It indicates that protein interactions change from repulsion to attraction. This is due to the counterions increasingly screening Coulombic repulsion when the ionic strength increases.

Table II. Inverse Debye-Huckel screening length κ, interaction parameter λ, and infinite dilution extrapolated diffusion coefficient D_0, for four NaCl molarities; the interaction parameter has been extracted from linear regression of measurements sets involving four different BSA concentrations in the range between 0.05 and 0.5 mM to allow an accurate determination to be made of the collective diffusion coefficient Dm as a function of volume fraction Φ

C_{ion} (M)	$\kappa(\times 10^9\ \text{m}^{-1})$	λ	D_0 ($\times$10e$-$7 cm^2/s)
0.05	0.73	1.12 ± 0.10	6.77 ± 0.03
0.10	1.04	-0.4 ± 0.11	6.75 ± 0.01
0.20	1.46	-1.15 ± 0.12	6.75 ± 0.02
0.50	2.31	-1.46 ± 0.10	6.73 ± 0.03

The interaction parameter λ was obtained by a simple linear regression of $D^{(c)}$ versus ϕ for each NaCl molarities. Higher order fits were not justified. The inverse Debye-Huckel screening length κ, the interaction parameter λ, and the monomer diffusion coefficient, extrapolated at infinite dilution, D_0, are given in Table II. The value of the free-particle diffusion coefficient, $D_0 = 6.75 \pm 0.05 \times 10^{-7}$ cm^2/s, extrapolated to zero salt molarity and zero volume fraction, agrees with the values reported by other investigator. The hydrodynamic radius resulting from Eq. (4) is 3.45 nm. A. K. Galgalas *et al* measured the BSA diffusion coefficient in 0.5g/L solution at 23 °C, pH 5 with DLS, they obtained 6.79×10^{-7} cm^2/s [19]. Nispa Meechai *et al* obtained hydrodynamic radius 3.58 nm at pH 7.4, I = 0.15 M solutions [9].

4.2. Molecular Parameters Z_P and H_A

The potential of mean force (PMF) outlined previously contains two adjustable parameters: the Hamaker constant, H_A, and the protein monomer valence, Z_P; both are obtained from the data at pH 5.4. For each ionic strength between 0.05 and 0.5 M, a curve was generated representing the set of $\{H_A, Z_P\}$ points that satisfy the constraint that the experimentally measured λ and the λ calculated from the PMF model are equal. This was achieved by setting H_A to a constant value and finding the value of Z_P that gave a calculated value λ equal to the experimentally measured value. These regressions were performed with MATLAB 6.5, a symbolic equation-solving software application. Each curve in Figure 2 was generated stepwise by repeating this procedure over small increments in H_A. The common intersection of these curves for different ionic strength occurs at $H_A/K_BT = 2.8$ and $Z_P = -9.0$ e. This procedure has been used previously to obtain molecular interaction parameters. Martin Muschol and Franz Rosenberger performed static and dynamic light-scattering measurements on lysozyme in solutions of solium chloride and sodium acetate at pH 4.7 with a total ionic strength from 0.05 M to 0.5 M. They calculated from DLS data the $H_A = 7.2k_BT$, $Z_P = 5.4$ [3]. Charles

