Liquid-Liquid Phase Separations in Aqueous Solutions of Globular Proteins

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A simple statistical-mechanical theory, known as the random-phase approximation, is applied to study liquid-liquid phase separations in solutions of globular proteins. Phase separation may be induced by addition of nonionic polymer or/and ordinary electrolytes. In this analysis, the osmotic-attraction mechanism, whereby the depletion of "solvent" particles between two proteins causes an attractive force, is primarily responsible for phase separation. For one-component models of protein solutions, the theory yields simple algebraic expressions for the equation of state and for the chemical potential of the protein. This analytical theory describes the observed solubility behavior of proteins, including the effect of protein and polymer size, protein charge and concentration, and concentration of simple electrolytes.

Introduction

Several experimental studies (Vrij, 1976; de Hek and Vrij, 1981; Vincent et al., 1980; Sperry et al., 1981; Atha and Ingham, 1981; Haire et al., 1984; Forciniti et al., 1991) and several theoretical studies (Asakura and Oosawa, 1954, 1958; Vrij, 1976; Joanny et al., 1979; Middaugh et al., 1979; de Hek and Vrij, 1981; Gast et al., 1983; Mahadevan and Hall, 1990, 1992; Lekkerkerker, 1990) have described phase separation of colloids and globular proteins by addition of a nonadsorbing polymer (for example, polyethylene glycol) to the aqueous solution; here nonadsorbing means that there are no attractive forces between polymer and protein. These studies are motivated by an increasing demand for pure proteins in pharmaceutical and related industries (Bjurstrom, 1985). In this context, charge-stabilized colloids, having electrochemical properties similar to those of aqueous globular proteins, play an important role. Interactions in colloidal dispersions are less complex than those in protein solutions; therefore, colloids may serve as model substances to test theoretical predictions for aqueous solutions of globular proteins. The one-component model applied in this work, frequently used to describe interactions in colloidal dispersions (Verwey and Overbeek, 1948; Asakura and Oosawa, 1958; Ottewill and Richardson, 1982; Gast et al., 1983; Grimson, 1983; Vincent and Hansen, 1984), may be useful for modeling aqueous solutions of globular proteins (Mahadevan and Hall, 1990, 1992; Vlachy and Prausnitz, 1992).

In an aqueous polymer solution, experimental observations indicate that the solubilities of proteins depend on their size: larger proteins precipitate at lower concentration of polyethylene glycol (PEG). Further, protein solubilities depend on the size of the polymer; the solubility of proteins decreases with rising molecular weight of PEG. The electrochemical properties of the solution are also important: protein solubility falls as solution pH approaches the protein's isoelectric point. Protein solubility also falls with the solution's rising ionic strength.

In their seminal work, Asakura and Oosawa (1954, 1958) have suggested that phase separation is caused by the depletion of polymer particles in the region between two colloids. The osmotic pressure exerted by the polymer molecules in the space between two colloidal particles is smaller than its bulk value; the result is a net osmotic attraction between the colloidal particles. This idea has been explored in several theoretical papers (Vrij, 1976; de Hek and Vrij, 1981; Gast et al., 1983; Mahadevan and Hall, 1990, 1992; Lekkerkerker, 1990); comparison with experimental data suggests that "osmotic" attraction is indeed the primary mechanism for phase separation in these systems. An excellent review of theoretical and experimental work done in this area of research has been presented by Russel et al. (1991).

Theoretical studies of polymer-induced phase separations in aqueous and nonaqueous colloidal suspensions (Gast et al., 1983) combine the osmotic attraction model and the pertur-

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bation theory of Barker and Henderson (1967). The theory predicts existence of a very dense (solid-like) flocculated phase. However, some experimental data (de Hek and Vrij, 1981) suggest liquid-liquid phase separation rather than flocculation. Nevertheless, the perturbation theory correctly predicts general trends observed in experimental studies.

Important theoretical and experimental studies of protein precipitation by nonionic polymer have been presented by Hall and coworkers (Mahadevan and Hall, 1990 and 1992; Forciniti et al., 1991). The effective protein-protein interaction due to the presence of polymer is related to the osmotic-attraction model of Asakura and Oosawa (1954, 1958). Again, the perturbation theory of Barker and Henderson (1967) is used to calculate the solubility curves for varying protein-polymer diameter ratio. Mahadevan and Hall (1990) have also been able to incorporate the effect of parameters such as pH and ionic strength in the theory. Recently the theory is extended to a mixture of two proteins in aqueous solution (Mahadevan and Hall, 1992). The theoretical predictions are in accord with experimental observations. The only disadvantage of the perturbation theory (Gast et al., 1983; Mahadevan and Hall, 1990, 1992) is that it requires substantial numerical work, which makes an extension to the multicomponent cases, such as study of protein fractionation, very difficult. Equally (numerically) demanding seems to be an extension of the model to regard the polymer molecules as separate species in the protein-polymer mixture.

In this article, we apply another statistical-mechanical theory, the random-phase approximation (RPA) (Evans and Sluckin, 1981; Grimson, 1983; Victor and Hansen, 1984) to study protein (or colloid) phase separation induced by addition of nonadsorbing polymer or simple electrolyte. An important attractive feature of RPA is its simplicity; the theory yields algebraic expressions for the osmotic pressure and chemical potential of the protein in the mixture. The study presented here is based on a one-component model, where the major contributions to the effective pair potential between two protein molecules are osmotic attraction (Asakura and Oosawa, 1954), electrostatic repulsion and dispersion interactions (Verwey and Overbeek, 1948). The theory predicts phase separation similar to vapor-liquid transition in simple liquids. The concentrations of protein in the coexistent dilute and dense phases have been calculated to obtain the protein partition coefficient as a function of protein size and concentration, pH of the solution (net charge on the protein) and electrolyte concentration.

