Osmotic Pressure of Associating Systems. I. Basic Theory*

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ABSTRACT: For two component, nonideal associating systems of the types $nP \rightleftarrows P_n$, $n = 2, 3, \ldots$, or $nP_1 \rightleftarrows qP_2 + mP_3 + \ldots$ the osmotic pressure equation has been developed. It is assumed that the activity coefficient for each associating species may be expressed as $\ln y_i = iBM_1c + O(c^2)$, $i = 1, 2, \ldots$. From the osmotic pressure data one can obtain the weight average molecular weight, $M_{w(c)}$, in ideal solutions, or the apparent weight average molecular weight, $M_{w\text{app}}$, in nonideal solutions. Furthermore, one can obtain the weight fraction of monomer in ideal solutions, or the apparent weight fraction of monomer in nonideal solutions, from the experimental data.

This and other relations obtained from the experimental data can be used to analyze ideal or nonideal associating systems. For three- (or multi-) component systems containing water, supporting electrolyte(s), and an ionizing, associating macromolecule, it is shown, with the proper definition of components, how one can develop a workable osmotic pressure equation that is formally the same as the osmotic pressure equation for a two-component system.

Detergents, soaps, and many proteins undergo reversible association reactions in aqueous solutions. There is much interest in these systems, and one will find many of the associating proteins discussed by Reithel (1963). Since the detergent or soap monomer has a low molecular weight (compared to the protein monomers), it will quite likely diffuse through the semipermeable membrane and shift the chemical equilibrium to dissociation. Thus, the discussion that follows is concerned with osmotic pressure (π) measurements on associating protein systems of the types

$$nP \rightleftarrows P_n \qquad (1)$$

or

$$nP_1 \rightleftarrows qP_2 + mP_3 + \ldots \qquad (2)$$

Previous treatments of osmotic pressure theory by Guntelberg and Linderstrøm-Lang (1949) and Steiner (1954) have been restricted to ideal systems. Kupke (1960) has written a very thorough review article on osmotic pressure measurements of protein solutions; one should consult his article for references to the literature on osmometry of protein solutions. Wagner and Moore (1959) have described the details and the interpretation of osmotic pressure measurements; much detail is given in this paper to the technique of osmometry.

In this paper the effect of nonideal behavior will be included, and it will be shown how one can evaluate equilibrium constants and nonideal effects. In previous communications, methods have been derived for analyzing ideal and nonideal associating systems by sedimentation equilibrium experiments (Adams, 1962; Adams and Fujita, 1963; Adams and Williams, 1964; Adams, 1965). With the development of high speed, membrane osmometers,[1] it may become easier to perform osmotic pressure experiments. Already osmotic pressure experiments using a high speed membrane osmometer on an associating protein (Banerjee and Lauffer, 1965), namely, tobacco mosaic virus protein, have been reported. In addition there may be the dual virtue of economy of the apparatus (compared to an ultracentrifuge) plus rapidity of analysis.

Nonionizing, Two-Component Systems

Osmotic Pressure Equation. Consideration will be given first to the two-component, incompressible system: water and a nonionizing, associating solute. On a molar basis, the condition for chemical equilibrium is

$$n\mu_1 = \mu_n, n = 2, 3, \ldots \qquad (3)$$

Here μ_i is the molar chemical potential of species i ($i = 1, 2, \ldots$). It will be assumed that the activity coefficients (y_i) on the c (g/dl) concentration scale can be described by the previous equation (Adams and Fujita, 1963; Adams and Williams, 1964; Adams, 1965).

$$\ln y_i = iBM_1c + O(c^2), i = 1, 2, \ldots \qquad (4)$$

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[1] Several high-speed membrane osmometers have been described. One version (Reiff and Yiengst, 1959) is designed for studies with aqueous solutions; another version (Rolfson and Coll, 1964) is designed for work with synthetic polymers in organic solvents.

An ideal solution will be defined as one for which $BM_1 = 0$. When equation (4) applies, then the total concentration, c, can be described as

$$c = c_1 + K_n c_1^n,\ n = 2, 3, \ldots \tag{5}$$

for the monomer-n-mer association, or

$$c = c_1 + K_2 c_1^2 + K_3 c_1^3 + \ldots \tag{5a}$$

for the monomer-dimer-trimer (-n-mer) association. As a consequence of equation (4) it should be noted that

$$c_n = K_n c_1^n,\ n = 2, 3, \ldots \tag{5b}$$

At constant temperature, the Gibbs-Duhem equation is

$$VdP = \sum_{i=1}^{n} n_i d\mu_i \tag{6}$$

Here V is the volume of solution, P the pressure, and n_i the number of moles of associating species i. For concentrations in g/dl, c, equation (6) can be arranged to give

