SOME CONSEQUENCES OF THE THEORY OF MEMBRANE EQUILIBRIA.

BY DAVID I. HITCHCOCK.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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The theory of membrane equilibria, as developed by Donnan,\(^1\) has been shown by Loeb\(^2\) to be the basis for a satisfactory explanation of the colloidal behavior of proteins. It seemed of interest to formulate, in more detail than Donnan has done, some of the consequences of this theory as applied to systems simpler than those studied by Loeb; namely, systems containing a non-diffusible base, acid, or ampholyte of known ionization constants.

The theory applies wherever one type of ion in a mixture of electrolytes is prevented from diffusing freely to all parts of the system, by some constraint such as a membrane impermeable to it but permeable to other ions. Donnan showed that in such systems there will, in general, be an unequal distribution of the diffusible ions between the solutions on the two sides of the membrane. The ratio of the activities of any diffusible ion in the two solutions must be equal to that of any other diffusible ion of the same sign and valence, and equal to the inverse ratio of the activities of any ion of the opposite sign but of the same valence. Where ratios of ions of different valence are compared, the same law holds if each ratio is raised to a power equal to the reciprocal of the valence of the ion.

As a consequence of this unequal distribution of ions, Donnan showed that a difference in electrical potential must exist between two such solutions, and that its magnitude must be given by the Nernst formula, being proportional to the logarithm of the ion ratio. He pointed out that a further consequence of this unequal distribution

\(^1\) Donnan, F. G., a, Z. Elektrochem., 1911, xvii, 572; b, Chem. Rev., 1924, i, 73.
must be a difference in osmotic pressure between the two solutions. Such differences in electrical potential and in osmotic pressure were measured by Loeb in experiments with various proteins, and were shown to be quantitatively in accord with the theory.

A derivation of Donnan's equations which is, perhaps, more rigorous, has been given by Hückel. He pointed out that the original equations in terms of ion concentrations are exact for ideal dilute solutions, but that for real solutions the total concentration of each ion must be multiplied by its activity coefficient to give the ionic activity. This was previously recognized by Donnan. Hückel also pointed out that, for non-ideal solutions, each concentration term in the osmotic pressure equation must be multiplied by an osmotic coefficient which is a function of the activity coefficient of the water in the solution.

In the equations which follow, ion concentrations are used in place of activities, with the understanding that the equations can be exact only for solutions so dilute that these quantities become identical. The equations are also based on the assumption of complete ionization for strong electrolytes. The cases to be considered are the addition of a strong acid or base to a system containing a weak non-diffusible base, acid, or ampholyte. The equations are based on (1) Donnan's equation for the ion ratio, (2) the constancy of the ion product for water, (3) the validity of the law of mass action for the ionization of the non-diffusible electrolyte, (4) the principle of electrical neutrality for each solution.

The equilibrium state for such systems may be represented by the following diagram, in which the capital letters represent molecular or ionic species, and the small letters their molar concentrations. The membrane is represented by the vertical line. The non-diffusible species are R, R^+, R^-.

3 Hückel, E., Zsigmondy Festschrift, Dresden, 1925, 204. (Ergänzungsband, Kolloid-Z., xxxvi, 204.)
The condition of electrical neutrality is implied in the values for the concentration of the ion $A^{p-}$. The other conditions are the Donnan equation, the ion product equation, and the ionization equations, which are as follows:

$$k_s = xu = yv;$$

$$k_a = \frac{yw}{c - w - z};$$

$$k_b = \frac{w}{c - w - z},$$

\(\lambda\) is Donnan's symbol for the ion ratio; \(k_s, k_a,\) and \(k_b\) have their usual significance. To these should be added the osmotic pressure equation

$$P = RTe$$

where \(e\) represents the difference in total concentration of molecules and ions in the two solutions. The equations which follow are all derived from these five, as applied to the special cases indicated.

