The Physical Basis of Osmosis in a Donnan Ionic System

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1 Abstract

The osmotic flow of water is driven by the repulsive force between semipermeable membrane and impermeable solute that creates a pressure drop at the interface between membrane and aqueous solution. An analysis of the physical principles involved is relatively simple for a neutral solute that is absolutely impermeable. Extension to partially permeable neutral solutes is more complicated but has been accomplished. In this paper

the analysis is further extended to include Donnan ionic systems. The electric forces due to the charges on the ions give rise to non-uniform ionic concentrations and trans-membrane electric voltages as well as pressure gradients, and the relationships among all of these Donnan effects have an impact on osmosis. The supplementary effect of an ion pump, acting to prevent the osmotic danger of cell swelling and rupture, is explored.

2 Introduction

The physical mechanism driving osmotic flow is a subject of continuing interest in biology,[1] but the now settled thermodynamic and statistical mechanical description of equilibrium osmotic pressure has been sufficient to satisfy physical chemists and soft matter physicists. When a reservoir of pure water is separated from an aqueous solution by a membrane permeable

to the water molecules but not to those of the solute, and the system is brought to equilibrium, it is observed that the pressure in the solution compartment is higher than in the compartment filled with pure water. The equilibrium pressure *difference* across the membrane is called the osmotic pressure. The initially empirically established relation that the osmotic pressure is equal to the product of RT (gas constant times temperature) and the sufficiently dilute solute molarity of the solution,[2] has long since been well understood as following from the equality of water chemical potentials across the membrane.[3,4] The virial series expansion of osmotic pressure for arbitrary solute concentrations was presented by McMillan and Mayer[5,6] as formally analogous to the virial expansion for the pressure of a real gas. It was Debye, however, who first consistently modeled and correctly analyzed the generation of equilibrium osmotic pres-

sure from the repulsive forces between the impermeable solute molecules and the membrane.[7] (Incidentally, to emphasize our focus in this paper on molecular level mechanism, we will use the Boltzmann constant k_B instead of the gas constant R, and then concentrations are expressed as number of molecules per unit volume. We also use q, the positive electric charge on a proton, in place of the Faraday, which is the electric charge on a mole of protons.)

In cellular physiology, the osmotically driven flow of water into the cell, and how the cell manages to prevent it, receives more attention than the build up of osmotic pressure.[8] In an osmosis experiment, as distinct from an osmotic pressure measurement, the pressures on both sides of the semipermeable membrane are maintained equal, and water is then observed to flow continuously from the reservoir of pure water into a well-stirred

solution compartment. The cells of higher organisms contain macromolecules that cannot pass through the cell membrane, and if there were no mechanism to regulate the osmotic flow of water into the cell, an uncontrolled increase of cell volume would ultimately burst the fragile membrane surrounding it.

Actually, osmotic flow occurs even if the solute is not absolutely impermeable, as long as its permeability through the membrane is more restricted than the passage of water molecules. The well-known Kedem-Katchalsky equations describe this more general situation on the basis of linear response thermodynamics,[9]

$$j_s = -\omega_s k_B T \Delta c_s + c_s(1-\sigma) J_v \tag{1}$$

$$J_v = -L_p(\Delta P - \sigma k_B T \Delta c_s) \tag{2}$$

In the first of these equations "s" refers to the single neutral solute species, and j_s is its flux across the membrane (number of molecules per second per unit area). The dilute solute

concentration difference between the two reservoirs is Δc_s, and c_s (number of molecules per unit volume) is an average of the two reservoir concentrations, a necessary feature of these linear equations, where both flows (j_s and $\boldsymbol{J}_v$) and forces (the pressure and concentration differences) are assumed small and therefore appear only to the first power. In the second term, $\boldsymbol{J}_v$ is the volume flux across the membrane (volume per second per unit area), which mainly consists of water flow in dilute conditions. The quantity ω_s is the "solute permeability". It is a thermodynamic coefficient, which means that its interpretation on the molecular level is not given, although in this case it clearly involves diffusion of the solute. The quantity σ is called the "reflection coefficient". It is also a thermodynamic coefficient, but some molecular interpretation is possible. Suppose $\omega_s = 0$ so that solute diffusion across the membrane does not occur. Then

the solute must be impermeable and therefore cannot be swept along by the bulk flow of water. The second term then implies that the reflection coefficient equals unity for an impermeable solute.

