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Mutual and self-diffusion in concentrated $\alpha$-crystallin protein dispersions. A dynamic light scattering study

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1. Introduction.

Colloidal dispersions are multi-component systems which often present a great variety of interesting phenomena [1] like crystallization or flocculation, for example. If the complete description of static or mean structural properties of simple colloidal dispersions can be considered as achieved [2], the same is not valid as far as dynamics is concerned. Colloidal particles interact not only through direct interparticle potentials, but also through solvent mediated forces, the hydrodynamic interactions. These velocity dependent forces play no role in the static properties of solutions. On the other hand, they are fundamental for the correct description of viscosity or diffusion phenomena. However, hydrodynamic interactions have only been calculated for a limited range of situations. Of particular interest we quote the 2-sphere mobility tensor calculated by Felderhof [3] in 1978. He used the solution of the linearized Navier-Stokes equation, for the field flow around a sphere, to which he applied a method of successive reflections. Although this calculus presents a great improvement relative to the well known Oseen approximation, for many applications 2-body hydrodynamics

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remains a weak approximation. The general treatment of 3 (or more)-body hydrodynamic interactions presents an overwhelming difficulty even with the use of great computers. It is however worth noting that treatments of many-body hydrodynamic interactions for hard-sphere systems have been recently proposed by Mazur and Saarloos [4] and Beenaker and Mazur [5].

In the lack of a general treatment of hydrodynamic interactions, allowing for the prediction of diffusion coefficients from mean to high concentrations, some authors have suggested the use of semi-empirical models such as the screened Navier Stokes equation [6]. In this formulation, 3 (or more)-body hydrodynamic effects are pictured as a concentration dependent screening of the 2-body interactions and one or two empirical parameters are required to fit the functional form of the screening length.

In this article we present mutual and self diffusion coefficients as measured for concentrated α-cristallin protein dispersions at 2 ionic strengths. These measurements are compared to models which take hydrodynamic forces into account, namely 2-body interactions and screened hydrodynamic interactions. α-cristallins are the main protein components of the eye lens. They are present at high concentrations in the lens cells and have been well characterized from a physicochemical standpoint [7]. α-cristallins are permanent quasi-spherical assemblies of ~50 subunits. They have a charge Z~50e, a hydrodynamic radius $R_h\approx 90$ Å and are naturally polydisperse.

We have previously detected the presence of three diffusive components in the quasi-elastic light scattering spectra [8] from α-cristallin dispersions. We have identified the fastest relaxation to a collective process and the following one to the presence of exchange modes due to the polydispersity of the dispersion. We have further proposed a semi empirical model, without adjustable parameters, capable of foreseeing the relative intensities of the exchange modes [9]. In contrast with this study of statics, we present here a systematic study of the dynamics of the collective and exchange modes. These modes are characterized by a mutual and a mean self diffusion coefficients. The diffusion coefficients have been obtained from dynamic light scattering experiments, analysed by the method of maximum entropy [10]. In the theoretical analysis of the present paper, we have used a screened Coulomb interaction potential model for the direct interactions between α-cristallins. This form of potential, together with the rescaled mean spherical approximation (RMSA) [11, 12] have already been successfully applied to the analysis of structural properties of α-cristallin dispersions [13, 14].

The aim of the present work is to stress the need for a theoretical development on the description of hydrodynamic interactions for simple colloidal dispersions, and to present new dynamic data on a system which is now well characterized, α-cristallins.

2. Experimental procedures.

2.1 Preparation of α-cristallin dispersion. α-cristallins were extracted from the periphery of fresh calf lenses and prepared as described in detail elsewhere [13]. Dispersions at various concentrations were obtained either at physiological physico-chemical conditions (pH 6.8, ionic strength $I=150$ mequiv) or, after being dialized for 2 days, at a lower ionic strength ($I=17$ mequiv). Care was taken to work with filtered buffers (0.22 μm Millipore HA filters) and prior to light scattering experiments, the dispersions were centrifuged for 30 min at 6 000 g in order to sediment any residual dusts.

2.2 Dynamic light scattering. Data acquisition and analysis. — The dynamic light scattering or photon correlation spectroscopy set-up is a classical homodyne one and has been described in detail elsewhere [14]. Two apparatus were used in order to build the time correlation function $C(t)$ of the photopulses:

$$C(m \Delta T) = \sum n(t_i) n(t_i + m \Delta T)$$

where $n(t_i)$ is the number of photopulses at time $t_i$ and during a sampling time $\Delta T$. The photopulses were fed to a 48 channels digital single bit correlator (ATNE = Application des Techniques Nouvelles en Electronique) used for sampling times from 2.5 μs to 40 μs.

