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Effects of polymer density fluctuations on depletion interactions

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Abstract. The strength of depletion interactions has been characterized by measuring the second virial coefficient, B_2, of suspensions of lysozyme molecules in the presence of non-adsorbing polymer poly(ethylene glycol) (PEG). The solution properties of the PEG are characterized for three molecular weights (1000, 6000 and 12 000) providing an opportunity for quantitative comparisons of measurements and theoretical predictions of B_2. We report non-monotonic changes in B_2 as the concentration and molecular weight of PEG are increased. The observed minimum in B_2 cannot be predicted by the standard depletion model of Asakura and Oosawa, and is closely associated with the proximity of the polymer solution lower critical solution temperature. The location and depth of the minima in B_2 are well captured by the thermal polymer reference interaction site model theory where the polymer mesh size is treated as a function of temperature.

1. Introduction

When non-adsorbing polymers are added to stable particle suspensions, the particles experience an induced 'depletion attraction' due to an unbalanced osmotic pressure arising from the exclusion of polymer molecules from a region between the particles. This phenomenon has significant scientific and technological consequences in diverse fields and has seen extensive investigation [1]. Many studies of the depletion phenomena have focused on the 'colloid limit' where the particle radius, R, greatly exceeds a statistically averaged measure of polymer size, the radius-of-gyration R_g. The first successful model to describe the depletion effect in the colloid limit was proposed by Asakura and Oosawa (AO) [2]. This model suggests that as two colloidal particles are brought to a centre to centre separation $r < 2(R + R_g)$, an excluded volume overlap develops which gives rise to an unbalance in osmotic pressure that can be modelled as an effective attraction between the larger particles [2]

$$U(r) = \begin{cases} \infty & r < 2R \\ -\dfrac{4}{3}\pi d^3 n_p kT \left(1 - \dfrac{3r}{4d} + \dfrac{r^3}{16d^3}\right) & 2R \leqslant r \leqslant 2d \\ 0 & 2d < r \end{cases} \tag{1}$$

where $d = (R + R_g)$ characterizes the distance of closest approach of a polymer and a particle, and n_p is the polymer number density. This approach approximates a flexible polymer chain as a rigid sphere of radius R_g, ignores polymer–polymer interactions and treats particle–particle and particle–polymer interactions as hard-core repulsions. The AO model has been employed to

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interpret direct measurements of depletion forces [3, 4], and as an effective pair decomposable potential to predict the phase behaviour of colloidal suspensions [1]. The AO potential has a spatial range of $2R_g$, and a strength determined by the ideal gas law for the polymer osmotic pressure. Although useful for many situations, the AO model has obvious limitations, e.g. it cannot address semidilute solutions where the macromolecule concentration exceeds the threshold c_p^* for polymer–polymer interpenetration, or variable polymer–solvent interactions ('solvent quality'). These limitations of the AO model have been addressed in a number of studies [5, 6] indicating that there is a competition between the strength and the extent of the depletion attraction as the polymer concentration is increased. However, these studies have focused primarily on depletion interactions between flat plates or the case where the polymer radius of gyration is substantially smaller than the particle radius.

Our interests in depletion phenomena lie in the influence of polymers in the separation and crystallization of proteins where often $R < R_g$, referred here to as the 'protein limit' [7]. Under these conditions, the concept of an excluded volume giving rise to an osmotic imbalance becomes complicated by the observation that as the particles shrink to a sufficiently small size, they can diffuse through a polymer coil. Thus from a theoretical point of view, understanding interactions between polymers and particles in this limit offers a new perspective on depletion phenomena.

The interaction of polymers and particles in the protein limit is of significance as low molecular weight polymers are often used in the formation of x-ray quality protein crystals. For protein crystallization, a commonly used nonionic, water soluble polymer is poly(ethylene glycol) (PEG) with the chemical formula $-(CH_2{-}CH_2{-}O)_N-$. PEG has the useful advantage of interacting weakly with protein molecules [7].

