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Osmotic Pressure, Protein Solutions and Active Transport. I

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The McMillan–Mayer solution theory is ideally suited for a rigorous discussion of systems in which at least one species, although present on both sides of a membrane, has different electrochemical potentials on the two sides. Examples are active transport across biological membranes and protein (or other macromolecular) solutions in which the protein molecule cannot pass through the separating membrane. The theory is developed in this paper for a single such "non-equilibrium" species. The same problem is also discussed approximately using the classical Donnan method. If point charges and the Debye–Hückel potential of average force are used in the McMillan–Mayer theory, the two methods give the same results for leading terms (because they both take into account electrical neutrality). Generally speaking, however, the McMillan–Mayer method has the distinct advantage, aside from rigor, of not being restricted to small concentrations of the "non-equilibrium" species. As far as protein (or macromolecular) solutions are concerned, the theoretical considerations outlined here suggest (1) a means of acquiring information about protein–protein interactions at finite protein concentrations, (2) a possible new osmotic method for molecular weight determinations, and (3) a procedure to obtain $\partial\pi/\partial\rho$ and $\partial^2\pi/\partial\rho^2$ as well as $\pi(\rho)$ from osmotic measurements, where π = osmotic pressure and ρ = concentration.

I. Introduction

The object of this paper is to discuss two further applications of the McMillan–Mayer solution theory[1]: (1) the osmotic pressure across a membrane when protein (or other non-diffusible species) is present on both sides of the membrane but at different concentrations; and (2) the osmotic pressure which accompanies the non-equilibrium distribution of an ion with respect to the two sides of a membrane engaged in active transport of the ion. In previous papers we have applied the McMillan–Mayer theory to the binding of ions or molecules on protein or other large molecules,[2] and to the Donnan membrane equilibrium.[3]

The McMillan–Mayer theory is ideally suited for a rigorous statistical mechanical discussion of the pressure difference across a membrane when one or more species have different electrochemical potentials on the two sides of the membrane. A special case is the usual situation[1,3] in which a non-diffusible species is present on one side of a membrane but is completely absent on the other side. In the applications of interest here, however, a certain species is present but has different electrochemical potentials on the two sides of the membrane, either (1) because the species cannot pass through the membrane or (2) because the membrane does work to maintain the electrochemical potential difference. The theory, to be outlined below, is formally the same in the two cases.

We confine ourselves in the present paper to the essential features of the problem, considering the case in which there is only one species with an electrochemical potential difference across the membrane. The biologically important situation in which there are two or more such species (*e.g.*, two proteins, one protein and one actively transported ion, or two "active" ions) and other extensions[4] of the theory will be reserved for part II.

II. Analysis

Let the activity of the sth molecular or ionic species on one side of a membrane be z_s and on the other z_s^*, where z_s is proportional to $e^{\mu_s/kT}$ (μ_s = electrochemical potential) with proportionality constant such that $z_s = \rho_s$ in a perfect gas at zero electrostatic potential (ψ), where ρ_s is the number density or concentration. The activity coefficient γ_s is defined as z_s/ρ_s, and therefore includes a contribution from the potential ψ, if it is non-zero. We let $\mathbf{z}$ and $\mathbf{z}^*$ represent the activity sets $z_1, z_2, \ldots$ and $z_1^*, z_2^*, \ldots$, respectively. A set of m_1 molecules or ions of species 1, m_2 of species 2, etc., is denoted by $\mathbf{m}$ and the coördinates of this set by $\{\mathbf{m}\}$. The coördinates of the i-th molecule of species s are represented by (i_s). Then we have the general relation for the pressure difference across the membrane,[1,5] expressed as a power series in the quantities $(z_s - z_s^*)/\gamma_s^*$

$$\exp\left\{\frac{[p(\mathbf{z}) - p(\mathbf{z}^*)]V}{kT}\right\} = \sum_{\mathbf{m} \geqslant 0}\left[\prod_s \frac{1}{m_s!}\left(\frac{z_s - z_s^*}{\gamma_s^*}\right)^{m_s}\right]\int \exp\left[-\frac{w^{(\mathbf{m})}(\{\mathbf{m}\}, \mathbf{z}^*)}{kT}\right] \mathrm{d}\{\mathbf{m}\} \quad (1)$$

where $w^{(\mathbf{m})}(\mathbf{z}^*)$ is the potential of the average force between molecules of the set $\mathbf{m}$ at the activity set $\mathbf{z}^*$. This type of expansion is clearly particularly appropriate for the problem at hand.

