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van der Waals phase transition in protein solutions

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The van der Waals equation of state for imperfect gases is applied to solutions of macromolecules, especially to explain the fluid–fluid phase transition in protein solutions, a phenomenon of much interest in relation to protein crystallization. The van der Waals b parameter corresponds to the total excluded volume per pair of molecules and can be calculated from independently known molecular properties. It is comprised of terms resulting from hard-sphere and net charge–charge interactions. The experimentally determined second virial coefficient B_2 can then be used to obtain the equilibrium constant for dimerization K_2, a phenomenologically accessible measure of the van der Waals a parameter. Sedimentation equilibrium is recommended as the technique for measuring B_2 most accurately. More general results are used to make a minor quantitative correction to the van der Waals prediction concerning the criterion for the fluid–fluid phase transition. Calculations of the effect of inert co-solutes on the phase transition may prove useful in choosing crystallization conditions.

1. Introduction

Most attempts to understand the complex process of protein crystallization begin with some consideration of the character and strength of the forces of interaction between individual molecules. The thermodynamic parameter that provides a direct measure of the net effect of such forces is the second virial coefficient determined from osmotic pressure measurements, light-scattering experiments, sedimentation-equilibrium studies and results obtained by using a range of other techniques. Indeed, the so-called 'crystallization slot' is characterized in terms of a narrow range of values of this parameter (George & Wilson, 1994; George *et al.*, 1997; Bonneté & Vivarès, 2002; Demoruelle *et al.*, 2002) and it has become quite routine to use the second virial coefficient as a diagnostic parameter for protein crystallization (Bonneté *et al.*, 1997; Haas *et al.*, 1999).

The second virial coefficient is a measure of the net average effect of the forces between an isolated pair of molecules in an infinite volume. It does not supply direct information about collective behaviour of molecules that results in a thermodynamic phase transition such as crystallization. It is therefore perhaps surprising that such a coarse measure of the overall strength of intermolecular forces is of much value in this context. However, it has been known since the time of van der Waals (1873) that combined consideration of weak attractive intermolecular forces and the finite size of molecules is sufficient to explain why a gas condenses to form a liquid when it is cooled. Indeed, by separating the opposing contributions to the second virial coefficient of intermolecular attraction and volume exclusion, van der Waals was able to predict the existence of the critical point characterizing the onset of condensation.

The second virial coefficient for macromolecules in solution must be defined with some care because of the different conditions under which the concentration of the solute can be varied (Winzor & Wills, 1994). Osmotic equilibrium often presents a convenient set of standard conditions to which changes in thermodynamic quantities can be referred and choice of this standard allows the establishment of a formal equivalence between the theories of imperfect gases and non-ideal solutions (Hill, 1959). In that regard, as we show here, van der

Waals theory is directly applicable to the description of a phase transition in the behaviour of solutions of macromolecules. While the predicted phase transition is of the fluid–fluid type rather than crystallization, such a transition serves as a good indicator of conditions favourable for crystallization. The protein apoferritin (Tanaka & Ataka, 2002) provides a pertinent example.

The connection between the second virial coefficient and the gas–liquid critical point, analogous to the fluid–fluid transition in solutions of macromolecules, has been considered in some detail by Vliegenthart & Lekkerkerker (2000). We adopt the view that the simplest possible interpretation will be of greatest benefit provided it has a sound conceptual basis and offers good comparative judgments of how solution conditions may be changed to assist crystallization. Therefore, rather than concentrating on the details of protein–protein and protein–polymer interaction potentials (Acedo & Santos, 2001; Rosenbaum *et al.*, 1999), we approach the problem in terms of van der Waals' approximate equation of state and enquire into the sensitivity of the predicted phase transition to varying laboratory conditions.