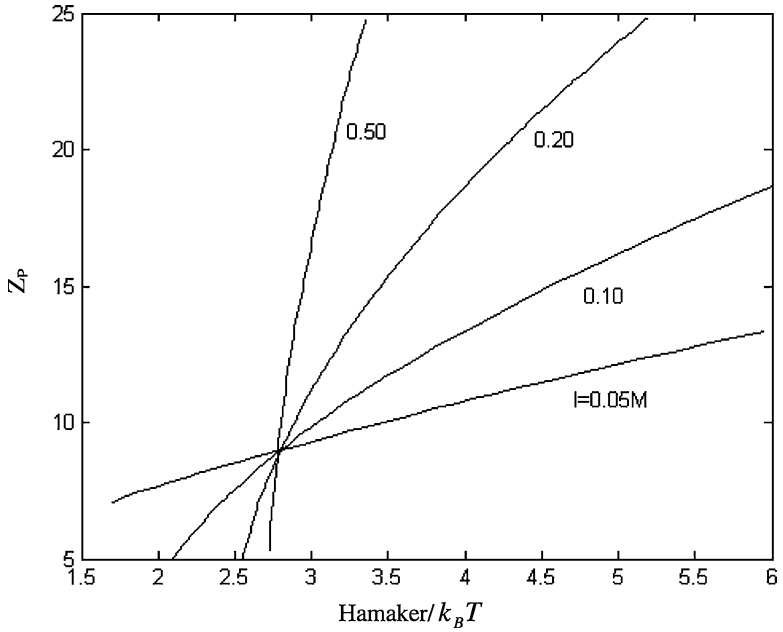

Figure 2. Hamker constant H_A versus protein charge Z_P. The plotted curves correspond to the measured values of the interaction parameter$^{\lambda}$ at the different degrees of screening. The curves have a common locus with coordinates $Z_P = 9.0e$ and $H_A = 2.8k_BT$.

M. Roth *et al* calculated out $H_A = 3.1$ of BSA by accounting for both the geometric irregularity of protein molecules and the material properties of the interacting media [20]; Jianzhou Wu *et al* obtained $H_A = 2.48k_BT$ by regressing osmotic pressure data using random phase approximation model in pH 5.4 BSA solution [7]. The charge is calculated as -9 e.u. This can be compared with the result of Vilker V. L. [21], who estimated the BSA charge numbers in 0.15 M sodium chloride aqueous solutions is -9.1 at pH 5.4 by calculation, which employed titration data.

The Hamaker constant for the interaction of two colloidal particles depends primarily on their chemical compositions, which determine the magnitude of their overall electronic polarizabilities. It also depends on the dielectric properties of the medium containing the particles. Hence H_A depends to some extent on the ionic strength of the protein solution. Furthermore, as solution pH changes, the net fixed proton charge of the protein changes; the effect of this change is taken into account by Eq. (4).

Figure 3a illustrates the magnitude of the contributions to the potential of mean force for protein monomers, as a function of particle separation r. At 0.05M ionic strength, electrostatic repulsion is strong. However, because of the strength of the attractive dispersion interaction at small separations, the overall potential becomes slightly attractive near contact. Figure 3b shows that, as ionic strength increase,