Now suppose all species have the same electrochemical potential on the two sides of the membrane except species 1: $z_1 \neq z_1^*$, $z_2 = z_2^*$, $z_3 = z_3^*$, etc. If we drop the subscript on species 1 (and refer to this species from now on as the "solute" species), eq. 1 becomes

$$\exp\left\{\frac{[p(\mathbf{z}) - p(\mathbf{z}^*)]V}{kT}\right\} = \sum_{m \geqslant 0} \frac{1}{m!}\left(\frac{z - z^*}{\gamma^*}\right)^m \int \exp\left[-\frac{w^{(m)}(\{m\}, \mathbf{z}^*)}{kT}\right] \mathrm{d}\{m\} \quad (2)$$

where here $\mathbf{z} = z, z_2^*, z_3^*, \ldots$ and $\mathbf{z}^* = \mathbf{z}^*, z_2^*, z_3^*, \ldots$ and the sum is over sets of solute molecules only. In the conventional osmotic pressure case $z^* = 0$.

On taking the logarithm of eq. 2, expanding in powers of $(z - z^*)/\gamma^*$, and using the thermodynamic relation

$$\frac{\rho}{z} = \left(\frac{\partial\, p/kT}{\partial z}\right)_{T,\, z_2,\, z_3,} \quad (3)$$

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[1] W. G. McMillan and J. E. Mayer, *J. Chem. Phys.*, **13**, 276 (1945).

[2] T. L. Hill, *ibid.*, **23**, 623, 2270 (1955).

[3] T. L. Hill, *ibid.*, **22**, 1251 (1954); Faraday Soc. Discussion on Membranes, 1956.

[4] Compare, for example, reference 3.

[5] T. L. Hill, "Statistical Mechanics," McGraw–Hill Book Co., New York, N. Y., 1956. See eq. 40.62.

we obtain[1,6]

$$\frac{\Pi}{kT} = \sum_{j\geqslant 1} b_j(\mathbf{z}^*)\left(\frac{\gamma}{\gamma^*}\rho - \rho^*\right)^j \tag{4}$$

and

$$\rho - \frac{\gamma^*}{\gamma}\rho^* = \sum_{j\geqslant 1} b_j(\mathbf{z}^*)\, j\left(\frac{\gamma}{\gamma^*}\rho - \rho^*\right)^j \tag{5}$$

where

$$\Pi = p(\mathbf{z}) - p(\mathbf{z}^*) \tag{6}$$

$$\begin{aligned} 1!\,Vb_1 &= Z_1 = V;\ b_1 = 1 \\ 2!\,Vb_2 &= Z_2 - Z_1^2 \\ 3!\,Vb_3 &= Z_3 - 3Z_1Z_2 + 2Z_1^3 \\ 4!\,Vb_4 &= Z_4 - 4Z_1Z_3 - 3Z_2^2 + 12Z_1^2Z_2 - 6Z_1^4, \text{ etc.} \end{aligned} \tag{7}$$

and

$$Z_m(\mathbf{z}^*) = \int \exp\left[\frac{-w^{(m)}(\mathbf{z}^*)}{kT}\right] \mathrm{d}\{m\} \tag{8}$$

Inversion of eq. 5 gives[1,6]

$$\frac{\gamma}{\gamma^*}\rho - \rho^* = \sum_{i\geqslant 1} a_i(\mathbf{z}^*)\left(\rho - \frac{\gamma^*}{\gamma}\rho^*\right)^i \tag{9}$$

where

$$\begin{aligned} a_1 &= 1,\ a_2 = -2b_2 \\ a_3 &= -3b_3 + 8b_2^2 \\ a_4 &= -4b_4 + 30b_2b_3 - 40b_2^3, \text{ etc.} \end{aligned} \tag{10}$$