2. van der Waals theory

The van der Waals equation of state for an imperfect gas,

$$\left(P + \frac{a}{v^2}\right)(v - b) = RT, \tag{1}$$

is an extension of the ideal gas equation $Pv = RT$ written in terms of the molar volume $v = V/n$; V is the volume of the system, n the number of moles of gas, P is the pressure and T is the temperature. The van der Waals parameters a and b make corrections for properties of real molecules ignored in the kinetic theory of a perfect gas. The a parameter measures the spatially averaged potential energy per mole of gas owing to weak short-range forces between the molecules,

$$a = -2\pi L^2 \int_{\sigma}^{\infty} u(r) r^2 \, \mathrm{d}r, \tag{2}$$

where L is Avogadro's number and the energy of interaction $u(r)$ of two molecules separated by a centre-to-centre distance r is assumed to be small compared with thermal energy. The b parameter adjusts the available volume by subtracting from the true volume the molar excluded volume for a pair of molecules,

$$b = \frac{2\pi L}{3}\sigma^3, \tag{3}$$

expressed in terms of the molecular diameter σ.

A more general formulation of the equation of state of an imperfect gas is the virial form

$$P = RTC(1 + B_2 C + B_3 C^2 + \ldots), \tag{4}$$

which allows the pressure to be approximated by the addition of terms of increasing order in the molar concentration $C = 1/v$. The van der Waals equation can be reforged in this way, giving, to first order in C,

$$P = RTC[1 + (b - a/RT)C + \ldots]. \tag{5}$$

What is pleasing about the virial expansion is that there is an exact statistical mechanical theory for the calculation of the coefficients B_2, B_3 *etc*. In the case of a spherically symmetric interaction between molecules, the expression for the second virial coefficient is

$$B_2 = -2\pi L \int_{0}^{\infty} \{\exp[-u(r)/kT] - 1\} r^2 \, \mathrm{d}r, \tag{6}$$

where k is Boltzmann's constant and $u(r)$ is the energy of interaction between two molecules as in (2). The van der Waals form can be retrieved by considering a potential of interaction such as the square well,

$$u(r) = \begin{cases} \infty & 0 < r < \sigma \\ -\varepsilon & \sigma < r < \lambda\sigma \\ 0 & \lambda\sigma < r < \infty \end{cases}. \tag{7}$$

where ε is the depth of the attractive energy well and $(\lambda - 1)\sigma$ is its width. Integration over the range $0 < r < \sigma$ of the hard-sphere interaction gives the excluded volume b (3). Under the approximation $\varepsilon << kT$, further integration over the range $\sigma < r < \lambda\sigma$ yields

$$a = \frac{2\pi L^2}{3}\sigma^3\varepsilon(\lambda^3 - 1) \tag{8}$$

for a weak square-well potential. Formally, the definition of a as a constant requires only that $u(r) << kT$ in the range $r > \sigma$ and that the integral over $u(r)$ converges as $r \rightarrow \infty$. The case of an anisotropic potential has been discussed by Kern & Frenkel (2003).

It must be emphasized that the second virial coefficient B_2 does not provide a direct measure of the relative magnitudes of the van der Waals parameters a and b; it measures only the difference $b - a/RT$. In the following, we consider how the cooperative interactions involved in phase transitions are affected by the relative magnitudes of a and b, reflecting a balance between forces of attraction and repulsion. We seek to improve on the 'crystallization slot' approach that relies on consideration of the net effect of opposing forces.

3. Solutions of macromolecules

These results can be applied directly to the description of macromolecules in solution. We consider a solution comprised of a macromolecular solute A at a molar concentration C_A and a solvent s. The osmotic pressure of the solution is an artificial comparative parameter defined as the pressure difference through which pure solvent must be raised at constant temperature to render its chemical potential μ_s equal to that of the solvent component in the solution,

$$\mu_s(P, C_A) = \mu_s(P - \Pi, 0) = \mu_s(P, 0) - \int_{P-\Pi}^{P} \bar{V}_s \, \mathrm{d}P. \tag{9}$$