In this article, we discuss effective interactions in the one-component model of the protein-polymer-electrolyte mixture. After introducing the necessary statistical-mechanical framework and the random-phase approximation, the equations for the osmotic pressure and chemical potential are derived. We also describe phase separations in protein-polymer and protein-polymer-electrolyte mixtures. Finally, we discuss the influence of the osmotic attraction force on the phase separation in protein solutions induced by addition of a simple electrolyte.

Model Interactions in Protein Solutions

Aqueous solutions of colloids or globular proteins are multicomponent systems which are too complicated for a complete description on the molecular level. However, many experimental properties of colloidal dispersions and aqueous solutions of globular proteins can be explained using a simple one-component model wherein a pseudo-solvent (electrolyte, water, or polymer) modifies interactions between the protein molecules (Verwey and Overbeek, 1948; Asakura and Oosawa, 1954; de Hek and Vrij, 1981; Gast et al., 1983; Mahadevan and Hall, 1990, 1992; Vlachy and Prausnitz, 1992).

According to this model, the mixture is described as a fluid of single-component macroparticles with diameter d_2 interacting via the potential $u(r)$ (Gast et al., 1983; Mahadevan and Hall, 1990, 1992):

$$u(r) = u_R(r) + u_A(r) + u_{OA}(r), \tag{1}$$

where $u_R(r)$ is the repulsive interaction, $u_A(r)$ is the attractive (dispersion) potential, and $u_{OA}(r)$ is the potential due to osmotic attraction derived by Asakura and Oosawa (1954, 1958). The screened Coulomb repulsion is given by:

$$\begin{aligned} &u_R(r) = \beta^{-1}(A/r)\exp(-\kappa r), && r > d_2, \\ &u_R(r) = \infty, && r < d_2, \\ &A = z_2^2 L_B \exp(\kappa d_2)/(1+\kappa d_2/2)^2 \\ &L_B = \beta e^2/(4\pi\epsilon_0\epsilon_r) \end{aligned} \tag{2}$$

In Eq. 2, r is the distance between macroion centers, $z_2 e$ is the charge on a polyion and $\beta = (k_B T)^{-1}$, T is the absolute temperature and k_B is Boltzmann's constant; κ^{-1} is the Debye screening length ($\kappa^2 = 8\pi L_B N_A I$; $I = 0.5(z_+^2 n_+ + z_-^2 n_-)$; I is ionic strength and N_A is Avogadro's number) and ϵ_r is the relative permittivity of the solution. All properties of water and electrolyte are subsumed in κ. An attractive van der Waals term, $u_A(r)$, is added to account for short-range attraction.

$$u_A(r) = -H/36(d_2/r)^6 \tag{3}$$

Equation 3, where H is the Hamaker constant, is the simplified form of the dispersion interaction potential (Grimson, 1983), which is only correct at large reduced distances r/d_2. Because dispersion interactions play a minor role in these phase separations, a limiting form of $u_A(r)$ is chosen to keep the theory analytical. The third term in Eq. 1, $u_{OA}(r)$, represents the osmotic attraction contribution to the total potential (Asakura and Oosawa, 1954, 1958). This term is essential to describe phase transitions induced by a nonadsorbing polymer (Vrij, 1976; de Hek and Vrij, 1981; Gast et al., 1983; Mahadevan and Hall, 1990, 1992), but it may also be important at higher concentrations of simple electrolytes (Vlachy and Prausnitz, 1992). For our approximate purposes, we use the potential proposed by Asakura and Oosawa (1954, 1958):

$$\begin{aligned} &u_{OA}(r) = \infty, && \text{for } r < d_2, \\ &u_{OA}(r) = -\frac{4}{3}\pi d_{23}^3 P^{\text{osm}}[1 - 3r/4d_{23} \\ &\qquad + r^3/16d_{23}^3], && \text{for } d_2 < r < 2d_{23}, \\ &u_{OA}(r) = 0, && \text{for } r > 2d_{23}, \end{aligned} \tag{4}$$

where osmotic pressure P^{osm} is approximated by $\rho_3 k_B T$ (Mahadevan and Hall, 1990, 1992). In Eq. 4, d_3 and ρ_3 are, respectively, the diameter and the number concentration of the particles (polymer or electrolyte) exerting the osmotic force and $d_{23} = (d_2 + d_3)/2$. For separations $r < (d_2 + d_3)$, polymer molecules cannot penetrate into the region between two macroions, and the depletion of polymers in this region causes a net attraction between the two macroions. The attraction potential vanishes for $r > (d_2 + d_3)$, when the concentration of polymer segments in the space between two proteins becomes equal to that in the bulk.

The derivation proposed by Asakura and Oosawa (1954) neglects the internal degrees of freedom of the polymer molecule: that is, the polymer coil is replaced by an impenetrable hard sphere. However, more rigorous theories (for example, Joanny et al., 1979; Russel et al., 1991) have confirmed the validity of this simple derivation. In our work here, the osmotic pressure is approximated by the ideal term $P^{osm} = \rho_3 k_B T$, while the polymer coil is characterized by d_3. The approximation for P^{osm} can easily be relaxed; a more realistic expression for osmotic pressure, based on experimental values for thermodynamic parameters (the second virial coefficient and the radius of gyration of the polymer as a function of molecular weight), has been used by Gast et al. (1983) and by Patel and Russel (1989). This way, account is taken of polymer-polymer (segment) interactions in addition to polymer-solvent interactions.