Finally, on substituting eq. 9 in eq. 4, we find

$$\frac{\Pi}{kT} = \rho - \frac{\gamma^*}{\gamma}\rho^* + \sum_{n\geqslant 2} B_n(\mathbf{z}^*)\left(\rho - \frac{\gamma^*}{\gamma}\rho^*\right)^n \tag{11}$$

where

$$B_n = -\frac{n-1}{n}\beta_{n-1} \tag{12}$$

and

$$\begin{aligned} \beta_1 &= 2b_2 \\ \beta_2 &= 3b_3 - 6b_2^2 \\ \beta_3 &= 4b_4 - 24b_2b_3 + (80/3)b_2^3, \text{ etc.} \end{aligned} \tag{13}$$

Equations 4 and 11 give alternative exact expressions for the osmotic pressure Π in terms of the potentials of average force $w^{(m)}$ $(\mathbf{z}^*)$, the concentrations ρ and ρ^*, and the activity coefficient ratio γ^*/γ. However, γ^*/γ is itself a function of $w^{(m)}$ $(\mathbf{z}^*)$, ρ and ρ^*.

Although eq. 4 and 11 are the most elegant ways of expanding Π/kT, it is also possible to express Π/kT in the more practical form of an expansion in powers of $\rho - \rho^*$ from which γ^*/γ has been eliminated. To do this, we begin by multiplying eq. 9 by γ^*/γ

$$\begin{aligned} \frac{\gamma^*}{\gamma} &= \frac{\zeta}{\sum\limits_{i\geqslant 1} a_i\zeta^i} = \frac{1}{1 + \sum\limits_{i\geqslant 2} a_i\zeta^{i-1}} \\ &= 1 + 2b_2\zeta + (3b_3 - 4b_2^2)\zeta^2 - (4b_4 - 18b_2b_3 + 16b_2^3)\zeta^3 + \cdots \end{aligned} \tag{14}$$

where

$$\zeta = \rho - \frac{\gamma^*}{\gamma}\rho^* \tag{15}$$

Now we multiply eq. 14 by $-\rho^*$, add ρ to both sides, and obtain

$$\rho - \rho^* = (1 + 2b_2\rho^*)\zeta + (3b_3 - 4b_2^2)\rho^*\zeta^2 + (4b_4 - 18b_2b_3 + 16b_2^3)\rho^*\zeta^3 + \cdots \tag{16}$$

The inverse of eq. 16 is

$$\zeta = \frac{\rho - \rho^*}{1 + 2b_2\rho^*} + \frac{\rho^*(4b_2^2 - 3b_3)(\rho - \rho^*)^2}{(1 + 2b_2\rho^*)^3} + \frac{\rho^*[18b_2b_3 - 4b_4 - 16b_2^3 + \rho^*(-12b_2^2b_3 + 18b_3^2 - 8b_2b_4)](\rho - \rho^*)^3}{(1 + 2b_2\rho^*)^5} + \cdots \tag{17}$$

Substitution of eq. 17 in eq. 11 then gives

$$\begin{aligned} \frac{\Pi}{kT} = \frac{\rho - \rho^*}{1 + 2b_2\rho^*} &+ \frac{[-b_2 + \rho^*(2b_2^2 - 3b_3)](\rho - \rho^*)^2}{(1 + 2b_2\rho^*)^3} \\ &+ \frac{[4b_2^2 - 2b_3 + \rho^*(-4b_4 - 8b_2^3 + 16b_2b_3) + \rho^{*2}(18b_2^3 - 8b_2b_4 - 8b_2{}^2b_3)](\rho - \rho^*)^3}{(1 + 2b_2\rho^*)^5} + \cdots \end{aligned} \tag{18}$$

which is the required result. Also, substitution of eq. 17 in eq. 14 or 15 leads to

$$\begin{aligned} \frac{\gamma^*}{\gamma} = 1 + \frac{2b_2(\rho - \rho^*)}{1 + 2b_2\rho^*} &+ \frac{(3b_3 - 4b_2^2)(\rho - \rho^*)^2}{(1 + 2b_2\rho^*)^3} \\ &+ \frac{[4b_4 - 18b_2b_3 + 16b_2^3 + \rho^*(8b_2b_4 - 18b_3^2 + 12b_2^2b_3)](\rho - \rho^*)^3}{(1 + 2b_2\rho^*)^5} + \cdots \end{aligned} \tag{19}$$