For greater sampling times, as the mean number of photopulses per sampling time becomes comparable to and greater than unity, one also needs an increasing number of bits in the correlator or a predivision, and therefore a loss, of the input signal. To cope with this problem we also programed a micro-computer to build correlation functions at 200 delay times quasi-logarithmically spaced for sampling times $\Delta T = 102\ \mu s$ and 6.6 ms. Moreover, the two apparatus could work in parallel and in real time. Measurements were pursued until a signal-to-noise ratio better than $10^5$ was attained. Each complete set of measurements thus consisted of 6 curves totalizing ~640 points spanning ~6 decades of time. Since each measurement overlapped the preceding one by at least 20 points, these were matched together by a least squares affinity plus translation prior to analysis.

The correlation curve analysis was performed through the Laplace inverse transform of the curves, assuming that the scattered field autocorrelation function, $g_1(t)$, can be simply written as an integral over a distribution of relaxation times, $G(\tau)$:
The Laplace inverse transforms were performed through the Maximum Entropy Method using the Cambridge algorithm as described in reference [8], allowing for the determination of smooth, positive and objective $G(\tau)$ distributions.

$G(\tau)$ were obtained in logarithmic time scale and presented a few peaks. Each peak was characterized by a properly defined mean time $\tau_i$, by integration between two consecutive minima:

$$\tau_i = \exp\left(\frac{\int G(\tau) \ln(\tau) d\ln(\tau)}{\int G(\ln(\tau)) d\ln(\tau)}\right).$$

2.3 INTERACTION POTENTIAL. COMPUTER CALCULATIONS. — The RMSA has been successfully applied to describe the structure of $\alpha$-crystallin dispersions [13, 14]. This model neglects departure from sphericity and polydispersity of macro-ions in a solvent. It also neglects Van der Waals attraction to consider the screened Coulomb potential $v(r)$, between a pair of particles:

$$v(r) = \begin{cases} \infty & \text{for } r < d \\ (\pi \varepsilon_0 \varepsilon d^2 \psi_0^2/r) \exp(-k(r-d)) & \text{for } r > d \\ \end{cases}$$

where $r$ is the distance between the centers of 2 particles of diameter $d$, $\varepsilon_0$ is the permittivity of free space, $\varepsilon$ is the dielectric constant of the solvent, $\psi_0$ is the surface potential and $k$ is the inverse Debye-Hückel screening length.

The surface potential is approximated by:

$$\psi_0 = \frac{Ze}{\pi \varepsilon_0 \varepsilon d(2 + kd)}$$

where the $(2 + kd)$ term reflects the classical mutual exclusion by the macro-ions of counterions from each other's volume and with $Ze$ as the effective charge of the macro-ion and the inverse Debye-Hückel screening length being given by:

$$k = \left(\frac{2e^4 N_A I}{\varepsilon \varepsilon_0 k_B T}\right)^{1/2}$$

with $N_A$ the Avogadro number, $e$ the electronic charge, $I$ the solvent ionic strength and $k_B T$ thermal energy.

The RMSA program of Hayter and Penfold [11] and Hansen and Hayter [12] was implemented in an HP 1000 computer. This algorithm calculates analytically the well known static structure factor $S(q)$ from the pair potential, equation (4). The pair distribution function $g(r)$ was also calculated as the Fourier transform [15] of $S(q)$:

$$g(r) = \frac{1}{\rho} \left(1 + \frac{1}{2 \pi^2} \int [S(q) - 1] \frac{\sin qr}{qr} q^2 dq\right)$$

where $\rho$ is the particle number density.

The pair distribution function and static structure factors were needed in order to calculate diffusion coefficients as explained in the next section.

2.4 VISCOSITY MEASUREMENTS. — The viscosity measurements were performed using a magnetorheometer [16] at the Centre d'Etudes Atomiques, Saclay (France). In this apparatus, a magnetized sphere of radius ~200 mm is suspended in the fluid to be measured by applying a magnetic field. The position of the sphere is kept fixed by a feedback circuit optically monitored. The viscosity is then obtained by measuring the solenoidal current while uniformly translating the test tube with the fluid.

The $\alpha$-crystallin viscosities at 2 ionic strengths ($I = 0.15$ equiv. and $I = 0.017$ equiv.) were measured at $T = 25.0^\circ$C at small shear rates.