$$dP = \sum_{i=1}^{n} c_i d\mu_i / 100 M_i \tag{6a}$$

Knowing the value of $c_1 d\mu_1/100M_1$, one can obtain the values for the other $c_i d\mu_i/100M_i$ with the aid of equations (3), (4), and (5) or (5a). The following treatment will use a monomer-dimer-trimer association as an example; the treatment can be applied to any association reactions described by equations (1) and (2), provided equation (4) is valid. Thus

$$\begin{aligned}\frac{c_1 d\mu_1}{100M_1} &= \frac{c_1}{100M_1}\left[\frac{RTdc_1}{c_1} + RTBM_1 dc + M_1 \bar{v}_1 dP\right] \\ &= \frac{RTdc_1}{100M_1} + \frac{c_1 RTBdc}{100} + \frac{c_1 \bar{v}_1 dP}{100}\end{aligned} \tag{7}$$

Since $d\mu_q = qd\mu_1$, $M_q = qM_1$, and $c_q = K_q c_1^q$, one finds

$$\begin{aligned}\sum_{i=1}^{n} \frac{c_i}{100M_i} d\mu_i = \frac{1}{100}\Bigg[&RT\left(\frac{dc_1}{M_1} + \frac{K_2 c_1 dc_1}{M_1} + \frac{K_3 c_1^2 dc_1}{M_1}\right) \\ &+ RTBcdc + c\bar{v}_1 dP\Bigg]\end{aligned} \tag{7a}$$

The number-average molecular weight, $M_{n(c)}$, is defined as

$$M_{n(c)} = \sum_i n_i M_i \Big/ \sum_i n_i = c \Big/ \sum_i c_i/M_i,\ i = 1, 2, \ldots \tag{7b}$$

It follows from equations (5a) and (5b) that

$$\frac{c}{M_{n(c)}} = \frac{c_1}{M_1} + \frac{K_2 c_1^2}{2M_1} + \frac{K_3 c_1^3}{3M_1} \tag{7c}$$

Thus equation (7a) may be written as

$$100dP(1 - c\bar{v}_1/100) = RTd(c/M_{n(c)}) + RTBcdc \tag{7d}$$

In order to integrate equation (7d) it is necessary to separate variables; this is accomplished by dividing both sides of the equation by $(1 - c\bar{v}_1/100)$ and then recalling that $1/(1 - c\bar{v}_1/100) = 1 + (c\bar{v}_1/100) + \ldots$. When one integrates the rearranged form of equation (7d), using the limits $P = P_0$ to $P = P_0 + \pi$ on the left hand side of the equation and $c = 0$ to $c = c$ on the right hand side, one obtains

$$\begin{aligned}\frac{100\pi}{RT} &= \frac{c}{M_{n(c)}} + \frac{Bc^2}{2} + \frac{B\bar{v}c^3}{300} + \frac{\bar{v}c^2}{200M_1} - \frac{K_2 c_1^3 \bar{v}}{300M_1} + \ldots \\ &= \frac{c}{M_{n(c)}} + \frac{Bc^2}{2} + \frac{\bar{v}c^2}{200M_1} + O(c^3)\end{aligned} \tag{8}$$

For convenience equation (8) may be rewritten as

$$\frac{100\pi}{RT} - \frac{\bar{v}c^2}{200M_1} \equiv \frac{c}{M_{n\,\mathrm{app}}} = \frac{c}{M_{n(c)}} + \frac{Bc^2}{2} \tag{8a}$$

It should be noted that it is assumed that $\bar{v}_1 = \bar{v}_2 = \bar{v}_3 = \bar{v}$. The quantity $\bar{v}$ is the partial specific volume of the associating solute.

In arriving at equation (8a) the terms in c^3 have been arbitrarily cut off for simplicity; the validity of this procedure will be dictated by experience. It is quite possible that in some systems the terms in c^3 may be large and hence cause one to obtain variable values of B; in this case one may have to apply a theory analogous to the one developed in the preceding paper (Adams, 1965) for two virial coefficients. Thus, letting

$$\ln y_i = iB_1 M_1 c + iB_2 M_1 c^2 + O(c^3),\ i = 1, 2, \ldots \tag{8b}$$

the final form of equation (8a) in this case would be

$$\frac{100\pi}{RT} = \frac{c}{M_{n(c)}} + \frac{B_1' c^2}{2} + \frac{B_2' c^3}{3} + \ldots \tag{8c}$$