Finally, we note that Eq. 4 is an approximation even for a mixture of hard spheres. More accurate calculations of Henderson (1988) show that the osmotic interaction potential is an oscillatory function of r, if the volume fraction of small spheres (solvent species) is high. The recent article of Heno and Regnault (1991) discusses this topic using integral-equation theories.

Structure and Stability of Simple Liquids

An important class of statistical-mechanical theories uses the Ornstein-Zernike equation:

$$h(r) = c(r) + \rho \int c(r')h(|r - r'|)dr' \qquad (5)$$

as a starting point to calculate thermodynamic properties for the system of interest (Hansen and McDonald, 1986). Equation 5 relates the total correlation function $h(r)$ to the direct correlation function $c(r)$. The correlation function $h(r)$ is related to the radial distribution function $g(r) = h(r) + 1$. The Fourier transform of $h(r)$ is given by:

$$h(k) = \int drc(r)\exp[ik \cdot r], \qquad (6)$$

where k is the wave vector; $h(k)$ can be determined by light-scattering or neutron-scattering experiments. These scattering data yield important information about the "structure," that is, correlations between the particles in charged colloidal dispersions, micellar solutions, and globular proteins (Ottewill and Richardson, 1982). An important quantity is the polyion-polyion structure factor $S(k)$:

$$S(k)^{-1} = [1 - \rho c(k)] \qquad (7)$$

Upon applying the Ornstein-Zernike equation, $S(k)$ can also be expressed in terms of $h(k)$. The connection between the structural properties (correlation functions) and thermodynamics comes in the limit as $k \to 0$ (Hansen and McDonald, 1986). The $k = 0$ limit is equivalent to integration over the volume: $C \equiv \lim_{k \to 0} c(k) = 4\pi \int c(r) r^2 dr$. For the one-component system (and only in this case), C [and $S(0)$] are related to the isothermal compressibility, χ_T (Hansen and McDonald, 1986):

$$(\rho \chi_T)^{-1} = (\partial P/\partial \rho)_T = \beta^{-1}[1 - \rho C] \qquad (8)$$

For colloidal solutions, described by a one-component model [McMillan-Mayer approximation (Hansen and McDonald, 1986)], χ_T is an osmotic compressibility which can be determined: experimentally (Ottewill and Richardson, 1982). Utilizing angular light scattering (scattering angle θ is proportional to the wave vector k), it is possible to obtain the structure factor $S(k)$ (Ottewill and Richardson, 1982). By extrapolation of $S(k)$ to $k \to 0$ ($\theta \to 0$), it is then possible to determine χ_T and, if this quantity is measured as a function of the colloid concentration, also the osmotic pressure.

Equation 8 is important for assessing the stability of a one-component system. The isothermal compressibility χ_T, reflecting concentration fluctuations in a liquid, becomes infinite when the system is approaching phase separation (Hansen and McDonald, 1986).

Random-Phase Approximation (RPA)

We apply the random-phase approximation (Evans and Sluckin, 1981; Grimson, 1983; Victor and Hansen, 1984) to study phase separation in a protein solution induced by addition of neutral polymer and/or simple electrolyte. This approximation has been used previously by Evans and Sluckin (1981) to study the liquid-vapor transition in the Lennard-Jones fluid and by Grimson (1983) for a system similar to ours.

RPA is a perturbation theory which approximates the direct correlation function $c(r)$ by:

$$c(r) = c_o(r) - \beta u_1(r), \qquad (9)$$

where $c_o(r)$ is the direct correlation function for the reference system and $u_1(r)$ is the perturbation part of the total potential $u(r)$. By taking the Fourier transforms of both sides of Eq. 9 in the $k = 0$ limit, we obtain:

$$C = C_o - \beta U_1 \qquad (10)$$

where $C_o = 4\pi \int c_o(r) r^2 dr$ and $U_1 = 4\pi \int u_1(r) r^2 dr$. From Eqs. 8 and 10 we obtain the equation of state in the form:

$$\beta P/\rho = \beta P_o/\rho + \rho \beta U_1/2, \qquad (11)$$

where P_o is the contribution of the reference system to the total pressure P and ρ is the number concentration of protein particles in a one-component system. Equation 11 is a version of the Van der Waals equation (Hansen and McDonald, 1986).

The Gibbs-Duhem equation relates the chemical potential of the protein μ to C through

$$\rho(\partial \mu/\partial \rho)_T = \beta^{-1}[1 - \rho C]. \qquad (12)$$

Integration of Eq. 12 yields a simple expression for the chemical potential of the protein in the random-phase approximation (Grimson, 1983):

$$\beta(\mu - \mu') = \ln \rho + \beta\mu_o + \rho\beta U_1 \tag{13}$$

In Eq. 13, $\beta\mu' = \ln(\Lambda^3)$ and $\Lambda^2 = \beta h_P^2/2\pi m$, where h_P is Planck's constant and m the mass of the molecule. Further, $\beta\mu_o$ is the contribution of the reference system to the chemical potential of the protein. The reference system is specified below.