or to

$$\frac{\mu^* - \mu}{kT} = \ln\frac{z^*}{z} = \ln\frac{\rho^*}{\rho} + \ln\frac{\gamma^*}{\gamma} \tag{20}$$

where, from eq. 19

$$\begin{aligned} \ln\frac{\gamma^*}{\gamma} = \frac{\beta_1(\rho - \rho^*)}{1 + 2b_2\rho^*} &+ \frac{(\beta_2 - 4b_2^3\rho^*)(\rho - \rho^*)^2}{(1 + 2b_2\rho^*)^3} \\ &+ \frac{\{\beta_3 + [8b_2b_4 + (80/3)b_2^4 - 18b_3^2]\rho^* + (32/3)b_2^5\rho^{*2}\}(\rho - \rho^*)^3}{(1 + 2b_2\rho^*)^5} + \cdots \end{aligned} \tag{21}$$

It is of course understood in eq. 14–21 that the b_j's and β_k's all refer to the activity set $\mathbf{z}^*$.

Equations 18 and 20 give Π and $\mu^* - \mu$ in terms of the properties of the $\mathbf{z}^*$ solution and of the concentration ρ of solute on the other ($\mathbf{z}$) side of the membrane. However, no other properties of the $\mathbf{z}$ solution are involved. For example, the membrane potential and the concentrations ρ_2, ρ_3, . . . are not discussed.

To be more specific, Π and $\mu^* - \mu$ are determined by ρ, ρ^* and the forces between solute molecules in the $\mathbf{z}^*$ solution (through the potentials of average

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(6) The argument is formally the same as that given in eqs. (37.91)–(37.102) of reference 5 for a one-component gas (*i.e.*, for the special case $z_2^* = z_3^* = \ldots = 0$).

force $w^{(2)}(\mathbf{z}^*), w^{(3)}(\mathbf{z}^*), \ldots$). If these potentials are known or can be approximated, Π and $\mu^* - \mu$ may be calculated. On the other hand, experimental measurement of Π can provide information concerning the $w^{(m)}(\mathbf{z}^*)$. It should be emphasized that the potentials of average force here do *not* refer to the solute at infinite dilution (as in the usual osmotic pressure situation[1,3], $\rho^* = 0$) but rather to the finite concentration ρ^*. Also, the expansions in eq. 18 and 20 are not restricted to small values of ρ^* since they have been developed in powers of $\rho - \rho^*$.

Suppose Π is measured experimentally for a fixed $\mathbf{z}^*$ solution (including ρ^*) but different values of ρ. Then we see from eq. 18 that if the data are plotted with $\Pi/(\rho - \rho^*)kT$ as ordinate and $\rho - \rho^*$ as abscissa, the intercept on the ordinate is $[1 + 2b_2(\mathbf{z}^*)\cdot\rho^*]^{-1}$ and the slope at $\rho - \rho^* = 0$ is

$$\frac{-b_2(\mathbf{z}^*) + \rho^*[2b_2(\mathbf{z}^*)^2 - 3b_3(\mathbf{z}^*)]}{[1 + 2b_2(\mathbf{z}^*)\rho^*]^3} \qquad (22)$$

In principle, if b_2 is found from the intercept, b_3 may then be deduced from the slope It should be noted that an extrapolation to $\rho - \rho^* = 0$ is in general not required in order to find the intercept, for values of ρ can be chosen so that Π and $\rho - \rho^*$ take on both positive and negative values in the neighborhood of $\rho - \rho^* = 0$.