$$B_1' = B_1 + \frac{\bar{v}}{100M_1}$$

$$B_2' = 2B_2 + \frac{B_1\bar{v}}{100} - \frac{K_2\bar{v}}{100M_1}$$

An analysis of associating systems requiring two virial coefficients is described in the preceding paper (Adams, 1965) on the sedimentation equilibrium of associating systems; the analysis for the osmotic pressure measurements would be similar. One might justify the use of only one virial coefficient in equation (8a) by noting that sometimes the terms making up B_2' in equation (8c) may add up to zero or that their sum may be small in comparison to B_1'. On the other hand some systems, such as those described by Adams and Williams (1964), may be so nonideal that the analysis that follows in this paper or that which was developed for sedimentation equilibrium in the preceding paper (Adams, 1965) will not apply at all.

Evaluation of the Equilibrium Constants and Nonideal Effects. Steiner (1954) has developed a very elegant method for analyzing ideal associating systems; he has shown that one can obtain the number fraction of monomer (z) from the equation

$$\ln z = \int_0^c \left(\frac{M_1}{M_{n(c)}} - 1\right) d \ln m \qquad (9)$$

For ideal systems, m, the total number of moles, is given by $c/M_{n(c)}$, i.e., $m = c/M_{n(c)}$; thus equation (9) becomes

$$\ln z = \int_0^c \left(\frac{M_1}{M_{n(c)}} - 1\right) d \ln (c/M_{n(c)}) \qquad (9a)$$

where $z = m_1/m$ = number fraction of monomer. At present we have not been able to apply equations (9) or (9a) to nonideal solutions: the analogous integral using $M_{n\,\text{app}}$ appears not to yield a physically useful result. However, it is possible to obtain f, the weight fraction of monomer, for ideal solutions, or f_a, the apparent weight fraction of monomer, for nonideal solutions. It has been shown (Adams and Filmer, 1965; Adams, 1965) that

$$cM_1/M_{n(c)} = \int_0^c \frac{M_1}{M_{w(c)}}\, dc \qquad (10)$$

or

$$cM_1/M_{n\,\text{app}} = \int_0^c \frac{M_1}{M_{w\,\text{app}}}\, dc \qquad (10a)$$

for the associating systems under consideration here. It then follows from equations (10) or (10a) that

$$M_1/M_{w(c)} = \frac{d}{dc}(cM_1/M_{n(c)})$$
$$= \frac{M_1}{M_{n(c)}} + c\frac{d}{dc}(M_1/M_{n(c)}) \qquad (11)$$

or

$$M_1/M_{w\,\text{app}} = \frac{d}{dc}(cM_1/M_{n\,\text{app}})$$
$$= \frac{M_1}{M_{n\,\text{app}}} + c\frac{d}{dc}(M_1/M_{n\,\text{app}}) \qquad (11a)$$

Thus one finds

$$\ln f = \int_0^c \left(\frac{M_1}{M_{w(c)}} - 1\right) d \ln c$$
$$= \int_0^c \left(\frac{M_1}{M_{n(c)}} - 1\right) d \ln c$$
$$+ \frac{M_1}{M_{n(c)}} - 1, f = c_1/c \qquad (12)$$

and

$$\ln f_a = \int_0^c \left(\frac{M_1}{M_{w\,\text{app}}} - 1\right) d \ln c$$
$$= \int_0^c \left(\frac{M_1}{M_{n\,\text{app}}} - 1\right) d \ln c$$
$$+ \frac{M_1}{M_{n\,\text{app}}} - 1, f_a = fe^{BM_1c} \qquad (12a)$$

One notes that equations (11) or (11a) show that the weight (or apparent weight) average molecular weight can be obtained from the number (or apparent number) average molecular weight data. From equation (12a) one obtains

$$\alpha \equiv cf_a = c_1e^{BM_1c} \qquad (12b)$$

In osmotic pressure experiments one observes that

$$\lim_{c\to 0} \frac{d}{dc}(M_1/M_{n\,\text{app}}) = (BM_1 - K_2)/2 = -L/2 \qquad (13)$$

$$\lim_{c\to 0} \frac{d}{d(c_1e^{BM_1c})}(M_1/M_{n\text{app}}) = -L/2 \qquad (13a)$$

In obtaining equations (13) and (13a) one should note that $\lim_{c\to 0} dc_1/dc = 1$.