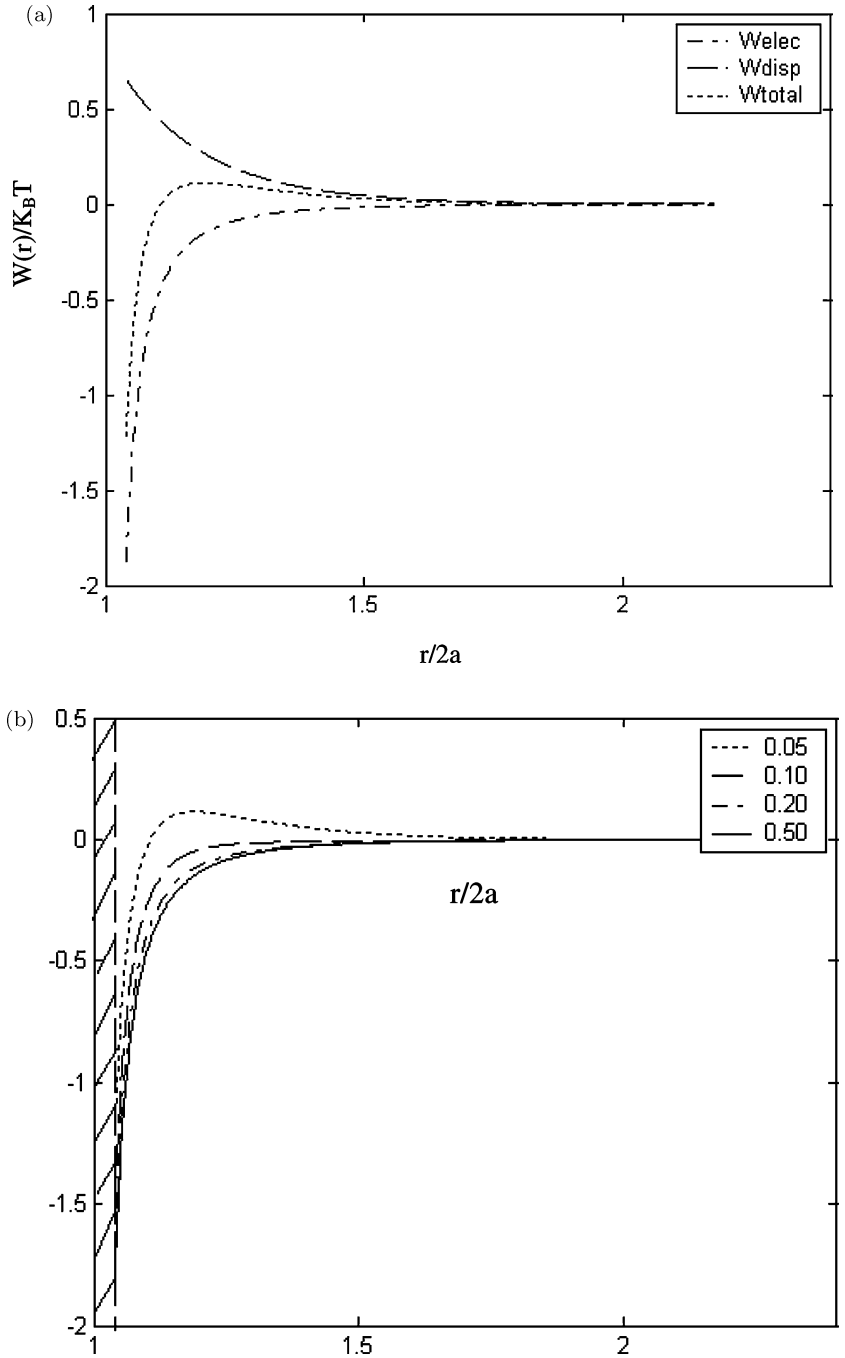

Figure 3. (a) Contribution to the potential of mean force between two protein monomers as a function of reduced distance for I = 0.05M. (b) Dependence of the potential of mean force on ionic strength. Parameters: $Z_P = -9.0e$, $H_A = 2.8k_BT$ and $a = 3.45$ nm.

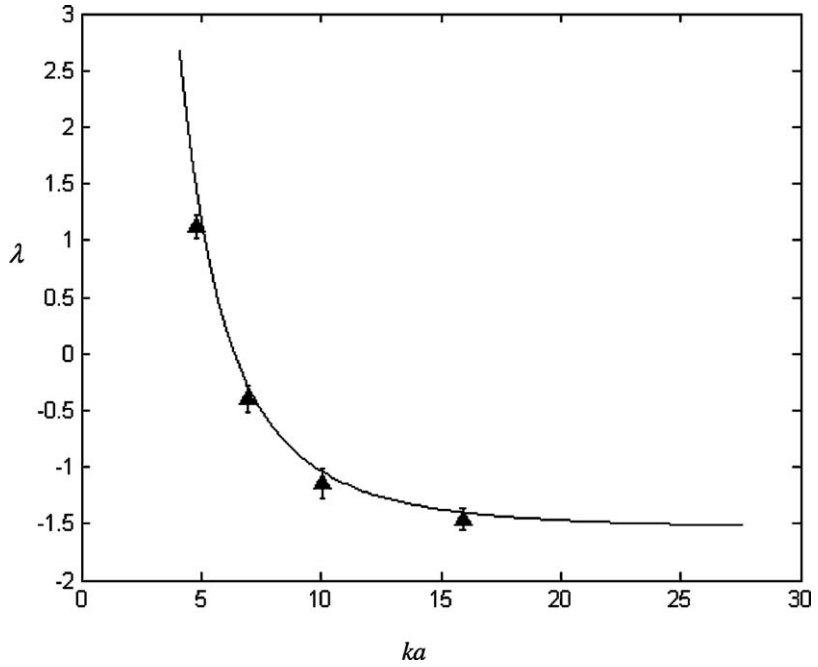

Figure 4. Interaction parameter λ as a function of dimensionless parameter κa. Parameters: $Z_P = 9.0e$, $H_A = 2.8k_BT$.

the repulsive barrier diminishes because of screening of the repulsive electrostatic contribution.

The interaction parameter, λ, is displayed in Figure 4 as a function of the dimensionless product κa. The solid line is calculate with the above determined values of protein charge and Hamaker constant and satisfies the observation. It drops from positive to negative values and reaches a constant value at about $\kappa a = 16$ that corresponds to a concentration of 0.5 M NaCl. As indicates that high ionic strength have completely screened electrostatic repulsion of protein molecular, and the interaction dominates by dispersion attraction.

