Anomalous colloidal stability of protein coated polystyrene latex beads studied by small angle light scattering

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Abstract

Results of small angle laser light scattering experiments carried out on polystyrene latex beads coated with lysate of Plasmodium falciparum (Pl) antigen (Ag) and human seropositive sera (1:10 ratio) are reported for various NaCl concentrations (0-300 mM). The protein coated beads showed time-dependent coagulation. The normalised intensity of scattered light $I(t)/I_0$ showed $I(t)/I_0 = 1 + (t/t)^\delta$ behaviour with the coagulation rate, $t$ and exponent, $\delta$ showing anomalous dependence on NaCl concentration. The coagulation rate exhibited strong increase up to NaCl concentration of 50 mM, above this and up to 300 mM the coagulation rate was found to remain independent of NaCl concentration yielding non-DLVO behaviour. The same was true for $\delta$ which increased from 1.04 ± 0.06 to 6.94 ± 0.07 as NaCl concentration was raised from 0 to 50 mM. Above 50 mM it remained constant with $\delta = 6.94 ± 0.07$. Results are discussed through Smoluchowski aggregation kinetics and theoretical construction of interparticle interaction potentials relevant to our problem.

Keywords: Colloidal stability; Small angle light scattering; Protein coated latex

1. Introduction

Presence of salt plays an important role in determining the stability of colloidal dispersions in solvent environment at a given temperature. The DLVO theory describes the total interaction potential between pairs of colloidal particles [1,2]. This theory recognises only the repulsive electrostatic energy and the attractive van der Waals dispersion energy between pairs of colloids. This over simplified picture of colloidal interaction shows two minima and one maxima in the potential energy curve as a function of distance. The maximum in the interaction energy curve represents an activation energy which if overcome by approaching colloids with high kinetic energy can give rise to coagulation, thus destabilising the dispersion. This formalism proves inadequate in describing several physical systems [3-8]. Though DLVO theory predicts enhanced coagulation of colloids at higher salt concentrations, same colloidal dispersions have been shown to be highly stable under these conditions.

Recently the role of structural or hydration forces have been investigated in some details and the DLVO formalism has been modified accordingly [9-12]. These additional forces were quantitatively measured by Israelachvili and Adams [11] in mica-aqueous KNO3 solutions. Consequently, it has been argued that the phenomena of wetting, adhesion and flotation etc. would be well accounted for if these repulsive hydration forces were included as a third component in the DLVO picture [12]. The repulsive feature of hydration forces can be visualised as follows. Every macro-ion sees short range ordering of the electrolytic solution in its vicinity. When two such surfaces mutually approach each other, the overlap of these ordered solvent layers occur which creates a mutual force between the approaching surfaces of macro-ions or colloids. Here the lateral correlation of ordered solvent layers is assigned a correlation length, $\xi$, and depending on the system parameters $\xi$ has a critical value $\xi_c$. For $\xi_c < \xi$, the
hydration force is repulsive and when $\xi_0 > \xi$, it is attractive [13]. This exponentially decaying force defines a decay length describing the spatial extent of the hydration force. Leikin and Kornyshev [13] have discussed the dehydration transition in a mean-field theory model with excellent details. Despite its simplicity, this model allows the prediction of dehydration transition, its physical characteristics, the role of structure of surfaces, impurities, and metastable defects in transitions.

This article describes the detailed investigation of colloidal stability of protein coated polystyrene beads studied at various NaCl concentrations. Anomalous stability of the colloidal solutions at higher salt concentrations were observed where DLVO theory predicts aggregation. This work is an offshoot of the Laser immunoassay analysis (LIA) work currently in progress in our laboratory [14].