At this point one is in a position to analyze a nonideal associating system. For the monomer-dimer-trimer system one takes $3cM_1/M_{n\,\text{app}}$ [refer to equations (7c) and (8a)] and subtracts the total concentration [see equation (5a)] to obtain

$$\frac{3cM_1}{M_{n\,\text{app}}} - c = 2c_1 + \frac{K_2c_1^2}{2} + \frac{3BM_1c^2}{2} \qquad (14)$$

Using equations (12b) and (13) or (13a) one obtains

$$\frac{3cM_1}{M_{n\,\text{app}}} - c = 2\alpha e^{-BM_1c}$$
$$+ \frac{(L + BM_1)}{2}\alpha^2e^{-2BM_1c} + \frac{3BM_1c^2}{2} \qquad (14a)$$

Equation (14a) has only one unknown, BM_1, so that it can be solved by successive approximations of the unknown. Once BM_1 has been obtained, one finds $2c_1$ from the first term on the right hand side of equation

(14a) and $K_2c_1{}^2$ (i.e., c_2) from the second term on the right hand side of equation (14a). One uses equation (8a) to obtain $M_{n(c)}$. Thus the analysis becomes identical with that used in sedimentation equilibrium experiments, and one is referred to the preceding paper (Adams, 1965) for a detailed description of the analysis used on associating systems described by equations (1) and (2). When equation (14a) is satisfied with $BM_1 = 0$, then the associating system is ideal.

Multicomponent, Ionizable Systems

The theory developed so far has been limited to a two-component, incompressible system; it will now be extended to a system containing an ionizable, associating macromolecule PX_z, water, and a supporting electrolyte, BX. For a discussion of ionizable, nonassociating macromolecular systems one is referred to the excellent review of Casassa and Eisenberg (1964). The symbols and terms used in this section are based on those used by Casassa and Eisenberg (1964) as well as those used in the preceding paper (Adams, 1965) on sedimentation equilibrium of associating systems.

Using the Eisenberg and Casassa (1960) definition of components, the monomeric species has been defined (Adams and Williams, 1964; Adams, 1965) as $PX_z - \Gamma z(BX)$, the dimeric species as $[PX_z - \Gamma z(BX)]_2$, and so on for other associating species which may be present. Again, a monomer-dimer-trimer association will be considered, although the treatment is valid for other associating systems described by equations (1) and (2). The starting equation will be the Gibbs-Duhem equation. For concentrations of the associating species on the W concentration scale, g/kg of solvent, one has[2]

$$dP = \sum_i \frac{W_{2i}{}^*}{V_m M_i{}^*}\, d\mu_{2i}{}^*,\ i = \text{monomer, dimer, trimer} \tag{15}$$

The asterisks denote that one has redefined the associating species according to the Eisenberg and Casassa (1960) definition of components. Because of the equilibrium relations it is only necessary to write down the expression of $(W_{2i}{}^*/V_m M_i{}^*)d\mu_i{}^*$ for the monomer. Thus for the monomer one has

$$\frac{W_{2M}{}^*}{V_m M_M{}^*}\, d\mu_{2M}{}^* = \frac{\hat{c}_{2M}{}^*}{M_M{}^*}\, d\mu_{2M}$$

$$= \frac{W_{2M}{}^*}{V_m M_M{}^*}\,(RT\, d\ln a_{2M}{}^* + M_M{}^*\bar{v}_2{}^* dP) \tag{15a}$$

$$d\ln a_{2M}{}^* = (\partial \ln a_{2M}{}^*/\partial c_M{}^*)_{T,P} dc_{2M}{}^*$$

In practice one measures concentrations on the c concentration scale, g/dl; thus it is necessary to convert from the W concentration scale to the c scale. This is done in the same manner as it is done for the sedimentation equilibrium equations (Adams, 1965). For convenience some of the relations used in converting the concentration scales are listed below.

$$\hat{c}_i = W_i/V_m,\ \hat{c}_i = \text{g/ml of species } i \tag{16}$$

$$c_i = 100\hat{c}_i,\ c_i = \text{g/dl of species } i \tag{16a}$$

$$RT\, d\ln a_{2M}{}^* = RT\,(dW_{2M}{}^*/W_{2M}{}^* + [\phi^0(z) + \gamma_{22}{}^{0*}]dW_2{}^*) \tag{16b}$$

$$V_m = V_m{}^0 + (dV_m/dW_2{}^*)^0 W_2{}^* + \ldots \tag{16c}$$

$$(dV_m/dW_2{}^*)^0 \cong \bar{v}_2{}^* \tag{16d}$$

$$\ln y_i{}^* = iBM_M{}^*c^* + 0(c^2),\quad i = 1(\text{monomer}), 2(\text{dimer}), 3(\text{trimer}) \tag{16e}$$

$$c^* = c_M{}^* + K_2 c_M{}^{*2} + K_3 c_M{}^{*3} \tag{16f}$$

Equation (16e) is an assumption and is analogous to equation (4). Using the equations above, (16) to (16f), and applying them to equations (15) and (15a), one obtains

$$\begin{aligned} 100dP(1 - c_2{}^*\bar{v}_2{}^*/100) &= RTd(c^*/M_{n(c)}{}^*) \\ &+ RT\bar{v}_2{}^*c_2{}^*(M_{w(c)}{}^*/M_M{}^*)d(c^*/M_{n(c)}{}^*) \\ &+ RT\,[\phi^0(z) + \gamma_{22}{}^{0*}]V_m{}^0 c_2{}^* dc_2{}^*/100M_M{}^* \end{aligned} \tag{17}$$