Here, $\bar{V}_s(P, T) = (\partial\mu_s/\partial P)_T$ is the partial molar volume of the solvent. For an ideal solution, use of the Gibbs–Duhem relation gives $\Pi = RTC_A$ analogous to the ideal gas equation and application of the van der Waals *ansatz* may be expected to yield

$$(\Pi + aC_A^2)\left(\frac{1}{C_A} - b\right) = RT. \tag{10}$$

In (10), aC_A^2 must represent the lowering of osmotic pressure below the level expected for an ideal solution owing to inter-molecular attraction and b should measure the molar excluded volume per pair of macromolecules (3). If the weak attraction between molecules results in a small equilibrium concentration C_2 of dimers present in the solution, then the expected reduction in the osmotic pressure will be $\Delta\Pi = RTC_2$ because for every dimer formed the number of osmotically active macromolecules in solution will be reduced by one. The constant K_2 for an ideal dimerization reaction defines the ratio of concentrations $K_2 = C_2/C_1^2$, where C_1 represents the molar concentration of remaining monomers $C_1 = CA - 2C_2$. In the case that $C_2 << C_1$, the approximation $C_1 \simeq C_A$ yields $\Delta\Pi = RTK_2C_A^2$, suggesting the exceedingly simple result

$$a = RTK_2 \tag{11}$$

for the van der Waals representation, correct to first order in C_2.

3.1. Virial expansion

A rigorous statistical mechanical description of the osmotic pressure of a solution of macromolecules yields the virial expansion

$$\Pi = RTC_A(1 + B_2C_A + B_3C_A^2 + \ldots), \quad (12)$$

where the coefficients B_2, B_3 *etc.* now reflect the potential of mean force for clusters of solute molecules under conditions of constant chemical potential of solvent (Hill, 1959). If the attractive interactions between solute molecules are of very short range, having effect only when the molecules are more or less in contact, then it is reasonable to think in terms of aggregates, dimers, trimers *etc.* of the basic macromolecules (monomers). The definition of aggregates arising as a result of forces between molecules of a single chemical component is an extra-thermodynamic exercise (Hill & Chen, 1973). The notional separation between 'associative forces' and 'non-associative forces' is completely arbitrary but it can facilitate interpretation of measurements of the physical properties of a system.

The osmotic pressure of a system containing multiple macromolecular species is given by

$$\Pi = RT\left(\sum_m C_m + \sum_{\{m,l\}} B_{ml}C_mC_l + \sum_{\{m,l,k\}} B_{mlk}C_mC_lC_k\right);$$
$$m, l, k \ldots \in \{1, 2 \ldots\}, \quad (13)$$

where the sets $\{m, l\}$ *etc.* run over all combinations rather than permutations of distinct indices and proper care must be taken to reckon with identity of indices in the definition of the virial coefficients (Hill & Chen, 1973; Wills & Winzor, 2002). Using '1' for monomer, '2' for dimer *etc.*, the formation of an aggregate A_m of m monomers A through the equilibrium process $mA \leftrightarrow A_m$ is governed by a constant

$$K_m = z_m/z_1^m, \quad (14)$$

where $z_m = \gamma_m C_m$ is the thermodynamic activity of A_m in the system and γ_m is the corresponding activity coefficient defined in terms of the chemical potential as

$$\mu_m(T, \mu_s, C_1, C_2 \ldots) = \mu_m^0(T, \mu_s) + RT \ln \gamma_m(C_1, C_2 \ldots)C_m. \quad (15)$$

By using appropriate expansions of the activity coefficients and $C_m \simeq K_mC_1^m$ as allowed, it is possible to rewrite (13) correctly as a sum of terms in increasing orders of the original base molar concentration of the solute component, $C_A = C_1 + 2C_2 + 3C_3 + \ldots$,