Equations 11 and 13 give the pressure and the chemical potential of a one-component fluid. To implement these equations, it remains to split the pair potential $u(r)$ into the reference part $u_o(r)$ and perturbation part $u_1(r)$. Following Grimson (1983), we choose a hard-sphere reference system:

$$\begin{aligned} &u_o(r) = \infty, \quad u_1(r) = u(r_{\min}) \quad \text{if } r < r_{\min}, \text{ and} \\ &u_o(r) = 0, \quad u_1(r) = u(r) \quad \text{if } r > r_{\min}, \end{aligned} \tag{14}$$

where $r_{\min}$ is the position of the first minimum in the potential. To avoid complications in the perturbation theory due to the "softening" of the hard-core when the screened-Coulomb potential (Eq. 2) is included in calculation, we use the additional approximation, $r_{\min} = d_2$ where subscript 2 refer to the protein (Grimson, 1983). As discussed by Victor and Hansen (1984), this is a valid approximation when the concentration of simple electrolyte is high (large κ).

Equation 14 indicates that the reference system is a hard-sphere fluid, where $c_o(r)$ in Eq. 8 is the direct correlation function and P_o and μ_o the pressure and chemical potential of the hard-sphere fluid. P_o is given by the Carnahan-Starling equation (Carnahan and Starling, 1970):

$$\beta P_o/\rho = (1 + \eta + \eta^2 - \eta^3)/(1 - \eta)^3, \tag{15}$$

where volume fraction of protein is $\eta \equiv \eta_2 = \pi\rho d_2^3/6$, and the hard-sphere contribution to the chemical potential of protein μ_o is given by:

$$\beta\mu_o = \eta(8 - 9\eta + 3\eta^2)/(1 - \eta)^3. \tag{16}$$

Finally, to obtain the total pressure and chemical potential for the system of interest here, Eqs. 11 and 13, we need to evaluate integral $U_1 = 4\pi\int u_1(r) r^2 dr$. From Eqs. 1-4 we obtain

$$\begin{aligned} \rho_2\beta U_1 = {} & (8\eta_2 z_2^2 L_B/d_2)[1 + 3/(\kappa d_2) + 3/(\kappa d_2)^2]/(1 + \kappa d_2/2)^2 \\ & - (4/9)\eta_2\beta H - \eta_{23}\eta_2[(2d_{23}/d_2)^3 \\ & - 3(d_2/2d_{23}) + 2(d_2/2d_{23})^3], \end{aligned} \tag{17}$$

where $\eta_{23} = 4\pi d_{23}^3\rho_3/3$.

The approximations of the RPA and its relation to other perturbation theories have been analyzed by Victor and Hansen (1984). They have shown that RPA, given by Eq. 9, can be obtained from a more exact theory for $c(r)$ (Eq. 18),

$$c(r) = c_o(r) - (1/2)\beta u_1(r)\partial^2[\eta^2 g_o(r;\eta_o)]/\partial\eta^2, \tag{18}$$

by making a mean-field approximation for the contribution of the perturbation to the thermodynamic properties, that is, by replacing $g_o(r;\eta_o)$ by 1 in Eq. 18. This approximation can easily be relaxed, giving a more accurate, but unfortunately, nonanalytical theory (Victor and Hansen, 1984). Equation 18 is a first-order thermodynamic perturbation theory which, in combination with Eq. 14, yields accurate results for thermodynamic properties of simple liquids (Weeks et al., 1971; Hansen and McDonald, 1986).

Phase Separations by Nonadsorbent Polymer

Nonaqueous solvents

We first consider phase separation of colloidal particles induced by polymer in a nonaqueous (preferably nonpolar) solvent where electrostatic effects are negligible. Phase separations in dispersions of spherical, lyophilic, monodisperse silica particles and polystyrene molecules in cyclohexane at theta temperatures were studied experimentally and theoretically by de Hek and Vrij (1981). They found that the concentration of polymer needed to induce liquid-liquid separation decreases: i) with rising molecular weight of polymer, ii) with rising diameter of colloidal particles, and iii) with increasing concentration of particles. We apply the random-phase approximation to the system studied by de Hek and Vrij (1981).

Only osmotic attraction and hard-sphere interactions (Eq. 4) are important in the nonaqueous systems studied by de Hek and Vrij (1981); accordingly, z_2 and H in Eq. 17 can be set to zero. Figure 1 presents the equation of state, that is, P as a function of the colloid volume fraction η_2, at several volume fractions of polymer η_3. At low values of η_3, the osmotic pressure is a single-valued increasing function of colloid η_2. Above a "critical" value of η_3, the curves exhibit familiar van der Waals loops; in a well-defined region, which corresponds to unstable states, P is a decreasing function of η_2 and the isothermal compressibility of the fluid is negative. The locus of points connecting the maxima and minima is the spinodal curve

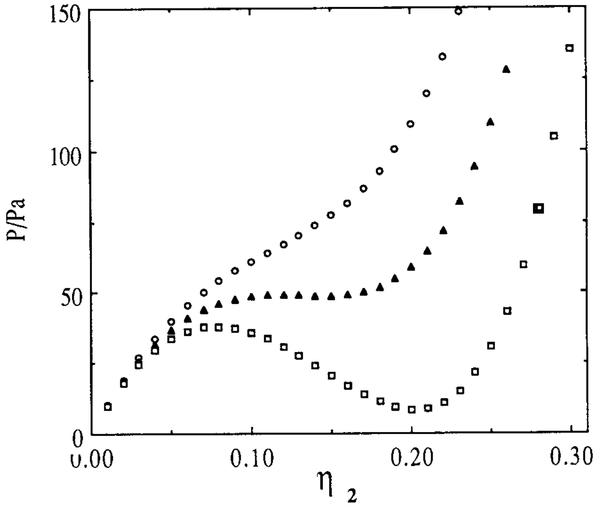

Figure 1. Osmotic pressure in colloid-polymer mixtures (Pascal units) as a function of the volume fraction of colloid η_2; $z_2 = 0$, $H = 0$, $d_2 = 9.0$ nm and $d_3 = 1.5$ nm.