On rearranging equation (17) and subsequently integrating from $P = P_0$ to $P = P_0 + \pi$ on the left-hand side and from $c = 0$ to $c = c$ on the right-hand side, one obtains

$$100\pi/RT = c^*/M_{n(c)}{}^* + B^*c^{*2}/2 + \bar{v}_2{}^*c_2{}^*/200M_M{}^* + 0(c^*)^3 \tag{18}$$

$$B^* = V_m{}^0\left[\phi^0(z) + \gamma_{22}{}^* + \left(\frac{1}{V_m}\frac{dV_m}{dW_2{}^*}\right)\right]/200M_M{}^*$$

It is convenient to rewrite equation (18) as

$$100\frac{\pi}{RT} - \frac{\bar{v}_2{}^*}{200M_M{}^*} \equiv \frac{c^*}{M_{n\,\text{app}}{}^*} = \frac{c^*}{M_{n(c)}{}^*} + \frac{B^*c^{*2}}{2} + 0(c^*)^3 \tag{18a}$$

It follows from equation (18a) that one can apply the same analysis to the three-component system, containing an ionizing, associating macromolecule, as is used for a two-component nonionizing system, providing one defines the components properly in the three- (or multi-) component system.

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[2] The quantity V_m is the volume of solution containing 1 kg of solvent.

Discussion

One advantage of the osmotic pressure experiment is that there is no salt redistribution due to centrifugal fields; thus at all protein concentrations one has the same concentration of supporting electrolytes, which are the concentrations obtained on redefinition of components by the Eisenberg and Casassa (1960) protocol. Although one can apply both the number and weight average molecular weights or their apparent values in osmotic pressure experiments, it must be realized that to obtain $M_{w(c)}$ or $M_{w\ \mathrm{app}}$ it is necessary to take a slope from the plot of $cM_1/M_{n\ \mathrm{app}}$ against c. Unfortunately one may incur error in the process of obtaining the derivative. With sedimentation equilibrium on the other hand, one already has the derivative: one obtains $M_1/M_{w(c)}$ or $M_1/M_{n\ \mathrm{app}}$ directly from the data; to obtain $M_{n(c)}$ or $M_{n\ \mathrm{app}}$ it is only necessary to obtain the area under the curve of a plot of $M_1/M_{w(c)}$, or $M_1/M_{w\ \mathrm{app}}$, against c. This procedure, numerical integration, usually introduces less error than does the process of numerical or graphical differentiation.

Presumably the theory developed here for neutral or ionizable associating systems can be extended to cases where the activity coefficient for the associating species is described by $\ln y_i = iB_1M_1c + iB_2M_1c^2 + 0(c^3)$, $i = 1, 2, \ldots$, as has been done with the sedimentation equilibrium theory (Adams, 1965). The theory for ionizable, associating macromolecules can be extended to systems containing two or more supporting electrolytes. In this case the final equation will have the same form as equation (18a). Instead of using the Eisenberg and Casassa (1960) definition of components, one could use the Scatchard and Bregman (1959) definition of components for three- (or multi-) component systems containing an ionizable, associating macromolecule.

A recent review by Nichol *et al.* (1964) discusses interacting protein systems and some of the techniques used to study them. Much emphasis is placed in their article on the Gilbert theory (Gilbert, 1955, 1959, 1963; Gilbert and Jenkins, 1959, 1963), which is used in sedimentation velocity and moving boundary electrophoresis studies on associating systems. This theory, as Gilbert (1955, 1963) and Nichol *et al.* (1964) point out, suffers from assumptions which are not always valid, the most serious being that there is no diffusion in a moving boundary. In sedimentation velocity experiments there are at least three false assumptions when one applies the Gilbert theory. Osmotic pressure and sedimentation equilibrium experiments are based on equilibrium thermodynamics, and they are free from the assumptions involved in the Gilbert theory. Furthermore, with osmometry or sedimentation equilibrium one can evaluate nonideal effects, if present; this cannot be done with the Gilbert theory.

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