$$\Pi = RTC_A[1 + (B_{11}^* - K_2)C_A + \ldots], \quad (16)$$

which gives, in relation to the original virial expansion,

$$B_2 = B_{11}^* - K_2. \quad (17)$$

Details of the derivation can be found elsewhere (Hill & Chen, 1973; Wills & Winzor, 2002). The modified virial coefficients B_{ml}^* *etc.* are specified in relation to integrals such as that in (6) as arising exclusively from the operation of notional non-associative forces between aggregates m and l. In particular, the coefficient B_{11}^* arises from non-associative forces acting between two monomers, $m = 1$ and $l = 1$, and the effect of associative forces, the van der Waals and other forces of attraction at close range between two monomers (Malfois *et al.*, 1996) is already taken into account through the definition of the dimerization constant K_2. As expected, (16) can be interpreted in terms of van der Waals theory (10), correct to first order in C_A, simply by making $a \equiv RTK_2$ and $b \equiv B_{11}^*$. Alternatively, $K_2 = \pi L\sigma^3/6\tau$ relates K_2 to the 'stickiness' parameter τ defined for the adhesive hard-sphere potential of Baxter (1968).

3.2. Electrostatic repulsion

Globular proteins usually carry a net charge when the pH differs appreciably from the protein's pI. This necessitates consideration of electrostatic repulsion between like molecules. Such generalized repulsion can be taken into account as non-associative forces which make a contribution to B_{11}^* in addition to that arising from the hard-sphere force between molecules. Electrostatic repulsion between proteins is often represented by a spherically symmetric DLVO potential of the form

$$u(r) = \begin{cases} \infty & 0 < r < \sigma \\ \dfrac{Q^2}{D(1 + \kappa\sigma/2)^2}\dfrac{\exp[-\kappa(r - \sigma)]}{r} & \sigma < r < \infty, \end{cases} \quad (18)$$

where Q is the surface charge on the molecule, D the dielectric constant of the medium and κ is the Debye–Hückel inverse-screening length of the electrolytic solvent medium. In the expression for the second virial coefficient, $\exp[-u(r)/kT]$ can be thought of as a ratio of Boltzmann factors expressing the ratio of the probability of finding two molecules with some finite energy of interaction $u(r)$ relative to the probability of finding them far apart where $u(r) = 0$ and $\exp[-u(r)/kT] = 1$. In that case, where $u(r) > 0$ the Mayer f-function $f(r) = \exp[-u(r)/kT] - 1$ is a measure of the probability that one particle is excluded from the space at a distance r from another particle. The integral over all space B_{11}^* is then the average of the f-function over all configurations and can be interpreted as the total excluded volume per pair of molecules. In the low-energy short screening-length limit, $f(r) = -u(r)/kT$ and $\kappa\sigma << 1$, we obtain through integration of (6) the familiar result

$$B_{11}^* = \frac{2\pi L}{3}\sigma^3 + \frac{Z^2}{4I}\left[\frac{1 + \kappa\sigma}{(1 + \kappa\sigma/2)^2}\right], \quad (19)$$

where Z is the number of electronic charges e on the protein and I is the solvent ionic strength, related to κ through $\kappa = (8\pi e^2 I/DkT)^{1/2}$. (19) has been shown to give an excellent description of the ionic strength dependence of the second virial coefficient for lysozyme at pH 4.5 (Wills *et al.*, 2000).

3.3. Use of sedimentation equilibrium

Microchip self-interaction chromatography can be used rapidly to obtain an estimate of the second virial coefficient through an empirically established correlation with the chromatographic k' parameter (Garcia *et al.*, 2003), but sedimentation equilibrium provides a way to measure B_2 accurately and unambiguously: through analysis of $c(r)$, the experimental trace of concentration *versus* radial distance (Wills & Winzor, 2002). The condition for sedimentation equilibrium is most conveniently written as $z_A(r) = z_A^0\psi(r)$, where z_A is the osmotic activity defined in relation to (15), z_A^0 is the nominal value of z_A at the centre of rotation, in practice a fitting parameter, and

$$\psi(r) = \exp[M_A(1 - \bar{\nu}_A\rho_s)\omega^2 r^2/2RT] \quad (20)$$

represents a rescaling of r in terms of the radial frequency ω, the solvent density ρ_s and the partial specific volume $\bar{\nu}_A$ and molar mass M_A of the protein. By fitting the experimental trace directly to the form

$$C_A(r) = z_A^0\psi(r) - 2B_2[z_A^0\psi(r)]^2 + \ldots, \quad (21)$$

B_2 can be determined quite accurately. Calculation of B_{11}^* based on (19) and prior knowledge of the molecular properties of the protein (σ, M_A, $\bar{\nu}_A$, Z) under the relevant solvent conditions (ρ_s, I, D) then allows the effect of attractive interactions to be determined as a value of K_2.