In this work we explore the effects of PEG on protein interactions by measuring the protein solution second virial coefficient, B_2,

$$B_2 = 2\pi \int_0^\infty r^2(1 - \mathrm{e}^{-V(r)/kT})\,\mathrm{d}r \tag{2}$$

where $V(r)$ is the protein-protein potential of mean force. In the absence of polymer, proteins are often modelled as spheres which interact via steric hard-core, electrostatic, and van der Waals forces (DLVO) [8]. In the presence of polymer, we assume that the polymer-induced depletion potential and DLVO potential can be summed. A naive application of the AO model (i.e. where one ignores the radical difference between the assumed conditions for which the AO model is derived and the protein limit investigated) fails both qualitatively and quantitatively as the polymer concentration and molecular weight are varied.

We also analyse our experimental results in terms of the analytic polymer reference interaction site model (PRISM) integral equation theory [9] of depletion forces which employs a random walk model of polymer conformation, adopts pure excluded volume particle–particle and segment–particle interactions, and accounts for variable effective segment–segment interactions. Based on the polymeric version of the Percus–Yevick closure, the attractive depletion potential between hard particles is given by:

$$U(r) = -kT \ln\left[1 + \frac{\pi z}{3}\left(\frac{R}{r}\right)\left(\frac{R}{\sigma}\right)\mathrm{e}^{-(r-2R)/\xi_\rho}\right] \tag{3}$$

where $\sigma = \sigma_0$ for $c_p < c_p^*$ and $\sigma_0(c_p/c_p^*)^{-1/8}$ in the semidilute regime [10], $z = \rho_p\sigma^3$ is the reduced polymer segment number density, the athermal mesh length is $\xi_\rho^{-1} = \xi_c^{-1} + (\pi z/3\sigma)$, where $\xi_c = R_g/\sqrt{2}$, N being the degree of polymerization. If this model is applied assuming that ξ_ρ is independent of temperature, the PRISM model fails to capture the essential features of the experimental results.

PEG/water solutions phase separate upon heating, giving rise to a lower critical solution temperature (LCST). As the phase boundary is approached, the mesh size grows significantly to its thermal analogue: $\xi_\rho^{th} = \xi_\rho(1 - T/T_s)^{-1/2}$, where $T_s(c_p)$ is the spinodal temperature for polymer–solvent phase separation. In this paper B_2 is determined in aqueous protein-PEG solutions as a function of three dimensionless variables: size ratio R_g/R, polymer concentration c_p/c_p^*, and reduced temperature T/T_c, where T_c is the critical temperature for polymer–solvent phase separation. We find that accounting for thermal fluctuations in screening length qualitatively captures our experimental results.

2. Results and discussions

Experiments were performed using globular protein lysozyme ($R = 1.7$ nm). Details of sample preparation and static light scattering methods of measuring B_2 are provided elsewhere [11, 12]. To interpret the osmotic pressure data, we have employed the analytic version of the microscopic PRISM integral equation theory [9], which has been shown to agree well with experiments on dilute and semidilute athermal polymer solutions [13], and also with scaling approaches [10]. The theoretical expression for the polymer solution osmotic pressure is:

$$\frac{\Pi(z)\sigma^3}{kT} = \frac{z}{N} + \frac{\pi}{36}\left(\frac{\sigma}{\xi_c}\right)z^2 + \frac{\pi^2 z^3}{324} \tag{4}$$

where all the terms are defined as in equation (3). Based on the monomer mass, the segmental degree of polymerization is $N = M_w/44$. The dilute solution (ideal) osmotic pressure data yields $\sigma_0 = 0.80 \pm 0.05$ nm, and $R_g = 1.43$, 3.54, and 5.38 nm for PEG 1000, 6000 and 12000 samples, respectively [12], in good agreement with prior studies [14].