The volume fractions of polymer η_3 are 0.20 (○), 0.225 (▲) and 0.25 (□).

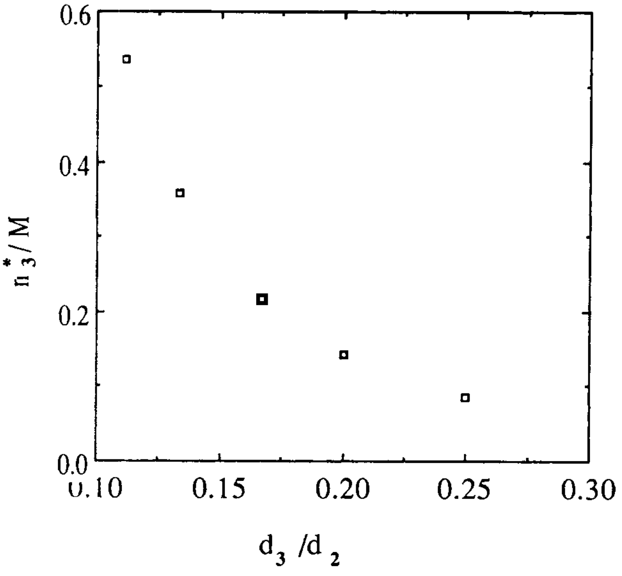

Figure 2. Molar concentration of polymer n_3^* needed for onset of phase-separation, calculated using Eq. 19, as a function of the polymer diameter d_3 at $\eta_2 = 0.1$.

In this calculation, $z_2 = 0$, $H = 0$, and $d_2 = 9.0$ nm.

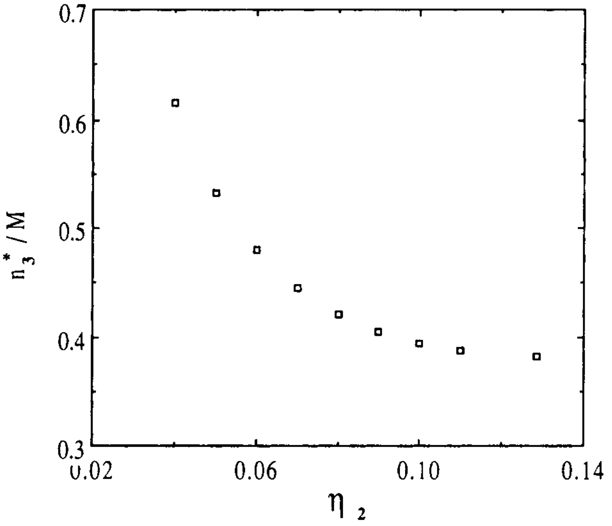

Figure 3. Molar concentration of polymer n_3^* needed for onset of phase-separation, calculated using Eq. 19, as a function of the volume fraction of colloid η_2.

Here, $z_2 = 0$ and $H = 0$; $d_2 = 8.0$ nm and $d_3 = 1.2$ nm.

which is sometimes used as a criterion for the onset of phase separation (de Hek and Vrij, 1981).

A one-component system is stable against spinodal decomposition if the isothermal compressibility is a positive quantity, that is, if $1 - \rho_2 C > 0$. The molar concentration of added polymer n_3^*, needed to destabilize the system (limiting concentration), may be calculated from the condition $\chi_T^{-1} = 1 - \rho_2 C = 0$. This spinodal criterion has been used by de Hek and Vrij (1981) to predict trends in phase separation in mixtures of colloidal silica spheres and polystyrene molecules in cyclohexane. For a polymer-induced phase separation in a colloid-electrolyte-polymer mixture, we obtain the analytical expression:

$$n_3^* = (3/4\pi d_{23}^3 N_A)\{\beta(\partial P_o/\partial \rho_2)_T - (4/9)\eta_2 \beta H + 8\eta_2 z_2^2 L_B/d_2$$
$$[1 + 3/(\kappa d_2) + 3/(\kappa d_2)^2]/(1 + \kappa d_2/2)^2\}/$$
$$\{\eta_2[(2d_{23}/d_2)^3 - 3(d_2/2d_{23}) + 2(d_2/2d_{23})^3\}, \quad (19)$$

where $\beta(\partial P_o/\partial \rho_2)_T = (1 + 4\eta_2 + 4\eta_2^2 - 4\eta_2^3)/(1 - \eta_2)^4$. Figures 2 and 3 show results for the limiting concentration of polymer needed for onset of phase separation n_3^* (H and z_2 are zero) as a function of polymer size d_3 and as a function of η_2, respectively. n_3^* decreases upon raising the polymer diameter (molecular weight) d_3 or by increasing the colloid concentration η_2. Equation 19 also predicts the decrease of the limiting polymer concentration upon raising the size of the colloid. All these trends have been observed in the measurements of de Hek and Vrij (1981).