4. van der Waals phase transition

The most important outcome of van der Waals theory of an imperfect gas is the prediction of a critical point below which the gas condenses to form a liquid. At the critical point the molar volume, pressure and temperature have values

$$v_c = 3b; \quad P_c = \frac{a}{27b^2}; \quad T_c = \frac{8a}{27Rb}. \tag{22}$$

The van der Waals parameters allow for a quite satisfactory first description of the condensation of simple gases. In practice, measured values of v_c and P_c are used to estimate the molecular quantities a and b rather than specifying these in terms of nominal parameters such as σ, ε and λ appearing in (3) and (8).

4.1. Protein solutions

Applying van der Waals theory to solutions of macromolecules we describe the fluid–fluid phase transition that often precedes protein crystallization. In the following, it is important to bear in mind that the empirically available second virial coefficient B_2 for a macromolecular solute is actually the difference between B_{11}^* and K_2 (17). Therefore, rather than concentrating on the absolute magnitude of B_2 in relation to predicting the conditions for protein crystallization (George & Wilson, 1994), we will focus attention on the relative contributions due to opposing associative and non-associative forces.

4.1.1. Exemplary protein. We first consider haemoglobin as an exemplary globular protein. Its effective hydrodynamic diameter is $\sigma = 6.26$ nm, giving a hard-sphere contribution of $B_{11}^{hs} \simeq 309\ \mathrm{l\ mol^{-1}}$ to the second virial coefficient. For a critical temperature $T_c \simeq 313$ K, sufficiently high to place laboratory conditions in the order–disorder equilibrium region of the phase diagram, a dimerization constant of magnitude $K_2 \simeq 1000\ \mathrm{l\ mol^{-1}}$ would be required, implying a critical osmotic pressure $\Pi_c \simeq 1.05$ kPa and a critical concentration $C_c = 1/v_c$ as high as $1.1\ \mathrm{m}M \simeq 70\ \mathrm{g\ l^{-1}}$. Is this prediction consistent with observation? Under conditions where haemoglobin is uncharged (pH 7.4 and 0.156 I), the dimerization constant has been determined to be only of the order $100\ \mathrm{l\ mol^{-1}}$ (Winzor & Wills, 2003). So, it is not surprising that under these conditions at a temperature of 293 K the protein has solubility beyond $1.6\ \mathrm{m}M \simeq 120\ \mathrm{g\ l^{-1}}$ with no evidence of phase separation. Clearly, the van der Waals prediction of a phase transition is consistent with these observations and we suggest that it may prove useful for calculating comparative estimates of the conditions for the fluid–fluid equilibrium region of the phase diagram for protein solutions.

4.1.2. Critical criterion. If we make the substitutions $a \equiv RTK_2 = RT(B_{11}^* - B_2)$ and $b = B_{11}^*$ in the expression for the van der Waals critical temperature (22), then the criterion $T < T_c$ for the fluid–fluid region of the phase diagram becomes

$$B_2 < \left(1 - \frac{27}{8}\right)B_{11}^*, \tag{23}$$

which dictates how strong the effects of attractive interactions must be to drive B_2 to a value sufficiently low for the effects of repulsive interactions to be overcome and phase separation to occur. However, we must take account of the fact that the van der Waals equation, although qualitatively correct in its prediction of a phase transition, is quantitatively incorrect. The considerations of Vliegenthart & Lekkerkerker (2000), who have investigated the critical point for a range of different intermolecular potentials, would appear to be of assistance. If we define the magnitudes of B_2 and B_{11}^* relative to the effective molar volume occupied by the protein in solution $v_0 = \pi L\sigma^3/6$ and make use of the the hard-sphere measure $B_{11}^* = 4v_0$ and the general criterion for phase separation $B_2 < -6v_0$, we obtain the modified result