The extent of the depletion interaction in the PRISM model is controlled by ξ_ρ. However, as the proteins carry a charge, electrostatic repulsions are also of significance. In addition, the proteins feel short-range attractions which we model here as arising from van der Waals interactions. The interaction potential in the presence of forces governing protein interactions in the absence of polymer has been characterized by measuring B_2 as a function of ionic strength. Assuming lysozyme can be modelled as a dielectric hard sphere of radius 1.7 nm [11], the protein charge q, and Hamaker coefficient, A, were extracted by fitting the B_2 data to the standard DLVO model [15]. In agreement with previous investigations, the DLVO model accurately describes our B_2 measurements over a wide range of ionic strengths with $A = 5$ kT, $\delta = 0.1$ nm, and $q = 10.5$ [11, 12].

To investigate the effect of added polymer on protein interactions, the lysozyme second virial coefficient B_2 was measured as a function of PEG concentration and molecular weight at a fixed ionic strength of 0.2 M (figure 1). For each polymer molecular weight, B_2 is a strikingly non-monotonic function of polymer concentration. The attractive minimum in B_2 at roughly $c_p \cong c_p^*$ becomes deeper, and shifts to lower polymer concentration, as the PEG molecular weight increases.

As seen in figure 2, increasing the ionic strength from 0.15 to 0.2 M results in nearly a factor of two decrease in the polymer-free second virial coefficient. This result is anticipated due to the screening of electrostatic repulsions. Despite the substantial change in the protein interaction in the absence of polymer, the qualitative dependence of B_2 on polymer concentration is unaffected by the change in ionic strength suggesting the physical mechanism giving rise to the non-monotonic behaviour in figure 1 is insensitive to modest changes in κR.

The spinodal curve for PEG 12000 was obtained by measuring the static structure factor in the limit of zero angle, $S(0)$, in the absence of protein. The mean field spinodal temperature, $T_s(c_p)$, was estimated by linear extrapolation to zero of the $1/S(0)$ (in arbitrary units) versus

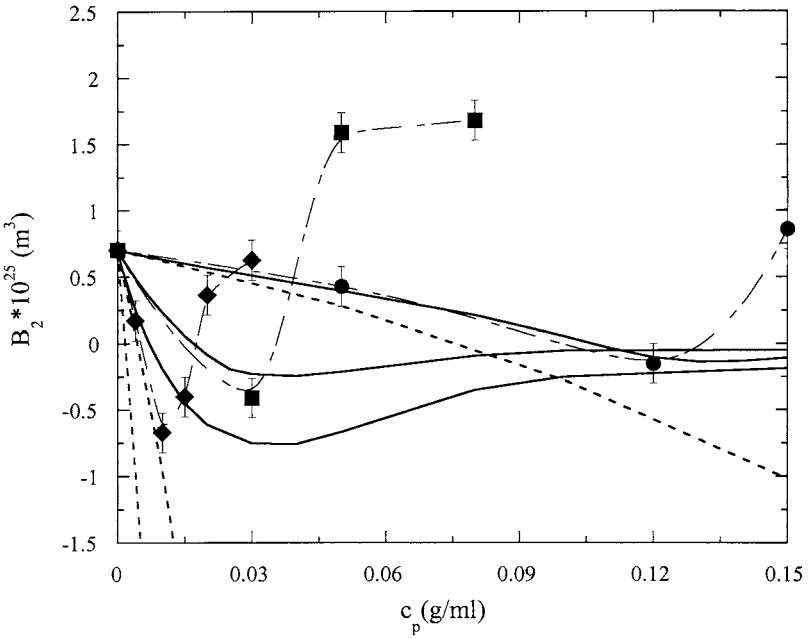

Figure 1. Comparison of the predictions of the AO model (broken curve) and thermal PRISM theory (full curve) to the experimental data (● denote data in the presence of PEG 1000, ■ PEG 6000 and ♦ PEG 12000) for lysozyme in presence of PEG at an ionic strength of 0.2 M ($T = 25\,^{\circ}$C). The chain curve through the data points are drawn as a guide to the eye.