3. Theory.

Quasi-elastic light scattering gives information on Fourier components of optical density fluctuations with a wave vector $q$ whose modulus is given by:

$$|q| = \frac{4 \pi n}{\lambda_0} \sin(\theta/2)$$

where $n$ is the mean refractive index of the scattering media, $\lambda_0$ is the vacuum incident (laser) light wavelength and $\theta$ is the scattering angle (angle between light incidence and observation directions).

In colloidal dispersions, fluctuations of optical density are mainly due to the Brownian diffusion of macroparticles. For spherical macroparticles at large enough dilution, these fluctuations are characterized by a relaxation time $\tau_0$ given by the free diffusion constant $D_0$ and the Stokes-Einstein relation by:

$$\tau_0 = \frac{1}{D_0 q^2} = \frac{6 \pi \eta R_h}{k_B T q^2}$$

where $\eta$ is the solvent viscosity and $R_h$ the hydrodynamic radius of the diffusing particles.

At higher particle concentrations, density fluctuations still decay as a diffusive process, characterized at short times by a diffusion constant $D_m(q)$ [17]

$$D_m(q) = D_0 \left(1 + \frac{H(q)}{S(q)}\right)$$

where $S(q)$ is the static structure factor, a function of the direct interaction potential alone, while $H(q)$ is a hydrodynamic term which also incorpo-
rates direct interactions implicitly, usually expressed as integrals involving \( g(r) \).

At the small wavevector limit, \( q \rightarrow 0 \), which corresponds to our measurements, \( D_m(0) \) is identified to the mutual or collective diffusion coefficient. It has however been argued by Pusey and Tough [17] and Weissman [18] that polydispersity would bring about some tracer signal to the light scattering spectra, allowing for the detection of exchange modes, ruled by self-diffusion. This has been indeed observed in photon correlation experiments involving \( \alpha \)-crystallins [8] and the static part, or amplitude of the light signal, due to the exchange modes, has been presented and analysed elsewhere [9].

Although equation (10) is strictly valid only in the short time limit [17] long time or memory effects do not contribute to change mutual diffusion as far as 2-body hydrodynamic interactions are concerned. The same is not true for tracer or self diffusion coefficients and, even at the lowest concentrations, memory effects should be considered.

To the lowest approximation the hydrodynamic term \( H(q) \) in equation (10) may be expressed in terms of 2 particle mobility tensors, \( \mu_{ij} \), relating the velocity \( \vec{u}_i \) of the particle \( i \) to the force \( \vec{F}_j \) applied to the particle \( j \):

\[
\vec{u}_i = \sum_{j=1}^{N} \mu_{ij} \cdot \vec{F}_j .
\]

The computation of self-diffusion coefficients takes a form similar to equation (10), where one needs to consider only the terms involving the self mobility tensors \( \mu_{ii} \) and the structure factor, \( S(q) \), is absent from the denominator [17].

The pair mobility tensors have been calculated by Felderhof [3] from the solution of the linearized Navier-Stokes equation:

\[
\begin{cases}
\nabla \cdot \vec{p} - n \Delta \vec{v} = 0 \\
\n\nabla \cdot \vec{v} = 0
\end{cases}
\]

where, from dimensional arguments, the inverse hydrodynamic screening length, \( K \), should have the form [6]:

\[
K = K_0 R_h^{-1} f(\phi)
\]

with \( f(\phi) \) a function of the dispersion volume fraction, \( \phi \), vanishing at infinite dilution. In the case of Brownian particles, a linear dependence has been found [6] \( f(\phi) = \phi \) so that a single empirical parameter, \( K_0 \), is left free.

Walrand et al. [6] have solved the screened Navier-Stokes equation, obtaining screened pair mobility tensors up to order \( r^{-7} \). They obtained hence expressions for the mutual and self diffusion coefficients for a dispersion of spherical particles as a function of the inverse hydrodynamic screening length. We omit here the presentation of these rather lengthy expressions and refer the interested reader to the original paper (Ref. [6]). Equivalent expressions using the non-screened 2-particle mobility tensors as calculated by Felderhof can be found in reference [17].

4. Results.

In a previous article [8] we have identified 3 diffusive components in the quasi-elastic light scattering spectra from \( \alpha \)-crystallin dispersions. The two fastest components have been identified to mutual and self diffusion respectively. In figure 1 we present mutual diffusion coefficients obtained for \( \alpha \)-crystallin dispersions at 2 ionic strengths after a systematic investigation up to a concentration \( c \sim 300 \text{ mg/ml} \). In the present paper we use a constant \( \phi/c \) ratio, corresponding to the low concentration limit [14], \( \phi/c = 2.0 \text{ ml/g} \). At higher concentrations, a decrease in \( \phi/c \) has been found [13]. However, a systematic correction would not change the bulk of conclusions.