In the second equation P is the ordinary physical pressure, the force per unit area exerted by material on one side of a plane on the material on the other side, and ΔP is the difference in pressures of the two reservoirs. The thermodynamic coefficient L_p is called the "hydraulic permeability". Its meaning is clear from the pressure term: a hydrostatic pressure difference causes volume flow toward the region of lower pressure. The second term is interesting, for when $\sigma = 1$, we see that bulk volume flow is prevented (it equals zero) by a pressure difference ΔP equal to $k_B T \Delta c_s$. In other words, $\sigma = 1$ means that the solute is impermeable to the membrane, and the "osmotic pressure"

$\Delta P = k_B T \Delta c_s$ (the van't Hoff equation) is required to halt bulk flow.

In the Kedem-Katchalsky equations the thermodynamic, as distinct from molecular, or mechanistic, level is made clear by the inclusion in them only of the properties of the two solution reservoirs, namely, their solute concentrations and pressures. The membrane is almost not there. It serves only as mathematical justification for maintenance of concentration and pressure differences. Nothing is said about the interaction of the membrane material with water or solutes.

When Debye presented his interpretation of osmotic pressure, he considered only van't Hoff's osmotic equilibrium, and he derived the van't Hoff equation from a molecular-level model and theoretical analysis.[7] In 1968 the author gave a detailed mechanistic analysis of a model generalizing Debye's idea.[10] If the

membrane is represented by a potential energy barrier to solute flow, infinitely high when the solute is absolutely impermeable but finite if the solute molecules can pass through, the Kedem-Katchalsky equations can be derived from a theoretical analysis on the molecular level. The Boltzmann exponential factors involving the energy barrier need not be linearized in the analysis, but must so be to produce the linear thermodynamic equations, along with the Onsager symmetry of cross coefficients.

The physical basis of osmotic flow across membranes containing bulk water ("porous" membranes) has been a controversial topic in membrane science, biology, and physiology, even though Vegard had long ago demonstrated convincingly that his osmotic flow measurements were best interpreted as driven by a hydrostatic pressure gradient throughout the membrane interior generated by a pressure drop at the solution-membrane interface.[11]

The author's 1968 paper contains a mechanistic derivation of the Vegard pressure profile. Mauro later showed that the measured rates of osmotic flow through membranes commonly used for water purification were greater by orders of magnitude than could be explained by Fick's law diffusion as observed with isotopic labeling,[12,13] Mauro also deduced theoretically a pressure drop at the solution-membrane interface with a local thermodynamic argument, which however does not go beyond the standard thermodynamic derivation of van't Hoff's law, and cannot expose the basis for the pressure drop in the repulsive membrane-solute molecular-level forces.[14] The author's 1968 paper[10] provides more insight by deriving the Vegard-Mauro pressure profile in terms of these forces.

A recent perspective article intended to be accessible to biologists reviews the Debye equilibrium model and its generalization

to osmotic flow.[1] In relatively open membranes containing water in bulk form, the Vegard-Debye pressure drop giving rise to pressure-driven flow is in all likelihood the mechanism for osmosis. But an important feature of this article is its argument that in the lipid bilayer of biological membranes also, where water exists only as separate molecules in the lipid, the Debye interfacial pressure drop and interior pressure gradient is also likely to be the driving force for flow. In this case pressure driven flow proceeds at the same rate as Fick's law diffusion.