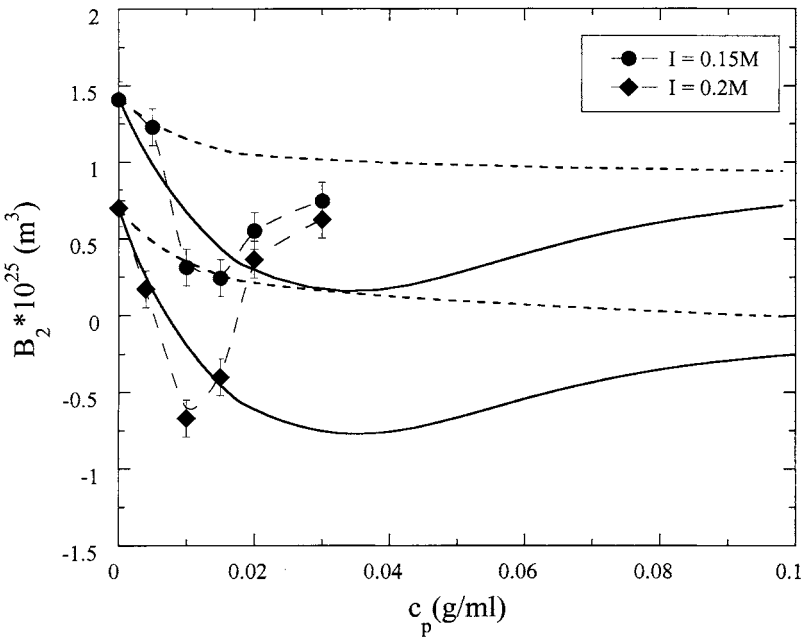

Figure 2. Second virial coefficient, B_2 (m^3), as a function of PEG 12000 concentration at ionic strengths of 0.15 and 0.2 M at 25 °C. Broken (full) curves are predictions of athermal (thermal) PRISM theory. The chain curves are a guide to the eye.

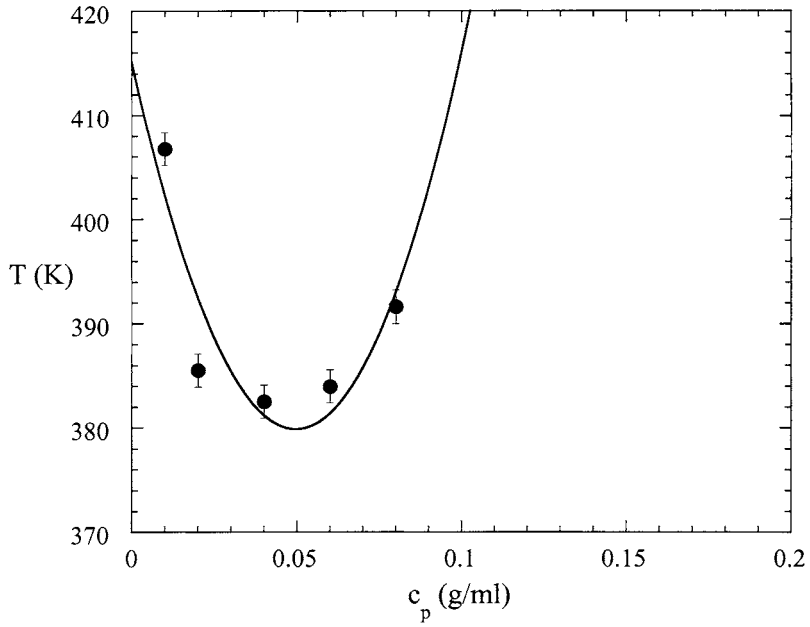

Figure 3. The spinodal curve for PEG 12000. The symbols mark the spinodal temperatures while the solid line represents a quadratic fit through the experimental data.

$1/T$ plot. From figure 3, we estimate that $T_c = 379$ K and the critical polymer concentration $c_{pc} \cong 0.04$ g cm^{-3}, close to c_p^*. Thus, the ratio $T/T_s = 0.78$ at 25 °C, yielding a thermal mesh size at c_{pc} of $\xi_\rho^{th} = 2.1\xi_\rho^{ath} \cong 7$ nm $\cong 4R$. As the polymer concentration increases or decreases from its critical value the thermal mesh length rapidly decreases towards its athermal value.