$$B_2 < \left(1 - \frac{5}{2}\right)B_{11}^*. \tag{24}$$

This criterion is based on a more robust description of the phase behaviour of protein solutions than van der Waals theory and has been found to be quantitatively consistent with the definition of the 'crystallization slot' (George & Wilson, 1994; George *et al.*, 1997; Vliegenthart & Lekkerkerker, 2000). When interpreted in terms of the 'stickiness' parameter τ defined for the Baxter potential (Baxter, 1968), the relationship

$$B_2 = \left(1 - \frac{1}{4\tau}\right)B_{11}^* \tag{25}$$

enables expression of the criterion as $\tau_c < 1/10$ (Dijkstra, 2002; Rosenbaum *et al.*, 1996) or, in our terms, $K_2 > 5\pi L\sigma^3/3$.

4.2. Use of inert precipitants

Finding the circumstances under which a fluid–fluid phase transition takes place in protein solutions obviously involves a compromise between attractive and repulsive forces of interaction between molecules. Phase separation is sometimes assisted by addition of an inert polymer or even a small co-solute such as a sugar. It is largely unnecessary to invoke ideas such as 'preferential solvation', 'depletion force' or 'osmotic stress' in order to understand such effects. If we imagine a hypothetical osmotic pressure experiment in which the membrane is permeable to the added substance P, then the virial expansion for the osmotic pressure becomes

$$\frac{\Pi}{RTC_A} = (1 + B_{1P}C_P) + \{B_{11}^* - K_2[1 + (2B_{1P} - B_{2P})C_P]\}C_A + \ldots. \tag{26}$$

This can be recast in the van der Waals form

$$\left(\Pi + \frac{\varphi^2 RT\Phi K_2}{v_{\mathrm{eff}}^2}\right)(v_{\mathrm{eff}} - \varphi^2 B_{11}^*) \simeq RT, \tag{27}$$

where $\varphi = 1/(1 + B_{1P}C_P)$ approximates $v_{\mathrm{eff}}/v = 1 - B_{1P}C_P$, the volume fraction effectively available to A taking into account exclusion due to P, and

$$\Phi = K_{\mathrm{eff}}/K_2 = [1 + (2B_{1P} - B_{2P})C_P] \tag{28}$$

defines the effective dimerization constant $K_{\mathrm{eff}} = C_2/C_1^2$ in the presence of P. The increase in the effective oligomerization constant through an excluded volume difference term has been understood for many years (Nichol *et al.*, 1981; Shearwin & Winzor, 1988; Patel *et al.*, 2002).

We see that addition of an inert substance to a solution of macromolecules may be expected to decrease the van der Waals critical volume by a factor φ and increase the critical temperature by a factor Φ. This latter effect may be highly significant in relation to protein crystallization, driving the critical point for the fluid–fluid phase transition to a temperature sufficiently high to guarantee that normal laboratory conditions fall within the fluid–fluid equilibrium region of the phase diagram.

4.2.1. Critical criterion. Substitution of the expressions $a = \varphi^2 RT\Phi K_{\mathrm{eff}}$ and $b = \varphi^2 B_{11}^*$ from (27) into (22) allows the van der Waals phase transition criterion to be written as

$$B_2 < \left(1 - \frac{27}{8\Phi}\right)B_{11}^*, \tag{29}$$

where Φ is the ratio $K_{\rm eff}/K_2$ defined in (28). If we further accept that the work of Vliegenthart & Lekkerkerker (2000) justifies a quantitative correction to the van der Waals result, we may prefer to use

$$B_2 < \left(1 - \frac{5}{2\Phi}\right) B_{11}^{*}. \qquad (30)$$

to calculate the expected effect of an inert substance P on the fluid–fluid critical point for a protein solution. A very recent study (Snoussi & Halle, 2005) has reported a value of $\Phi = 5.5 \times 10^5$ for a protein self-association (decamer formation) reaction in the presence of 14% (volume fraction) dextran.