Aqueous solutions with low or moderate electrolyte concentrations

We now turn to phase separation in water where electrostatic effects and dispersion forces cannot be neglected. Protein phase separations may be induced by addition of a nonadsorbent polymer to aqueous protein solutions containing ordinary electrolyte. If the molar concentration (n) of low-molecular electrolyte is not high ($n < 0.8$ M), we can use Eq. 19 to predict the limiting polymer concentration as a function of electrolyte concentration (or ionic strength I) and pH (z_2). For high electrolyte concentrations, Eq. 19 in its present form is not applicable, because the osmotic attractive force due to the presence of electrolyte may become important (Vlachy and Prausnitz, 1992), in addition to the polymer contribution. The case of high salt concentration (no polymer present) is discussed in the next section.

Figure 4 presents n_3^* as a function of the electrolyte concentration in the range $0.05\ \text{M} \leq n \leq 0.6$ M, at two values of d_3. The limiting polymer concentration is a decreasing function of n. Equation 19 predicts an increase of n_3^* with increasing charge on the protein z_2. These results reflect the functional form of the potential, Eqs. 1-4; by decreasing the electrostatic repulsion between the charged particles, osmotic attraction increases producing a decrease of solubility. In agreement with experimental data, proteins exhibit minimum solubility at the isoelectric point. The influence of the dispersion interactions can also be found from Eq. 19; as expected, a higher value of the Hamaker constant yields a lower value of the limiting polymer concentration.

Equation 19, based on the condition $1 - \rho_2 C = 0$, can be used only to predict general trends with respect to the parameters of the model. We expect phase separation to occur for the values of η_2 between the spinodal and binodal (coexistent) curve; the binodal curve connects the points in both phases having equal P, T and μ_2. To locate the coexistence points, we need to calculate the chemical potential of protein μ_2 given by Eq. 13.

Using Eqs. 11, 13 and 15-17, we can calculate the volume fractions of the protein in the dilute $\eta_2(v)$ and in the dense phase $\eta_2(1)$; the two phases have equal chemical potential μ_2, pressure and temperature. We are particularly interested in the

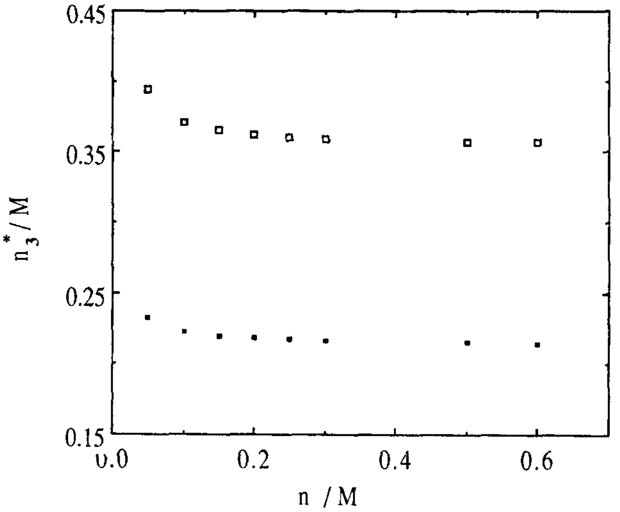

Figure 4. Molar concentration of polymer n_3^* needed for onset of phase-separation, calculated using Eq. 19, as a function of electrolyte concentration n/M (M in mol/dm^3) for $d_3 = 1.2$ nm (□) and $d_3 = 1.5$ nm (■).

In this calculation, $z_2 = 5$, $\beta H = 5$, $d_2 = 8.0$ nm and $\eta_2 = 0.1$.

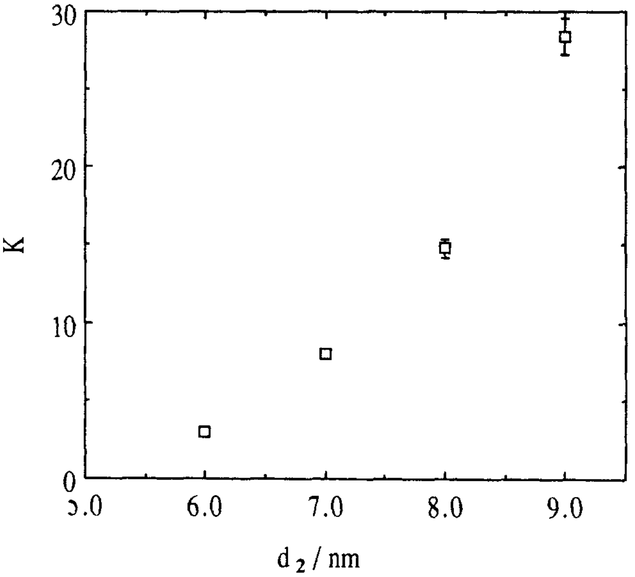

Figure 5. Phase separation induced by addition of neutral polymer: partition coefficient K as a function of d_2.

Other parameters are: $z_2 = 5$, $\beta H = 5$, $d_3 = 1.5$ nm and $n = 0.6$ M.

partition coefficient K, here defined as $K = \eta_2(l)/\eta_2(v)$. We want to determine how K varies as a function of protein and polymer size (d_2 and d_3), electrolyte concentration n and other parameters of the model.

The important parameter in these studies is the diameter ratio d_2/d_3. Typical ratios for protein-PEG mixtures are presented in Table 1 of the article by Mahadevan and Hall (1990). The size of a polymer coil (d_3) depends on its molecular weight and on the "quality" of solvent, as discussed by numerous authors (for example, Flory, 1983). For example, PEG with a molecular weight 600 has $d_3 \approx 1.44$ nm and PEG-1000 has $d_3 \approx 1.9$ nm.