**I. Addition of a Monobasic Acid to a Non-Diffusible Base.**

Here the values of \(w, k_s, m,\) and \(n\) are zero, while \(p = 1\). Equation (1) becomes

$$\lambda = \frac{x}{y} = \frac{u}{u} = \frac{y + z - v}{z - u} = \frac{y + z}{z},$$

(The last member results from the application of the laws of proportion to the two preceding it.) Since \(x = \lambda y\), this may be written
For this case, by substituting \( \frac{k_w}{y} \) for \( v \) and \( K \) for \( \frac{k_w}{k_b} \), equation (4) becomes

\[
\frac{x}{c - z} = \frac{y}{K} \quad \text{or} \quad r = \frac{cy}{K + y}
\]

From equations (7) and (8),

\[
\lambda = \sqrt{1 + \frac{c}{K + y}}
\]

From this it appears that as the concentration of hydrogen ion is increased the value of the ion ratio must always decrease, approaching unity as a limit. Accordingly the initial rise in the membrane potential which Loeb observed on adding acid to isoelectric protein would not occur in the case of a non-amphoteric base.

This is not in agreement with the conclusion reached by Donnan\(^{1,2} \) for this case, because he assumed that in the absence of acid the ionization of the base could be neglected. His reasoning would be strictly correct for the case of a non-diffusible substance forming no ions whatever in pure water, but forming ions by combination with H\(^+\) ion from the added acid. This condition is approximately fulfilled by the amphoteric proteins studied by Loeb, which furnish no excess of positive or negative ions at their isoelectric points, and hence do show the initial rise of the membrane potential on the addition of acid. If, however, a non-amphoteric base is appreciably ionized in pure water, its membrane potential should have a high value and could only be decreased by the addition of acid.

This can be made clear by proving that the derivatives \( \frac{d\lambda}{dx} \) and \( \frac{d\lambda}{dy} \) must always be negative. Inspection of equation (9) shows that \( \frac{d\lambda}{dy} \) is always negative. Since \( \lambda = \frac{x}{y} \), \( \frac{d\lambda}{dy} = \frac{1}{y} \frac{dx}{dy} - \frac{x}{y^2} \). Since \( \frac{d\lambda}{dy} \) is negative, \( \frac{dx}{dy} > \frac{x}{y} \). But \( \frac{d\lambda}{dx} = \frac{1}{y} - \frac{x}{y^2} \frac{dy}{dx} \). If this derivative is posi-
tive or zero, then \( \frac{1}{\gamma} \frac{d\gamma}{d\lambda} \geq \frac{x}{\gamma} \frac{dx}{d\lambda} \) and \( \frac{dx}{d\lambda} \geq \frac{x}{\gamma} \). But this has just been shown to be false, and therefore \( \frac{d\lambda}{dx} \) must be negative. Moreover \( \frac{dx}{d\lambda} \) and \( \frac{dy}{dy} \) are always positive, since they are quotients of the two negative derivatives \( \frac{d\lambda}{dx} \) and \( \frac{d\lambda}{dy} \).

For the special case when the acid and base are present in equivalent amounts, equation (9) assumes a simpler form. If the volumes of the two solutions are kept equal, this condition of equivalence gives the equation

\[ x + y + z = c + u + v. \]

Since the solution containing such a salt is acid, the value of \( v \) is negligible as compared with \( y \), and since \( \lambda > 1 \), \( u \) is even smaller than \( v \) and may likewise be neglected. Thus the equation becomes, approximately,

\[ c = x + y + z. \quad (10) \]

From equations (8) and (10),

\[ c = \frac{(K + y)z - y(x + y)}{y} = x + y + z, \quad \text{and} \quad z = \frac{y(x + y)}{K}. \]

From equation (6), \( z = \frac{x^2 - y}{y} = \frac{(x - y)(x + y)}{y} \). Equating these values of \( z, \frac{y}{K} = \frac{x - y}{y} = \lambda - 1, \) and

\[ \lambda = 1 + \frac{y}{K}. \quad (11) \]

The osmotic pressure difference in the general case of a system containing a non-diffusible base and a strong acid should be given, as Donnan\(^1\)\(^b\) has pointed out, by equation (5) in the form

\[ P = RT\epsilon = RT(c + 2y + z - 2x). \quad (12) \]

Thus \( \epsilon - c \) represents the difference between the concentrations of diffusible ions in the two solutions, and it is on this difference that the
changes in osmotic pressure with pH are dependent. The nature of these changes with pH may be inferred from the sign of the derivative of $e - c$.