2. Materials and methods

The assay was performed using lysate of Plasmodium falciparum (PF) as antigen. The antigen and the human sera were kind gift from Malaria Research Centre, New Delhi, India. The antigen (Ag) was coated on polystyrene beads of average nominal diameter of 96 ± 3 nm (Sigma Chemical Company, USA) by incubating it overnight with the beads in 50 mM bicarbonate buffer, pH 9.6 (5 µl of 6 mg/ml Pf per 1000 µl, 1% aqueous suspension of polystyrene beads). The antigen coated latex and the seropositive sample (1:1) was incubated for 2 h before sealing in the quartz capillary cell, volume ≈ 40 µl. After coating, the average hydrodynamic diameter of the latex beads was found to be 120 ± 5 nm (measured by photon correlation spectroscopy, details follow). Before carrying out these experiments, these samples were tested by ELISA and agglutination assay, monitored by absorbance/transmittance at 492 and 340 nm, respectively. Samples that tested clearly positive by both these methods were only used for present work. Samples were sonicated to breakup initial clusters. The light scattering studies were carried out on a home made light scattering goniometer with a He/Ne laser source (AEROTECH, USA) that had a power output of 35 mW and a wavelength of 632.8 nm, attached to an autocorrelator (BI-9000 AT, Brookhaven Instrument Corporation, USA). The total intensity measurement of the scattered light was performed at a scattering angle of 10° and the count rate histogram was monitored in real time using the software provided by the company. In photon correlation measurements, the autocorrelation function of the intensity of the scattered light signal was measured on freshly sonicated sample (assumed to be at $t = 0$) and we did recover the average monomer size of the colloids using CONTIN data reduction software (120 ± 5 nm). The software used for this analysis (providing by the company) gives directly the distribution of diameter of particles as function of relative particle number density. The final colloidal sizes were measured when the coagulation presumably saturated, i.e. after 6 h. In any time-dependent coagulation experiment it is impossible to follow the size growth of aggregating particles continuously using correlation spectroscopy, since each correlogram necessitates at least 30 min of accumulation time for reliable statistics to build up, unlike in the case of total intensity measurements. All experiments were carried out at room temperature (25 ± 2°C). Experiments were repeated several times to ensure data reproducibility. Reported values are averages taken over several such experimental runs.

2.1. Smoluchowski aggregation kinetics

Smoluchowski discussed the coagulation of particles through random collision in the dispersion medium within the framework of a free diffusion model [15,16]. The time-dependent evolution of size distribution $n_s(\tau)$ was given as

$$\frac{\partial n_s}{\partial \tau} = \sum_{i+j=k} n_i A_{ij} n_j - 2 \sum_{j=1} n_j A_{kj} n_k$$

(1)

Here the particles $i$ and $j$ collide to form particle $k$ with a certain probability, called the sticking coefficient, $e$. Smoluchowski defined the parameter $\gamma$ as the probability that the $i$-mer and $j$-mer bind irreversibly upon collision, and $e$ was assumed to be independent of $i$ and $j$. Here, the reduced time $\tau$ is defined through the rate constant $\gamma$ as $\tau = \gamma t$, with

$$\gamma = \gamma_0 e$$

(2)

For freely diffusing spherical particles, the collision rate $\gamma_0$ was shown to be

$$\gamma_0 = 8 \pi D_0 R_0 c_0$$

(3)

where, $D_0$, $R_0$, and $c_0$ are monomer diffusion coefficient, hydrodynamic radius and number concentration respectively. The transition matrix $A_{ij}$ gives the probability that $i$ and $j$ collide per unit time by diffusion alone. Smoluchowski estimated this as

$$A_{ij} = (D_i + D_j)(R_i + R_j)/4D_0 R_0$$

(4)

$$D_i R_i = k_B T/6 \pi \eta$$

(5)

and Eq. (5) is independent of $i$, Smoluchowski approximated $A \approx 1$. Here $\eta$ is the solvent viscosity at absolute temperature $T$. This allowed Smoluchowski to solve Eq. (1) and obtain cluster size distribution as a function of reduced time $\tau$ as
\[ n_i(\tau) = \frac{e^{-1}}{(1 + e)^{i-1}} \quad \text{for} \quad i = 1, 2, 3, \ldots \]  

(6)

The average intensity of scattered light \( I_s \) from such a solution is given by

\[ I_s = I_0 \sum n_i i^2 \]  

(7)

where \( I_0 \) is the intensity of light scattered by the sample at time \( t = 0 \) when all particles were monomers. Eq. (7) can be summed using Eq. (6) to yield

\[ I_s(t) = I_0 (1 + 16\pi a D_0 R_0 \kappa_0) \]  

(8)