The infinite dilution limit of the diffusion constants is \( D_0 = 2.23 \times 10^{-7} \text{ cm}^2/\text{s} \) for both ionic strengths, corresponding to an alpha-crystallin hydrodynamic radius \( R_h = 94.3 \text{ Å} \), cf. equation (9). At the physiological ionic strength, \( I = 150 \text{ mequiv} \), the data follows smoothly a general increasing tendency. At \( I = 17 \text{ mequiv} \), we notice that mutual diffusion coefficients increase much faster but they fall with age of preparation as indicated in the figure, with a tendency to stabilize after a few weeks. This behaviour could be interpreted from the knowledge of the quaternary structure of \( \alpha \)-crystallins as follows. Figure 2 pictures a schematized view of an \( \alpha \)-crystallin protein showing its subunit composition as deduced in reference [7]. Such a conformation cages much unbounded water and small ions. As a matter of fact, less than half the hydrodynamic volume (taken as the sphere with the \( \alpha \)-crystallin hydrodynamic radius) is occupied by the protein subunits.
Fig. 1. — Mutual diffusion coefficients for alpha-crystallin dispersions: (□) $I = 0.15$ equiv. (physiological ionic strength); (●) $I = 0.017$ equiv. Measurements within a month of preparation; (○) $I = 0.017$ equiv. Measurements after 2 months of preparation; (———) 2-body hydrodynamic interactions model calculation; (-----) linear extrapolation from the origin of the 2-body hydrodynamic interactions model; (...) screened hydrodynamic interactions model with $K_0 = 0.72$ for $I = 0.15$ equiv and $K_0 = 0.90$ for $I = 0.017$ equiv. (see Eq. (10) and text).

Fig. 2. — Schematic representation of the model developed in reference [7] for the quaternary structure of alpha-crystallin proteins.

After the dialysis to the weak ionic strength (see experimental procedures), small ions inside the protein may be slowly and progressively released until a new equilibrium is reached at a higher ionic strength. Another possible interpretation is a slow protein oxidation, accompanied by a loss of charge [20]. This would indicate an insufficient amount of antioxidation agent in the buffer.

In figure 1 we also draw the curves corresponding to the 2-body hydrodynamic interaction model (solid lines) and the screened hydrodynamic interaction model for the best screening parameter ($K_0 = 0.72$ for $I = 0.15$ equiv and $K_0 = 0.90$ for $I = 0.017$ equiv), cf. equation (14), which fits the data (dotted lines) at each ionic strength. The linear extrapolations of the mutual diffusion coefficients from the origin are also shown as dashed lines.

Self-diffusion coefficients, as obtained from the maximum entropy analysis of correlation curves are presented in figure 3. In order to cope with a variation spanning 4 decades, we present self-diffusion coefficients in a logarithmic scale. Note that there is a lower limit at which the exchange modes are detectable at $c \sim 80$ mg/ml. This is due to the noise limited resolution of the 2 decays (collective and exchange modes) which reach the same value at infinite dilution. Also note that contrary to the mutual diffusion coefficients, the self-diffusion coefficients at each of the 2 ionic strengths decrease and cannot be distinguished. It is remarkable to notice how closely the self-diffusion coefficients follow the inverse macroscopic viscosity of the dispersion. The relative inverse viscosity curve for $\alpha$-crystallin dispersions is drawn in figure 3 (continuous line) for comparison. Figure 3 also presents the results of

Fig. 3. — Self-diffusion coefficients for alpha-crystallin dispersions at $I = 0.15$ equiv. (squares) and $I = 0.017$ equiv. (solid circles): (———) relative inverse viscosity for alpha-crystallin dispersions, $(\eta / \eta_o)^{-1}$. Ionic strength did not appreciable affect viscosity; (-----) 2-body hydrodynamic interactions model calculation of self diffusion coefficient (short time limit); (...) screened hydrodynamic interactions model with $K_0 = 0.72$. The effect of ionic strength in the calculated self-diffusion coefficients is of the order of the line thickness.
calculations using the 2-body hydrodynamic interaction model (dashed line) and the screened hydrodynamic interaction model (dotted line) with the screening constant \( K_0 = 0.72 \). The difference between the calculated values of the self-diffusion coefficients at the 2 ionic strengths is also small, of the order of line thickness in figure 3.