From equation (6), $y + z = \frac{x}{y}$, and $\lambda = \frac{x}{y}$; hence

$$e - c = y + z - 2x + y = y(\lambda - 1),$$

(13)

$$\frac{d(e - c)}{dy} = \frac{de}{dy} = (\lambda - 1) \left( \lambda - 1 + 2y \frac{dx}{dy} \right),$$

(14)

From equation (9) it is evident that $\lambda > 1$ (except that when $y$ becomes infinite, $\lambda = 1$); hence $\lambda - 1$ is positive. But since $\frac{dx}{dy}$ is negative, the value of $\frac{de}{dy}$ will be positive if $\lambda - 1 > -2y \frac{dx}{dy}$, negative if $\lambda - 1 < -2y \frac{dx}{dy}$, and zero if $\lambda - 1 = -2y \frac{dx}{dy}$. From equation (9), by differentiation,

$$\frac{dx}{dy} = -\frac{c}{2y(K + y)^2}.$$  

Therefore $\frac{de}{dy}$ is positive when $\lambda - 1 > \frac{cy}{\lambda (K + y)^2}$. But since, by equation (9),

$$\frac{cy}{(K + y)^2} = \frac{(\lambda^2 - 1)y}{K + y},$$  

this condition becomes $(\lambda - 1)\lambda

> \frac{(\lambda^2 - 1)y}{K + y}, \frac{(\lambda + 1)y}{K + y} > \frac{\lambda + 1}{\lambda} > \frac{1}{\lambda}$ or $\lambda > \frac{y}{K}$. Also

$\frac{de}{dy}$ is negative when $\lambda < \frac{y}{K}$ and zero when $\lambda = \frac{y}{K}$. In the latter case

$y = \sqrt{Kx}$. This is the condition for a maximum and not a minimum of osmotic pressure, for when $y$ is small, $\lambda$ has its largest value, as has already been pointed out. Hence an increase in $y$ will cause $\frac{de}{dy}$ and hence $\frac{dP}{dy}$ to have decreasing positive values, to pass through zero, and finally to have increasing negative values. Thus a rise and fall of the osmotic pressure with increasing acid, such as Loeb observed
with proteins, should also be found in the case of a non-amphoteric base.

II. Addition of a Monoacid Base to a Non-Diffusible Acid.

In this case the equations assume a form similar to those already given if negative ions are substituted for positive, \( \frac{1}{\lambda} \) for \( \lambda \), and \( k_a \) for \( k_b \). They may be written as follows:

\[
\frac{1}{\lambda} = \frac{u}{v} - \frac{y}{z} = \frac{v + w - y}{u - z} = \frac{v + w}{u} ;
\]

\[
\frac{1}{\lambda} = \sqrt{1 + \frac{w}{v}} ;
\]

\[
\frac{w}{c - w} = \frac{v}{K'} \quad \text{or} \quad w = \frac{c v}{K' + v} ;
\]

\[
\frac{1}{\lambda} = \sqrt{1 + \frac{c}{K' + v}} .
\]

Here \( K' \) is written in place of \( \frac{k_{ao}}{k_a} \). Equation (9a) shows that as the alkalinity is increased the value of \( \frac{1}{\lambda} \) must decrease, or \( \lambda \) must increase, approaching 1 as a limit. The remaining equations for this case will be omitted, as they are exactly similar to those for the non-diffusible base if the above substitutions are made.

III. Addition of a Monobasic Acid to a Non-Diffusible Ampholyte.

In this case the values of \( m \) and \( n \) are zero, while \( p = 1 \). Equation (1) becomes

\[
\lambda = \frac{x}{y} = \frac{y + z - w - v}{x - w} = \frac{y + z - w}{x} .
\]

Substituting \( \lambda y \) for \( x \),

\[
\lambda = \sqrt{1 + \frac{z - w}{y}} .
\]
By solving equations (3) and (4) for \( z \) and \( w \), substituting \( \frac{k_a}{y} \) for \( v \) and \( \frac{K}{v} \) for \( w \), it follows that

\[
z = \frac{k_a y}{k_b y + k_a + k_c} = \frac{c y^2}{y^2 + Ky + Kk_a}
\]