From the point of view of molecular theory, $b_2(\mathbf{z}^*)$ calculated from the intercept is related to $w^{(2)}(\mathbf{z}^*)$ through eq. 7 and 8. Explicitly

$$2Vb_2 = \int e^{-w^{(2)}/kT}\mathrm{d}(1)\mathrm{d}(2) - V^2 \qquad (23)$$

Let the subscript x refer to spatial coördinates and θ to rotational (internal and external) coördinates.[5] Define the spatial potential of average force $w_{\mathrm{x}}{}^{(2)}$ by

$$e^{-w_{\mathrm{x}}{}^{(2)}/kT} = \int e^{-w^{(2)}/kT}\mathrm{d}(1)_\theta\mathrm{d}(2)_\theta \qquad (24)$$

Then

$$2Vb_2 = \int e^{-w_{\mathrm{x}}{}^{(2)}/kT}\mathrm{d}(1)_{\mathrm{x}}\mathrm{d}(2)_{\mathrm{x}} - V^2$$

or, finally

$$b_2(\mathbf{z}^*) = 2\pi\int_0^\infty \left\{\exp\left[-\frac{w_{\mathrm{x}}{}^{(2)}(r, \mathbf{z}^*)}{kT}\right] - 1\right\} r^2\,\mathrm{d}r \qquad (25)$$

where r is the distance between the centers of mass of two solute molecules.

From the point of view of thermodynamics, the slope and intercept also have an obvious interpretation. Define

$$\begin{aligned}\pi(\mathbf{z}) &= p(z, z_2^*, z_3^*, \cdots) - p(0, z_2^*, z_3^*, \cdots)\\ \pi(\mathbf{z}^*) &= p(z^*, z_2^*, z_3^*, \cdots) - p(0, z_2^*, z_3^*, \cdots)\end{aligned} \qquad (26)$$

That is, these are conventional osmotic pressures. Then

$$\Pi = \pi(\mathbf{z}) - \pi(\mathbf{z}^*) = p(\mathbf{z}) - p(\mathbf{z}^*)$$

If we expand $\pi(\rho)$ in a Taylor series about $\rho = \rho^*$, we have

$$\Pi = \pi(\rho) - \pi(\rho^*) = (\rho - \rho^*)\left(\frac{\partial\pi}{\partial\rho}\right)_{\rho=\rho^*} + \frac{1}{2}(\rho - \rho^*)^2\left(\frac{\partial^2\pi}{\partial\rho^2}\right)_{\rho=\rho^*} + \cdots \qquad (27)$$

Comparison with eq. 18 yields

$$\text{intercept} = \frac{1}{1 + 2b_2(\mathbf{z}^*)\rho^*} = \frac{1}{kT}\left(\frac{\partial\pi}{\partial\rho}\right)_{=\rho^*, T, z_2^*, z_3^*, \ldots} \qquad (28)$$

$$= \frac{\rho^*}{kT}\left(\frac{\partial\mu}{\partial\rho}\right)_{\rho=\rho^*, T, z_2^*, z_3^*, \ldots} \qquad (29)$$

and

$$\text{slope} = \text{eq. } 22 = \frac{1}{2kT}\left(\frac{\partial^2\pi}{\partial\rho^2}\right)_{\rho=\rho^*, T, z_2^*, z_3^*, \ldots} \qquad (30)$$

where[7] eq. 29 follows from thermodynamics or from eq. 20. Thus a single measurement in the conventional osmotic system $\rho^*|0$ (vertical line = membrane) gives $\pi(\rho^*)$ while the intercept in the plot described above for the osmotic system $\rho^*|\rho$ provides $\partial\pi/\partial\rho$ and the slope $\partial^2\pi/\partial\rho^2$, both at $\rho = \rho^*$.

It seems quite possible that measurement of Π in the osmotic system $\rho^*|\rho$ may furnish a more accurate, though more elaborate, method of molecular weight determination than use of the conventional system $\rho^*|0$. Thus, suppose the intercept in eq. 28 (using now the weight concentration c instead of the number concentration ρ) is obtained for several values of c^*. Then a plot of intercept *versus* c^*, extrapolated to $c^* = 0$, will itself give an intercept which is

$$\frac{1}{RT}\left(\frac{\partial\pi}{\partial c}\right)_{c=0} = \frac{1}{M} \qquad (31)$$

where M is the molecular weight.