Figure 5 shows results for the partition coefficient K as a function of the size of the protein. Other parameters are: $z_2 = 5$, electrolyte concentration $n = 0.6$ M, $d_3 = 1.5$ nm and $\beta H = 5$. In accord with Eq. 19, K increases upon raising d_2 while other parameters are kept constant. Although not shown here (cf. Figure 2), partition coefficient K decreases upon raising d_3, the size of polymer. The results obtained by the random-phase approximation are consistent with those from other theoretical (Mahadevan and Hall, 1990) and experimental studies (Atha and Ingham, 1981).

Phase Separation Induced by Electrolyte: Influence of the Osmotic Attraction

We now consider phase separation caused by addition of electrolyte (no polymer present). Liquid-liquid transitions in colloidal systems have been observed experimentally by Cowel and Vincent (1982). The experimental work has been followed by theoretical studies of Grimson (1983), Victor and Hansen (1984), and Kovačič and Vlachy (1991). The observed "liquid-vapor" type of phase separation is believed to be a result of the sensitive balance between attractive van der Waals forces (Eq. 3) and repulsive electrostatic interactions (Eqs. 2) (Grimson, 1983; Victor and Hansen, 1984). The concentration of simple electrolyte plays here a role similar to that of temperature in the liquid-vapor transition of simple liquids.

Previous studies (Grimson, 1983; Victor and Hansen, 1984; Kovačič and Vlachy, 1991) are based on the one-component model which does not take into account the effect of the finite size of ions. In our recent study (Vlachy and Prausnitz, 1992), we have demonstrated that, for high electrolyte concentrations ($n > 1$ M), the osmotic attraction term given by Eq. 4 must be included in Eq. 1 to reproduce correctly the results of the more realistic multicomponent model. Our present study indicates that the osmotic-attraction term may provide an important driving force for the phase separation induced by the addition of low-molecular electrolyte.

Since the mechanism of phase separation induced by addition of electrolyte is not known, we compare the results for two suggested models. The first one is that of Grimson (1983); we call it the "dispersion-interaction" model, which models the interactions in the colloidal dispersions as a sum of the screened Coulomb potential (Eq. 2) and the attractive van der Waals (Eq. 3) potential. When this model is used with the random-phase approximation, it yields the following results (Grimson, 1983): i) the magnitude of z_2 has little effect on the shape of the coexistence curves; ii) the value of the Hamaker constant is crucial in determining the phase diagram; and iii) the size of the interacting species has marginal (colloid, d_2) or no (ion, d_3) effect on the shape of the coexistence curve and therefore on the partition coefficient K.

The second model is essentially the same as that described earlier. The interaction potential between the two colloid particles in the colloid-electrolyte mixture is given by Eq. 1. The contribution of dispersion interactions (Eq. 3) is assumed to be less important here; the primary driving force for phase transition is osmotic attraction (Asakura and Oosawa, 1954). Concentration ρ_3 in Eq. 4 is now the number concentration of simple ions in the solution. Calculated partition coefficients K are therefore similar to those obtained in the previous sec-

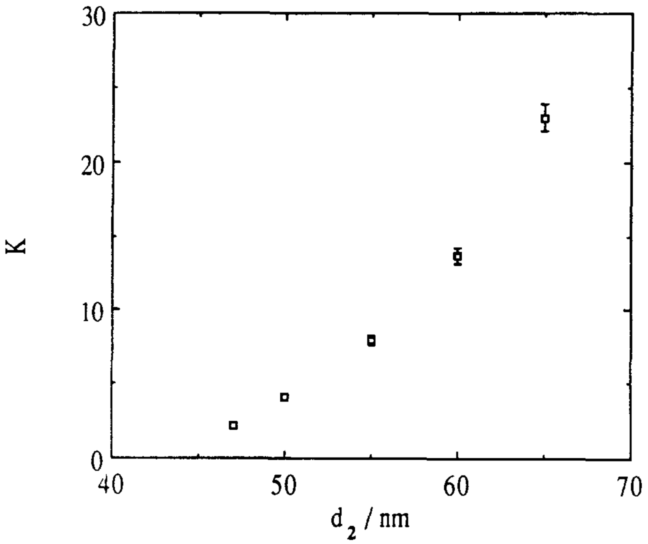

Figure 6. Phase separation induced by addition of electrolyte: partition coefficient *K* as a function of d_2.

Other parameters are: $z_2 = 5$, $\beta H = 5$, $n = 3.0$ M and ionic size $d_3 = 0.4$ nm.

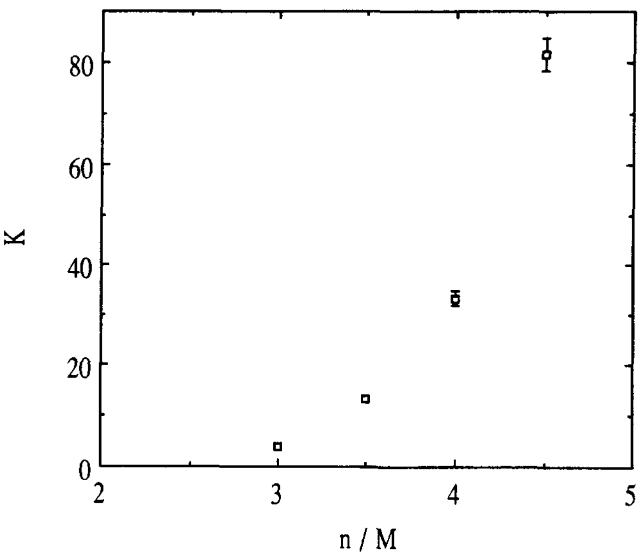

Figure 7. Phase separation induced by addition of electrolyte: partition coefficient *K* as a function of electrolyte molar concentration *n/M*.