5. Discussion.

The repulsive electrostatic nature of the interactions between alpha-crystallins is in conformity with the increase in the mutual diffusion coefficient at lower ionic strength, in figure 1. This behaviour can be qualitatively understood in terms of a simple model of effective hard spheres: particles will have a greater effective radius at smaller ionic strength, as electrostatic interactions are less screened. The greater the effective radius at a given mean concentration, the faster a concentration fluctuation will diffuse through the medium; the effective diameter of the particle can be seen as a shortcut in the « transmission of information ». The model of slow ionic strength equilibrium by protein liberation of small caged ions (and also the loss of charge by oxidation) is coherent with this picture: as could be expected, diffusion coefficients stabilize at intermediate values between physiological and low ionic strength values.

That self-diffusion coefficients are almost insensitive to direct interactions is at first sight surprising (see Fig. 3). Physically we expect that increasing repulsion will retard self-diffusion. In order to justify the small sensitivity of self-diffusion to ionic strength, relative to the large variation found for mutual diffusion, we recall that the structure factor \( S(q) \), defined solely in terms of direct interactions, is present in the definition of mutual diffusion equation (10) while it is not for self-diffusion. In the absence of hydrodynamic interactions, one would then expect a constant self-diffusion coefficient. On the other hand, for short-range direct interactions, as is the case here (the Debye screening length is much smaller than the hydrodynamic radius [14]), the hydrodynamic term \( H(q) \) is weakly sensitive to direct interactions up to intermediate concentrations (mobility tensors and hydrodynamic forces are long ranged, decaying as the inverse of distance).

One important point about self-diffusion is that our measurements are made in the long time \( q \to 0 \) limit while theory is for the short time \( q \to \infty \) limit. This difference is a consequence of memory effects resulting from passing the time scale during which direct interactions between particles occur [17]. In other words, the movements of surrounding particles should have been taken into account. These movements retard self-diffusion so that it is normal to measure diffusion coefficients lower than those predicted by the short-time limit calculations even at small concentrations, where the 2-body hydrodynamic interactions model should apply.

It is remarkable how well self-diffusion coefficients follow the inverse relative viscosity curve (Fig. 3). Although this behaviour has already been observed by Cebula et al. [21] in microemulsions and by Kops-Werkhoven and Fijnaut [22] in silica dispersions, to our knowledge there is no theory to explain it.

Returning to mutual diffusion, it has already been shown for \( \alpha \)-crystallin dispersions [14] that the predictions of the 2-body hydrodynamic interactions model work well up to a concentration \( c = 20 \text{ mg/ml} \), corresponding to a volume fraction \( \phi = 0.04 \). Here it is clear that the model fails for \( \phi > 0.1 \). As a matter of fact, considering only pair interactions means that only the first-order term in the virial expansion is correct [3]. Beenaker and Mazur [5] have shown that, for hard spheres, considering 3-body hydrodynamics more or less cancels the \( \phi^2 \) term leading to a fast decrease of \( D_m \). By some curious coincidence, we found that the mutual diffusion coefficients closely follow the predicted linear behaviour up to high concentrations (Fig. 1).

We have also used the screened hydrodynamic interaction model to fit mutual diffusion data. However we needed different constants \( K_0 \) to cope with each ionic strength, i.e., \( K_0 = 0.72 \) and \( K_0 = 0.90 \). These values should be compared to the value \( K_0 = 0.98 \) found by Walrand et al. [6] for micellar and hard sphere solutions. Close to \( \phi = 0.3 \) the hydrodynamic term \( H(0) \) in equation (6) becomes comparable to \( -1 \) and the mutual diffusion coefficient diverges very fast. The self-diffusion coefficient behaves more smoothly but a very small screening would be needed to follow the data. In figure 3 we have shown the prediction for \( K_0 = 0.72 \).

6. Conclusions.

We have presented mutual and (long time limit) self-diffusion coefficients for alpha crystallin protein dispersions at two ionic strengths. These data could not be accounted for by the 2-body hydrodynamic interaction model in most of the concentration range. In the lack of a more exact model taking into account many body interactions, we fitted mutual diffusion coefficients with a semi-empirical model for screened hydrodynamic interactions. However, we could neither find a universal screening constant, nor fit the data up from intermediate concentrations.

Mutual diffusion coefficients follow very closely up to the highest concentrations the linear behaviour predicted at infinite dilution, while self-diffusion coefficients decrease as the inverse viscosity. Neither
of these behaviours is predicted by theory, although they have been observed in some other systems. We hope that these measurements may stimulate theoretical progress in the description of many-body hydrodynamic interactions and that they can be analysed more rigorously in the future.

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