Protein interactions can be described quantitatively by a two-body potential of mean force; three-body and higher interactions become important at a higher concentrations. Selection of the proper form of the potential of the mean force requires knowledge of the dominant physical interactions between proteins in equilibrium solutions. In low-ionic-strength solutions, proteins interact primarily through a balance of electrostatic repulsion and attractive dispersion forces. In high ionic strength solutions, where salt-induced protein precipitation occurs, macromolecular Coulombic interactions are essentially screened and the overall interactions are attractive. Theories that predict the concentration dependence of the diffusion coefficient of spherical particles have been given by Batchelor, by Felderhoff, and by Phillies [16–18]. Batchelor was the first to obtain a theoretical expression for

the mutual coefficient that incorporates exact numerical knowledge of the two-body hydrodynamic interaction between particles [16]. Subsequently, Federhoff formulated a description based on the Smoluchowski equation that extend to general potential interaction and allows mixed stick-slip boundary conditions at the surfaces of the spheres [17]. Phillies carried out the first calculation of the second order correlation term to D_m and found that three-body hydrodynamic interactions contribute significantly to this term [18].

A large number of solution parameters that play a crucial role in deciding the crystal growth. Crystallization of biomolecules is a process involving nucleation and crystal growth. This process is determined to a large extent by the effective interaction between the molecules and the kinetic factors that control the nucleation and growth. The driving force for both nucleation and crystal growth is supersaturation, i.e., the concentration of solute in the solution above equilibrium solubility. Lowering temperature, increasing ionic strength, adjusting pH and increasing protein concentration can increase supersaturation.

The analysis of light scattering data on systems of interacting particles can only be carried out meaningfully if care is exercised in the computation of interactions. For charged proteins, these interactions are dominated by Coulombic repulsion. As the ionic strength is increased, Coulombic interactions decrease and Van der Waals interactions prevail; particles collide with each other and build dimers or higher aggregates. This effect manifests itself as an increment of the scattered intensity and as a decrease in the collective diffusion coefficient. Under optimal crystallization conditions, energetic barriers should be as low as possible to allow dimerization and further nucleation to take place.

In the present work we have adopted a simple treatment for interpreting the DLS results and obtaining estimates of the charge and of the Hamaker constant of BSA. This is a very important step toward the diagnostics of protein crystallization, since we can assess the instability threshold for a given precipitating agent. While Van der Walls interactions do not influence the diffusion coefficient of stable suspensions during nucleation, the system is far from equilibrium and it is expected that Van der Waals forces will be operative when Coulombic forces expire.

In the limit of infinite dilution, at pH 5.4, BSA behaves as monomer with diffusion coefficient of $D_s = 6.74\ e{-}7$ cm^2/s, bears a net charge $Z_P = -9.0e$ and a Hamaker constant $H_A = 2.8k_BT$. Both values fit well the experimentally determined interaction parameter λ when the latter is determined as a function of κa. These molecular parameters will be useful for computer simulations concerning the aggregation and crystal formation processes.

The DLVO framework, used here to model the interactions, should be considered only as a rough, first order approximate. It neglects both non-Coulombic ion–ion repulsion and ion–ion correlations, and overestimates the importance of the Van der Waals interactions, since it is assumed that they are not modified by the solvent.

We have shown that the concentration dependence of the diffusion coefficient of monomeric species in aggregation experiments under optimal or suboptimal

crystallization conditions may be used as a primary tool for obtaining information concerning the future of the solution. At least in experiments with BSA there is a strong correlation between the determined interaction parameter, λ, and the ability of the solutions to produce well diffracting crystals. These studies have to be extended to other proteins that serve as candidates for studying protein crystallization.

5. Conclusions

Dynamic light scattering has been used to study the interactions of BSA in aqueous of sodium chloride at 27 °C. The concentration dependence of diffusion coefficient is interpreted in the context of a two-body potential of mean force that includes repulsive hard-sphere and Coulombic interactions and attractive dispersion. Analyses of data for different ionic strength allow regression of the effective monomer charge of $-9.0\ e$ and Hamaker constant of $2.8k_{\mathrm{B}}T$. From the results, we find that DLS is actually an effective technique to study protein interactions.

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