(17)

and

\[
w = \frac{k_c y}{k_b y + k_c + k_a} = \frac{K k_a}{y^2 + Ky + Kk_a}
\]

(18)

Hence

\[
z - w = \frac{c(y^2 - I^3)}{y^2 + Ky + I^2}
\]

(19)

if \( I^3 = Kk_a = \frac{k_a k_w}{k_b} \), which means that \( I \) is the value of \( y \) at the isoelectric point of the ampholyte. From equations 16 and 19,

\[
\lambda = \sqrt{1 + \frac{c(y^2 - I^3)}{y(y^2 + Ky + I^2)}}
\]

(20)

(This becomes identical with equation (9) if \( k_a = 0 \).) From this it appears that at the isoelectric point, where \( y = I, \lambda = 1 \). On the acid side, where \( y > I, \lambda > 1 \), but in excess acid, where \( I^3 \) becomes negligible in comparison to \( y^2 \), then

\[
\lambda = \sqrt{1 + \frac{c}{Ky + y}}
\]

which is equation (9). Hence in excess acid the value of \( \lambda \) decreases with increase in \( y \) and again approaches 1 as a limit. (It is to be remembered that \( \lambda \) cannot be negative, since it is the ratio of two positive quantities, \( x \) and \( y \).) Thus as increasing amounts of acid are added to the pure ampholyte solution, the value of \( \lambda \) must rise from 1 to a maximum and again decrease toward 1. This predicts a rise and fall of the membrane potentials, such as were observed by Loeb when increasing amounts of acid were added to isoelectric protein solutions.
The value of $y$ corresponding to the maximum value of $\lambda$ can be calculated by differentiating equation (20) and placing the derivative equal to zero. The resulting equation of condition is

$$y^4 - 1^4 = 4Iy^3 + 2I^2Ky,$$  \hspace{1cm} (21)

and this can be solved graphically$^4$ for $y$ if the values of $K$ and $I$ (or of $k_a, k_b$, and $k_c$) are known.

A rough test of this equation was made in the case of gelatin. It was shown in a previous paper$^5$ that the combination curve of gelatin with hydrochloric acid was approximately equivalent to the ionization curve of a monoacid base having $K = \frac{k_w}{k_b} = 2.4 \times 10^{-4}$. Using this value, together with $I = 2 \times 10^{-5}$ (pH = 4.7), a solution of equation (20) gave the value $y = 6.7 \times 10^{-2}$, or pH = 4.17. Loeb's observed maximum value of the membrane potential occurred at pH 4.0.

The osmotic pressure may be calculated, as before, by taking the algebraic sum of the concentrations.

$$P = RTe = RT(c + 2y + s - w - 2z).$$ \hspace{1cm} (22)

By combining this with equation (16), it follows that

$$e - e = y (\lambda - 1)^2,$$

which is the same as equation (13).

With regard to the location of the maximum of osmotic pressure, the reasoning already given for the case of a non-amphoteric base will apply if the maximum occurs at such a high H$^+$ ion concentration that $w = 0$, a condition which makes equation (16) equivalent to equations (7) and (9). If this is the case, then $y = \sqrt{Kx}$, log $K = 2$ log $y - \log x$, or $pK = 2py - px$ (where $p$ is Sörensken's symbol for $- \log$).

Loeb's data for gelatin chloride show that the maximum osmotic pressure was observed where $py = 3.33$ and $px = 2.87$. Hence $pK = 6.66 - 2.87 = 3.79$, while the value obtained from the writer's combination curve for gelatin and hydrochloric acid was $pK = 3.625$.

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The maximum value of Loeb's "Donnan correction" or calculated osmotic pressure, which depends on \( \ln (\lambda - 1)^2 \), occurred where \( \psi y = 3.25 \) and \( \psi x = 2.81 \); hence the value for \( \psi K \) should be \( 6.50 - 2.81 = 3.69 \). This indicates that the simplified theory applies fairly well even in the case of a complex ampholyte such as gelatin.