This can be written as

\[ I_s(t) = I_0 (1 + Ft) \]  

(9)

where \( F \) is the aggregation rate constant. This predicts the linear growth of aggregates with time. Independently, using the Rayleigh–Gans–Debye summation of phase differences between geometric centres, the average flocc structural factor \( S(k) \) was deduced \cite{17,18} as

\[ S(k) = 1 + (2/c) \sum A_j n_j(k) \]  

(10)

where \( A_j \) is the configurational factors evaluated by Lips et al. \cite{19} for \( 2 \leq j \leq 13 \), for linear and close packed aggregates. Eq. (9) and Eq. (10) are identical expressions. Lips and Willis \cite{19} have given a similar solution as Eq. (9) with \( F \) replaced as \( 2c_0 K_n \), where \( K_n \) was defined as the aggregation rate constant. However, some systems have shown non-linear time dependent growth given as

\[ I_s(t) = I_0 [1 + (Ft)^\delta] \]  

(11)

However, Eq. (11) is a more generalised expression, though empirical, where both \( F \) and the exponent \( \delta \) govern the growth kinetics of aggregating particles. The physical situation \( \delta = 1 \) would correspond to an actual Smoluchowski process. Here we do not distinguish between colloid–colloid aggregation that this theory is normally applied to, as compared to our case, where antigen coated beads grow in size because of adsorption of antibody present in the sera. The colloid–colloid aggregation was found to be absent as seen from the particle size distribution of the collected data.

3. Results and discussions

3.1. Aggregation features

The time-dependent coagulation of protein coated polystyrene particles at various salt concentrations are shown in Figs. 1 and 2. The curves were least squares
fitted to Eq. (11) and the values of \( \Gamma \) and \( \delta \) were obtained. These are plotted in Figs. 3 and 4. It was observed that \( \delta \) increased from a value of 1.04 \( \pm \) 0.06 at no salt to 3.75 \( \pm \) 0.05 at 50 mM NaCl concentration. For 100, 200 and 300 mM salt concentrations the values obtained were \( \Gamma = 3.49 \pm 0.06 \) and \( \delta = 6.94 \pm 0.07 \), and these remained constant which violates the predictions of DLVO theory. The parameter \( \Gamma \) is a reaction constant term and the highest rate of \( \Gamma \) observed at 50 mM salt concentration can be taken as reference to distinguish between DLVO and non-DLVO regions of reaction kinetics. This parameter had values 2.24 \( \pm \) 0.05, 3.29 \( \pm \) 0.06, 3.40 \( \pm \) 0.06 and 3.49 \( \pm \) 0.06 at salt concentrations equal to 0, 10, 20 and 50 mM, respectively. Within experimental error \( \Gamma \) remained fixed at 3.49 \( \pm \) 0.06 for salt concentrations equal to and above 50 mM. We define \( \Gamma_i = 3.49 \) and introduce equilibrium rate constant \( k_e \) as

\[
k_e = \Gamma / \Gamma_i
\]

and equivalently the equilibrium exponent constant \( \delta_e \) as

\[
\delta_e = \delta / \delta_i
\]

with \( \delta_i = 6.94 \), corresponding to a 50 mM NaCl solution. It is interesting to notice that when salt concentration is 0 mM, the coagulation exponent was observed to be \( \approx 1.04 \) which is close to the Smoluchowski value of 1. And when NaCl was added to the system at different concentrations non-linearity in the Smoluchowski aggregation crept in a significant way as indicated by growing \( \delta \)-parameter. This growth of the exponent, however, got arrested above NaCl concentration of 50 mM. This is again an anomalous behaviour. In a time dependent coagulation process, the sticking coefficient \( \epsilon \) defined by Eq. (2) itself becomes time dependent and consequently exact solution of Eq. (1) becomes extremely difficult. In such a scenario the linear time dependence of intensity of scattered light given by Eq. (10) loses its validity and a non-linear time dependence of \( I(t) \) is bound to arise [24,25]. The results shown in Fig. 1 are a reflection of this phenomena. Again Smoluchowski model rests on the intrinsic assumption that the aggregating particles are driven by pure diffusion. In the present system the colloids have charged surfaces with finite surface potentials hence Eq. (10) is unlikely to be applicable as it is. The values of hydrodynamic radii measured at saturation (\( \approx \) after 6 h) are plotted in Fig. 5. This qualitatively indicates the same features as reflected by the \( I(t) \) data. It was not possible to continuously monitor the changes in radii values of aggregating colloids alike in the case of \( I(t) \) measurements because instantaneous measurement of intensity correlation function is not possible. Correlators need a finite data acquisition time to build up a good statistics during which the hydrodynamic radii is continuously changing. However, the initial and saturation values of radii of aggregates are used as input parameters in deducing the interparticle interaction potential.