When ρ and ρ^* are both small, expansions of Π and γ^*/γ in powers of ρ and ρ^* are of interest. We find, from eq. 18

$$\begin{aligned}\frac{\Pi}{kT} = \rho - \rho^* + B_2(\rho - \rho^*)(\rho + \rho^*) + (\rho - \rho^*)(B_3\rho^2 + {}&\\ b_3\rho\rho^* + b_3\rho^{*2}) + (\rho - \rho^*)[B_4\rho^3 + (2b_4 - B_4 - {}&\\ 8b_2^3)\rho^2\rho^* - b_4\rho\rho^{*2} - b_4\rho^{*3}] + \cdots&\end{aligned} \qquad (32)$$

and from eq. 19

$$\begin{aligned}\frac{\gamma^*}{\gamma} = 1 + 2b_2(\rho - \rho^*) + (\rho - \rho^*)[(3b_3 - {}&\\ 4b_2^2)\rho - 3b_3\rho^*] + (\rho - \rho^*)[(4b_4 - 18b_2b_3 + 16b_2^3)\rho^2 + {}&\\ (-8b_4 + 18b_2b_3 - 8b_2^3)\rho\rho^* + 4b_4\rho^{*2}] + \ldots&\end{aligned} \qquad (33)$$

In both of these equations, the B_n's and b_j's are evaluated at $\mathbf{z}^*$ (and are therefore themselves functions of ρ^*).

Donnan Method.—We supplement the above discussion by a simple and approximate treatment of the same problem using the method of Donnan.[3] Let the $\mathbf{z}^*$ side of the system contain an electrolyte solution, the j-th species of which has a valence q_j and a concentration ρ_j^*. In addition, on this side, is solute with valence q and concentration ρ^*. On the other ($\mathbf{z}$) side the j-th species has a concentration ρ_j and the solute a concentration ρ. Let $\Psi = \psi(\mathbf{z}) - \psi(\mathbf{z}^*)$ be the membrane potential. Assuming the solutions are dilute and all ions are point charges (so pV terms can be ignored), we have, from the equality of electrochemical potentials on the two sides of the membrane

$$\rho_j = \rho_j^* e^{-yq_j} = \rho_j^*\left(1 - yq_j + \frac{1}{2}y^2q_j^2 - \cdots\right) \qquad (34)$$

where $y = \epsilon\Psi/kT$ and $\epsilon = |\text{electronic charge}|$ We also have the neutrality conditions

$$\sum_j \rho_j^* q_j + \rho^* q = 0 \qquad (35)$$

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[7] The relation between the "cluster integral" b_2 and $\partial\pi/\partial\rho$ has been derived previously by Kirkwood and Buff from fluctuation theory: J. G. Kirkwood and F. P. Buff, *J. Chem. Phys.*, **19**, 774 (1951). See also eq. 25 above and eq. 21.4 and 40.45 of ref. 5.

and

$$\sum_j \rho_j q_j + \rho q = 0 \tag{36}$$

The osmotic pressure is

$$\frac{\Pi}{kT} = \left(\sum_j \rho_j + \rho\right) - \left(\sum_j \rho_j^* + \rho^*\right) \tag{37}$$

Using eq. 34 and 35 we find

$$\sum_j \rho_j = \sum_j \rho_j^* + y\rho^* q + \frac{1}{2} y^2 \sum_j \rho_j^* q_j^2 + \cdots$$

so that

$$\frac{\Pi}{kT} = \rho - \rho^* + y\rho^* q + \frac{1}{2} y^2 \sum_j \rho_j^* q_j^2 + \cdots \tag{38}$$

Substitution of eq. 34 in eq. 36 leads to

$$y = \frac{\epsilon\Psi}{kT} = \frac{(\rho - \rho^*)q}{\sum_j \rho_j^* q_j^2} + \cdots \tag{39}$$

Finally, if we put eq. 39 in eq. 38

$$\frac{\Pi}{kT} = \rho - \rho^* + \frac{q^2(\rho - \rho^*)(\rho + \rho^*)}{2\sum_j \rho_j^* q_j^2} + \cdots \tag{40}$$

Expansion in powers of ρ and ρ^* is appropriate here because the solutions are assumed dilute.