The complete equation of condition for the maximum of osmotic pressure for an ampholyte with added acid is so complex as to be practically useless. It may be written

\[
\lambda(\lambda - 1) = \frac{c}{\psi} \frac{y^4 - 4I^2y^2 - 2I^2Ky - I^4}{(\gamma^2 + K\gamma + I^2)^2}.
\]

**IV. Addition of a Monoacid Base to a Non-Diffusible Ampholyte.**

In this case \( q = 1 \), while the concentration of the ion \( A^- \) is zero. Hence \( w + v = y + z + n \) and \( u = x + m \). Equation (1) becomes

\[
\frac{1}{\lambda} = \frac{u}{x} = \frac{w}{v} = \frac{y}{z} = \frac{u + v - y - z}{u - x} = \frac{v + w - z}{u}.
\]

Substituting \( \frac{v}{\lambda} \) for \( u \),

\[
\frac{1}{\lambda} = \sqrt{\frac{1 + (w - z)}{v}} = \sqrt{\frac{1 + (w - z)\gamma}{k_\psi}}.
\]

Combining this with equation (19),

\[
\frac{1}{\lambda} = \sqrt{\frac{1 + cy(I^2 - y^2)}{k_\psi(y^2 + Ky + I^2)}}.
\]

(This becomes identical with equation (9a) if \( k_\psi = 0 \).) At the isoelectric point, \( y = I \), and \( \frac{1}{\lambda} = 1 = \lambda \). With increasing alkali, \( y \) becomes smaller than \( I \), so that the fraction in equation (25) has finite positive values and \( \frac{1}{\lambda} > 1 \) or \( \lambda < 1 \). With more alkali \( y^2 \) becomes negligible, and equation (25) takes the form

\[
\frac{1}{\lambda} = \sqrt{1 + \frac{k_\psi y}{k_\psi(k_n + y)}},
\]
which is identical with equation (9a). Finally, as \( y \) is decreased still more, \( \frac{1}{\lambda} \) decreases, approaching 1 as a limit. Thus with increasing alkali the values of \( \lambda \), starting at 1, decrease, pass through a minimum, and finally increase, approaching 1.

The position of the minimum can be calculated as before by differentiation. Again the calculation involves the graphical solution of the equation of condition, which is

\[
y^4 + 2Ky^3 + 4Ky^4 = I^4.
\]

This equation probably could not be applied, even roughly, to experiments with proteins, since their titration curves show that they have, in general, two sets of acid groups, or two mean acidic ionization constants.

In this case the osmotic pressure is given by

\[
P = RT\varepsilon = RT(c + 2v + w - z - 2u).
\]

From equation (24),

\[
v + w - z = \frac{y}{\lambda^3}.
\]

Hence

\[
\varepsilon - c = \varepsilon \left( \frac{1}{\lambda^3} - \frac{2}{\lambda} + 1 \right) = \varepsilon \left( \frac{1}{\lambda} - 1 \right)^2.
\]

Again the complete equation of condition for the maximum of osmotic pressure is very complicated, but if the maximum occurs at a sufficiently high alkalinity so that \( z \) becomes negligible, the calculation may be made as for a non-amphoteric acid. Thus equation (24) becomes identical with equations (7a) and (9a). By reasoning similar to that in Section I it can be shown that for this case the condition for the maximum of osmotic pressure is that \( \frac{y}{K} \cdot \frac{y}{K} = k = \sqrt{k_a} \).

V. Addition of Acid and Salt to a Non-Diffusible Base.

In systems containing ions of different valence, the equations differ in form according to the valence of the ions. Considering first the case of a non-diffusible base ROH with the addition of a monobasic acid, as HCl, and salts having the same anion but cations of variable
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valence $q$, the conditions imposed upon equation (1) are that $p = 1$ and $w = 0$. Accordingly

$$\lambda = \frac{x}{y} = \frac{v}{u} = \sqrt[2m+n]{\frac{y+z+n-v}{x+m-u}} = \frac{y+z+n}{x+m}.$$  (29)

For the special cases where there is no salt added, and where the salt has a cation of valence 1, 2, or 3, the following equations give the value of the ion ratio:

- **ROH, HCl:**
  $$\lambda = \sqrt[1+z-w]/y = \sqrt[1+z-w]/y.$$  (7)