Combining equations (1) and (2), the predictions of the AO model for $B_2(c_p)$ can be deduced with no free parameters. As shown in figure 1, the AO potential predicts a monotonic decrease of B_2 with c_p, and significantly overestimates the attractive depletion strength even at low polymer densities. The failure of the AO model is perhaps not surprising given its treatment of the polymer solution as an ideal suspension of small macromolecules modelled as impenetrable hard spheres.

The hard particle reduced second virial coefficient based on the PRISM theory is obtained by substituting equation (3) into equation (2) as

$$\frac{B_2}{B_2^{HS}} = 1 - \frac{\pi z}{4}\left(\frac{\xi_\rho^{th}}{\sigma}\right)\left(1 + \frac{\xi_\rho^{th}}{2R}\right) \tag{5}$$

where B_2 depends sensitively on the ratio of thermal polymer mesh size to protein radius, ξ_ρ^{th}/R, which itself is a function of polymer concentration, reduced temperature $T/T_s(c_p)$, and N. In the 'protein limit' of $\xi_\rho^{th} \gg R$, the second attractive term in equation (5) is dominant. If R_g/R is sufficiently large, the theory predicts B_2 is a non-monotonic function of polymer concentration, even in the athermal limit [16]. This arises from a competition between the strength of the depletion attractions which increase monotonically with polymer density, and their spatial range which decreases with c_p. Equation (5) predicts the minimum in B_2 will monotonically deepen and shift to smaller polymer concentrations as N is increased. The location of the minimum occurs at the semidilute crossover c_p^* for athermal solutions,

and correlates with the N-dependent critical polymer concentration ($c_{pc} > c_p^*$) as the spinodal boundary is approached. As the polymer solution approaches phase separation, ξ_ρ^{th} grows, which can result in a pronounced minimum in B_2 as polymer concentration is increased even rather far from T_c [16].

Combining equations (2) and (3) with $V(r) = U(r) + U_{DLVO}(r)$ and all the characterization data, the predictions of thermal PRISM theory can be evaluated for PEG 12000 with *no adjustable parameters*. In the athermal limit (figure 2), no minimum in B_2 appears because R_g/R does not exceed the required threshold [16]. Predictions of thermal PRISM theory are compared with the experimental data in figures 2 and 3. The experimentally obtained T_s was fit to a quadratic form: $T_s(c_p) = T_c + b(c_p - c_{pc})^2$ with $T_c = 379$ K, $b = 1.5 \times 10^4$ K (g mol^{-1})$^{-2}$, and $c_{pc} = 0.04$ g cm^{-3}. Spinodal curves for PEG 1000 and 6000 solutions were indirectly estimated with a similar quadratic form of T_s by setting $c_{pc} = c_p^*$, and $T_c = 422$ K and 388 K for PEG 1000 and 6000, respectively, in good agreement with prior measurements [17]. As seen in figure 1, thermal PRISM theory is successful in describing the depth and qualitative location of the minimum, and its dependence on polymer molecular weight and ionic strength (figure 2). This suggests the strong N-dependence of B_2, and its nonmonotonic variation with c_p, as primarily due to thermal polymer concentration fluctuations. However, the theory, and/or simple polymer–protein model employed, fails to account for the steep repulsive upturn of B_2 at higher concentrations.

3. Summary

The strength of depletion interactions between a small globular protein and relatively large water-soluble polymers has been characterized as a function of polymer concentration, molecular weight, and solution ionic strength. The central result of our studies is the observation that the protein second virial coefficient passes through a sharp minimum with increasing polymer concentration. This minimum deepens with polymer chain length and heating, and occurs close to the N-dependent semidilute crossover or critical polymer concentration. The classic AO depletion model fails to capture these observations, but predictions of polymer integral equation theory are in good qualitative, and reasonable quantitative, agreement with the data. This agreement suggests that the strength of polymer-mediated attractive forces between small proteins can be tuned by rational manipulation of the mesh size of polymer solutions by modest pre-transitional thermal polymer concentration fluctuations even 75–100 °C away from the critical temperature. This effect may be exploited in a variety of scientific and technological contexts, including the important problem of optimizing protein crystallization conditions.

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