III. Discussion

For approximate numerical purposes, the pressure difference Π for a system $\rho^*|\rho$, as in active transport, might be computed from the leading term in eq. 18. For example, if we treat all ions as point charges and use the Debye–Hückel $w_x^{(2)}(\mathbf{z}^*)$ in eq. 25

$$\begin{aligned} e^{-w_x^{(2)}/kT} - 1 &= -\frac{w_x^{(2)}}{kT} + \cdots \\ &= -\frac{q^2\epsilon^2 e^{-\kappa r}}{DrkT} + \cdots \end{aligned} \tag{41}$$

where

$$\kappa^2 = \frac{4\pi\epsilon^2\left(\sum_j \rho_j^* q_j^2 + \rho^* q^2\right)}{DkT} \tag{42}$$

Then

$$b_2(\mathbf{z}^*) = -\frac{q^2}{2\left(\sum_j \rho_j^* q_j^2 + \rho^* q^2\right)} \tag{43}$$

and

$$\frac{\Pi}{kT} = \frac{(\rho - \rho^*)\left(\sum_j \rho_j^* q_j^2 + \rho^* q^2\right)}{\sum_j \rho_j^* q_j^2} \tag{44}$$

Also, from eq. 21

$$\ln \frac{\gamma^*}{\gamma} = -\frac{q^2(\rho - \rho^*)}{\sum_j \rho_j^* q_j^2} \tag{45}$$

For comparison with the Donnan results above, we use eq. 32 and 43 and retain only quadratic terms in ρ and ρ^* (noting that ρ^* occurs in b_2). The result is identical with eq. 40. The expression for γ^*/γ in eq. 45 is already in the form of an expansion in powers of ρ and ρ^* (to linear terms). Equation 45 is consistent with the leading Donnan term in eq. 39 since, as we have already pointed out, γ^*/γ includes the contribution of the potentials $\psi(\mathbf{z})$ and $\psi(\mathbf{z}^*)$. Thus, with this simplest of models, the McMillan–Mayer method, using the Debye–Hückel potential of average force, gives leading terms which agree with those of the Donnan method, calculated *without* employment of the Debye–Hückel theory. As we have shown elsewhere,[3] the basic reason for this agreement is that both methods take electrical neutrality into account properly.

In treating the system[3] $\rho^*|0$, both McMillan–Mayer and Donnan methods use expansions in powers of ρ^*. In the present application, however, the McMillan–Mayer method has a distinct advantage in that, as remarked above, ρ^* need not be small, since expansions in power of $\rho - \rho^*$ are available. On the other hand, although in principle the Donnan method could be made rigorous for any ρ^*, in its present state of development and for practical purposes it is restricted to expansions in powers of ρ and ρ^*.

Appendix

We wish to point out here that, in addition to eq. 1, another general equation exists which might also find use in studies on systems of the type under discussion. This equation for Π has the advantage of not being an expansion but the disadvantages of being approximate, in general, and of involving, relatively inextricably, the properties of the **z** solution as well as the **z*** solution. If we make the so-called superposition approximation for the **z*** state, that is, that

$$w^{(\mathrm{m})}(\mathbf{z}^*) - \Sigma w^{(1)}(\mathbf{z}^*) = \Sigma w^{(2)}(\mathbf{z}^*) \tag{46}$$

where the first sum is over all molecules in the set **m** and the second sum is over all pairs of molecules in the set **m**, then[8]

$$\begin{aligned} \frac{p(\mathbf{z}) - p(\mathbf{z}^*)}{kT} &= \sum_s \left(\rho_s - \frac{\gamma_s^*}{\gamma_s}\rho_s^*\right) \\ &- \frac{1}{6VkT}\sum_{s,s'} \Bigg\{ \left(\rho_s - \frac{\gamma_s^*}{\gamma_s}\rho_s^*\right)\left(\rho_{s'} - \frac{\gamma_{s'}^*}{\gamma_{s'}}\rho_{s'}^*\right) \times \\ &\int r\frac{\partial w_{ss'}^{(2)}(\mathbf{z}^*)}{\partial r} \exp\left[-\frac{w_{ss'}^{(2)}(\mathbf{z})}{kT}\right] \mathrm{d}(1_s)\mathrm{d}(1_{s'}) \Bigg\} \end{aligned} \tag{47}$$

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(8) See eq. 40.110 of ref. 5.