- **ROH, HCl, NaCl:**
  $$\lambda = \sqrt[2+y+n]/y = \sqrt[2+y+n]/y.$$  (30)

- **ROH, HCl, CaCl$_2$:**
  $$\lambda = \sqrt[3+y+l]/y = \sqrt[3+y+l]/y.$$  (31)

- **ROH, HCl, LaCl$_3$:**
  $$\lambda = \sqrt[3+y+l]/y = \sqrt[3+y+l]/y.$$  (32)

If the values of $\lambda$ for these systems are compared for identical values of $y$ and $n$, it will be observed that the addition of any salt decreases the value of $\lambda$. This is in accord with the observations of Loeb in the case of gelatin and other proteins. Loeb also found, in comparing the effect of the three salts mentioned at the same equivalent concentrations and the same pH, that the values of $\lambda$ were identical. Equations (30), (31), and (32) can be identical only when $\lambda = 1$ or $m = n$. It is possible that some differences due to the valence of the cation might be detected with more refined experimental methods.

If sulfuric acid and sulfates are used in place of hydrochloric acid and chlorides, the value of $p$ is 2, and equation (1) becomes

$$\lambda = \frac{x}{y} = \frac{v}{u} = \sqrt[2m+n]{\frac{y+z+n-v}{x+m-u}}.$$  (33)

If the hydroxyl ion concentrations are neglected, the following equations should give the ion ratio for the special cases indicated:

- **ROH, H$_2$SO$_4$:**
  $$\lambda = \sqrt[1+z-w]/y = \sqrt[1+z-w]/y.$$  (34)
ROH, H₂SO₄, Na₂SO₄: \[ \lambda = \sqrt[3]{\frac{1 + \frac{s}{y + n}}{y + n}} = \sqrt[3]{\frac{p + \frac{z + n}{y + n}}{y + n}}; \] (35)

ROH, H₂SO₄, MgSO₄: \[ \lambda = \sqrt[3]{\frac{p + \frac{z + n}{y + \lambda n}}{y + \lambda n}} = \sqrt[3]{\frac{p + \frac{z + n}{y + \sqrt{mn}}}{y + \sqrt{mn}}}; \] (36)

ROH, H₂SO₄, La₂(SO₄)₃: \[ \lambda = \sqrt[3]{\frac{p + \frac{z + n}{y + \lambda^2 n}}{y + \lambda^2 n}} = \sqrt[3]{\frac{p + \frac{z + n}{y + \sqrt{m^2 n}}}{y + \sqrt{m^2 n}}}. \] (37)

These equations are similar to the preceding group in the expressions under the radical signs, and accordingly here again there should theoretically be some differences in \( \lambda \) due to the valence of the cation. Such differences, however, must be slight in comparison with the difference caused by taking the cube root instead of the square root, which is due to the valence of the anion. This difference, as Loeb has pointed out, makes the value of the membrane potential in the case of the bivalent anion just two-thirds of that observed in the case of the univalent anion.

The eight equations just mentioned assume the following general form, if \( p \) represents the valence of the common anion and \( q \) the valence of the cation of the salt:

\[ \lambda = \sqrt[3]{\frac{p + \frac{s + n}{y + \lambda^q - 1 n}}{y + \lambda^q - 1 n}} = \sqrt[3]{\frac{p + \frac{s + n}{y + \sqrt{m^q - 1 n}}}{y + \sqrt{m^q - 1 n}}}. \] (38)

**SUMMARY.**

In applying Donnan's theory of membrane equilibria to systems where the non-diffusible ion is furnished by a weak acid, base, or ampholyte, certain new relations have been derived. Equations have been deduced which give the ion ratio and the apparent osmotic pressure as functions of the concentration and ionization constant of the weak electrolyte, and of the hydrogen ion concentration in its solution. The conditions for maximum values of these two properties have been formulated. It is pointed out that the progressive addition of acid to a system containing a non-diffusible weak base should not cause the value of the membrane potential to rise, pass through a maximum, and fall, but should only cause it to diminish. It is shown that the theory predicts slight differences in the effect of salts on the ion ratio in such systems, the effect increasing with the valence of the cation.