3.2. Interparticle interaction potential

Five types of forces between colloidal particles are known to exist [9]. These are: (i) repulsive forces from the overlap of electrical double layers (\( u_0 \)); (ii) long range London–van der Waal attraction forces, also called dispersion forces (\( u_d \)); (iii) steric forces, from interaction of colloids adsorbed at particle surface; (iv) structural and Brownian forces from interaction with solvent molecules, also called hydration forces (\( u_h \)); and (v) hydrodynamic forces. For dilute solutions where the
interparticle distance is larger than typically ten solvent diameters (≈6 nm), the stability is primarily decided by London–van der Waal attraction, and double layer repulsion between mutually approaching colloidal particles. This forms the basis of DLVO model. When the hydration forces are included, the net interaction potential \( u_0(r) \) between pairs of spherical particles \( i \) and \( j \), having same radii ‘a’ and separated by a distance ‘r’ becomes [9]

\[
u_0(r) = u_R(r) + u_a(r) + u_h(r)
\]

where the suffixes \( R \), \( A \) and \( h \) correspond to repulsive, attractive and hydration part of the total potential. Other contributions arising from ‘steric’ interactions and short range solvent-mediated structural forces can be ignored for the present system, since these play a significant role only for concentrated colloidal dispersions. In our case the Debye–Hückel screening length \( k^{-1} \) is typically ≈ a few nanometers whereas the minimum colloidal radius ‘a’ is ≈ 60 nm. Hence, under this physical condition all the terms in \( u_0(r) \) can be written explicitly if all the system parameters are accessible which unfortunately is not true for our system [9, 20–23]. As the aggregation proceeds, the pair-wise interaction potential defined by Eq. (14) changes continuously due to the change in aggregate size. The time evolution of \( u_0(r, t) \) could not be plotted because of lack of information about aggregate sizes at different instances of time due to reasons already cited. The interaction features discussed should be taken purely as indicative. One can use the values cited in [26] as inputs in calculating \( u_0(r, t) \). The DLVO theory implies reduction in the activation energy for higher salt concentrations inferring lower stability. Appearance of a strong activation energy barrier at a particle salt concentration can imply anomalous stability at high electrolyte concentrations. We shall discuss the features of \( u_0(r, t) \) no more because the exact quantitative nature of colloidal charge surface is unknown to us.

4. Conclusions

The time-dependent coagulation of protein coated polystyrene latex beads showed anomalous stability at increased NaCl concentrations. Giles and Lips [27] have observed similar behaviour earlier in a different physical system. Our results concur with their observation that rapid coagulation in the presence of electrolyte produced a close packed ordered structure at low electrolyte concentrations, but a liquid like, disordered structure at high ionic strength. The interparticle interaction potential \( u_0(r) \) can be evaluated through the knowledge of pair correlation function which in turn is a Fourier transform of the measured static structure factor \( S(k) \) given by Eq. (10). The Ornstein–Zernike equation relates the pair correlation function and \( u_0(r) \) [28]. However, the statistical errors introduced at every stage while taking numerical integrals and Fourier transforms limit the usefulness of such a recipe. The colloidal stability is proposed as arising from the interplay of London–van der Waal attraction, double layer repulsion and hydration forces. In a similar work reported on a different system, it was proposed that hydration forces when added as an additional component to the DLVO model, can well describe the observed anomaly [29]. Though the observation of a second minimum in the interaction potential, which has been argued to promote reversible coagulation still remains contentious. Nieuwenhuis and Vanrij directly determined \( n(r) \) from total pair correlation function and have rejected the second minimum as it was thought to be spurious [30, 31].

References