Other parameters are: $z_2 = 5$, $\beta H = 5$, $d_2 = 5.0$ nm, and ionic size $d_3 = 0.4$ nm.

tion. In contrast to the first, "dispersion interaction" model, we found that the shape of the coexistence curve (and partition coefficient K) depends strongly on the sizes of interacting species. Figure 6, K plotted as a function of d_2, shows that the partition coefficient K increases strongly with rising size of the protein; larger particles separate more efficiently. Figure 7 presents the partition coefficient K as a function of electrolyte concentration n (ionic diameter is $d_3 = 0.4$ nm), showing the strong effect of electrolyte concentration on phase separation. Also, in accord with the results of the previous section, larger ions yield better separation. These calculations indicate that the two suggested models for phase separation yield qualitatively different behavior with respect to some of the pertinent parameters.

Conclusions

The statistical-mechanical random-phase approximation has been applied to simple models for phase separation caused by addition of nonadsorbing polymer to solutions of globular proteins. The effective protein-protein interaction due to the presence of the polymer is related to the osmotic-attraction potential of Asakura and Oosawa (1954, 1958). The virtue of the random-phase approximation is its simplicity. The theory yields analytical equations for the pressure and chemical potential; therefore, the partitioning of the colloid (protein) between the two coexisting phases can be easily studied for a variety of experimental conditions. Although the theory is simple, it reproduces all the experimentally observed dependencies with respect to protein size, polymer size, protein concentration, polymer concentration, electrolyte concentration and charge on the precipitating particles.

We show that, under certain conditions, the osmotic-attraction mechanism plays a significant role in electrolyte-induced separation. The two different mechanisms, one based on dispersion interactions (Grimson, 1983) and the other on osmotic attraction (Asakura and Oosawa, 1958), yield qualitatively different results with respect to the parameters of the model including protein and electrolyte size. The relevance of this or other models (Taratutta et al., 1990) of phase separation for protein solutions can be assessed by comparison with experiment. Comparison with some experimental data taken in this laboratory (Shih et al., 1992) indicates that the simple theory suggested here is in essential agreement with protein-precipitation experimental results. However, other experimental results (Arakawa et al., 1990) indicate that the solubility of protein in aqueous electrolyte solution, governed by the interactions between solvent components and proteins, may not obey simple electrostatic theory. The effects of electrolyte on the stability of protein are not always entirely electrostatic in nature; little dependence on protein charge has been found in some cases (Arakawa et al., 1990). The model, given by Eqs. 2 and 3, is not able to describe effects due to restructuring of solvent around charged groups on the protein.

The random-phase approximation used in this article may not be as accurate as the perturbation theory of Barker and Henderson (1967) used by Gast et al. (1983) and Mahadevan and Hall (1990, 1992). However, the random-phase approximation also has some advantages vs. numerically much more demanding perturbation theory: i) it can be easily generalized to a multicomponent case to treat a mixture of proteins; ii) the polymer can be included as a separate species into the model; and iii) protein-association, known to be an important feature of many proteins, can be incorporated into the RPA theory. All these refinements require little additional numerical work. However, an obvious first step in improving the theory would be to replace the mean-field approximation in Eq. 18 [$g_o(r;\eta_o) = 1$] with a more realistic approximation. Victor and Hansen (1984) have discussed the approximations in the random-phase theory and suggested a more accurate approach which, unfortunately, is not analytical. Evans and Sluckin (1981) found that RPA gives a reasonable estimate of the critical point for Lennard-Jones fluid; it is somewhat less accurate than the Percus-Yevick approximation. The accuracies of different statistical-mechanical approximations can be evaluated by comparison with computer simulation data but these are not now available.

On the other hand, it may not be worthwhile now to use a more demanding statistical-mechanical theory until the pertinent forces responsible for protein-protein and protein-ion interactions are better identified. In their present form, even the more advanced perturbation theories neglect specific salt effects and protein-protein association leading to semi-stable dimers and higher aggregates; these forces are known to be important for at least some protein solutions. An increase in mathematical sophistication is not helpful unless that increase corresponds to a comparable improvement in our quantitative understanding of intermolecular forces.

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Notation

$c(r)$ = direct correlation function
d = diameter of a molecule, m
e = elementary charge, C
$g(r)$ = radial distribution function
H = Hamaker's constant, J
$h(r)$ = total correlation function
h_P = Planck's constant
I = ionic strength of solution, mol/dm^3
K = partition coefficient
k = wave vector, m^{-1}
k_B = Boltzmann's constant, J/K
L_B = Bjerum's length, m
N_A = Avogadro's number of molecules, mol^{-1}
m = mass of the molecule
n = molar concentration, mol/dm^3
P = osmotic pressure, Pa
r = intermolecular distance, m
$S(k)$ = structure factor
T = absolute temperature, K
$u(r)$ = intermolecular pair potential, J
z = valence of ion

Greek letters

$\beta = (k_B T)^{-1}$
ϵ_r = relative permittivity
ϵ_o = permittivity in vacuum, C/Vm
η = volume fraction
θ = scattering angle
κ = inverse Debye length, m^{-1}
μ = chemical potential, J/mol
ρ = number concentration, m^{-3}
χ_T = isothermal compressibility, Pa^{-1}

Subscripts

o = reference system
1 = perturbation
2 = protein
3 = polymer

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