4.2.2. Calculation of excluded volumes. The quantity Φ can be calculated for molecules with certain general shapes. If molecules of both the protein A and the inert molecule P added to the protein solution are compact enough to be modelled as effective spheres, the protein dimer can be represented as a dumbbell. Then, following the calculation of Wills & Winzor (2001) the excluded volume quantities needed to define the quantity Φ are given by the formulae

$$B_{1P} = \frac{\pi L}{6}(\sigma_1 + \sigma_P)^3;$$
$$B_{2P} = \frac{\pi L}{12}(4\sigma_1^3 + 12\sigma_1^2\sigma_P + 9\sigma_1\sigma_P^2 + 2\sigma_P^3) \qquad (31)$$

and

$$2B_{1P} - B_{2P} = \frac{\pi L}{6}\sigma_P^2(3\sigma_1 + 2\sigma_P), \qquad (32)$$

where σ_1 and σ_P are the effective diameters of the protein monomer and a molecule of the added inert solute P, respectively. (31) and (32) can even be used in the case that P is a chain polymer (Nichol *et al.*, 1981), especially when a suitable measure of the effective diameter as a function of polymer molecular weight is available (Tanaka & Ataka, 2002).

Alternatively, the excluded volume for spherical proteins interacting with random-chain polymers can been expressed in the form

$$B_{AP} = \frac{2\pi\sigma_A^3 L}{3}\left[\frac{1}{4} + \left(\frac{3}{2\pi}\right)^{1/2}\frac{l_P}{\sigma_A} + \frac{1}{2}\left(\frac{l_P}{\sigma_A}\right)^2\right], \qquad (33)$$

where σ_A is the diameter of the protein and l_P is the root-mean-square end-to-end length of the polymer chain. This equation has been found to give a good representation of B_{AP} for protein–polymer interactions (Wills *et al.*, 1995; Chatterjee & Schweizer, 1998*a*), although there is minor uncertainty concerning the coefficients of the various terms in (33) (Chatterjee & Schweizer, 1998*b*). By applying the equivalent sphere approximation $\sigma_2 = 2^{1/3}\sigma_1$ to the dimer, one can use (33) to calculate the difference $2B_{1P} - B_{2P}$ needed to obtain Φ. The results of Tuinier *et al.* (2000) are likely to be useful for a more robust calculation of B_{2P}, the excluded volume for a random polymer interacting with a dumbbell comprised of spherical monomers.

5. Concluding remarks

We have applied the van der Waals equation of state for imperfect gases to solutions of macromolecules. The equation provides an understanding of the opposing roles of attractive and repulsive intermolecular forces in determining the deviation of the osmotic pressure from the ideal relation $\Pi = RTC_A$. When cast in terms of the second virial coefficient B_2, the van der Waals a parameter has a direct relation to the equilibrium constant for dimerization K_2 (11) and the b parameter corresponds to the total excluded volume per pair of molecules B_{11}^{*} comprised of terms resulting from hard-sphere and net charge–charge interactions (19). The phenomenological second virial coefficient can be determined experimentally by using a wide variety of techniques. Sedimentation equilibrium conveniently produces the most accurate results, but microchip self-interaction chromatography can provide useful estimates more rapidly. The total excluded volume can be calculated from independent knowledge of molecular parameters, allowing K_2 to be determined as an empirical measure of the net effect of intermolecular attraction (17).

The van der Waals equation of state successfully predicts the existence of the critical point that characterizes the fluid–fluid phase transition in protein solutions, a phenomenon of much interest in relation to protein crystallization. More general results (Vliegenthart & Lekkerkerker, 2000) can be used to make a minor quantitative correction to van der Waals' prediction concerning the criterion for the phase transition. The same correction can be applied to predict the quantitative effect of inert cosolutes on the fluid–fluid phase transition in protein solutions. (30) may prove useful in designing modifications to experimental conditions under which proteins may be induced to crystallize by allowing the effect of inert polymers to be assessed quantitatively.

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