In biological systems the impermeable solutes responsible for the osmotic danger to cells are not electrostatically neutral.[8] Instead, proteins like albumin and globulins with a net charge, usually anionic (negative), trapped in cells by their large size, are the impermeable solutes whose tendency to dilution by increasing water content can disrupt plasma membranes unless

sufficiently regulated. In physical and biochemistry also, Donnan ionic effects are present whenever membrane compartmentalization involves charged solutes.[4,15–17] There are three observably distinct Donnan equilibrium effects.[18] The presence of an impermeable ion causes an osmotic pressure difference across the membrane. A Donnan system always contains small permeable ions in addition to the impermeable species, and at equilibrium, the permeable ions are not distributed uniformly across the membrane. Finally, an electrical voltage (a difference in electric potential) is present across the membrane at equilibrium. These three effects are well understood on a thermodynamic level as arising from the equality of electrochemical potentials across the membrane.[18]

The three Donnan effects are present in biological nonequilibrium situations also.[8] Osmosis in biological systems is always in

the context of ionic species, both permeable and impermeable. The cellular membrane voltage is at the center of molecular neuroscience, and small permeable ions like sodium, potassium, and chloride ions, are always unevenly distributed between the cell and the extracellular fluid. Of course, these biological features are partly powered by active energy-expending processes like the hydrolysis of ATP, but passive Donnan mechanisms are present also, and a currently incomplete understanding of Donnan systems on the molecular level provides the main motivation for this paper.

To clarify the processes occurring in a Donnan nonequilibrium system, thermodynamic considerations, specifically, electrochemical potentials, will not appear in the analysis. Instead, the starting point is the Nernst-Planck equation,[8,19] supplemented by a volume flow term, and by terms representing

the mechanical forces between solute molecules and membrane at the interfaces with the aqueous reservoirs on either side of the membrane.[1] By "mechanical forces" is meant those forces due to the interactions of solute molecules with molecular groupings comprising the membrane material. These forces are understood to be additive with the electric Coulomb forces due to the electric charges on the ions, which are distinguished separately. It is assumed that the membrane material does not contain ionized groups. This paper can be viewed as the extension to ionic systems of corresponding considerations for non-electrolytes[1].

3 The Donnan System

We analyze the prototype Donnan system. A semipermeable membrane separates two infinite aqueous reservoirs. The reservoir on the left is called the outer reservoir, and the quanti-

ties pertaining to it are designated by "out". The reservoir on the right is the inner reservoir, designated, for the sake of biological flavor, by "cell". The outer reservoir contains monovalent "+" and "−" ions, permeable to the membrane. Together, these ions are the ions of a salt, for example NaCl, and the salt concentration is c_s^{out}. (Note that here and in everything that follows, "s" means "salt"). Electroneutrality requires that $c_+^{\text{out}} = c_-^{\text{out}} = c_s^{\text{out}}$. The units of concentration are number of ions per unit volume for the separate ions, and for the salt, the number of fictitious salt "molecules" per unit volume. The cell reservoir also contains + and − ions of the same species as in the outer reservoir. But additionally, it contains an impermeable anion with negative charge $-z$, with z a positive number, and concentration c_z. This anion is accompanied by z dissociated + ions of the same species as the + ions from the salt, so that the

total concentration of + ions in the cell reservoir is $zc_z + c_s^{\text{cell}}$. Of course the + ions as counterions to the impermeable anion are indistinguishable from those of the salt, but it will become clear why it is a good idea to be aware of their distinct origins. The concentration of − ions in the cell is just c_s^{cell}.

In a Donnan system at equilibrium the pressure in the cell reservoir is greater than in the outer one, and the pressure difference is the Donnan osmotic pressure. In this paper we study the Donnan system when the osmotic pressure is not allowed to build up. Instead, the pressures in both reservoirs are maintained as equal. Also, the salt concentrations in the reservoirs have dilute but otherwise arbitrary values. The system is then not in equilibrium. There is a nonzero volume flux $\boldsymbol{J}_v$, mainly water, and nonzero ionic fluxes j_+ and j_- across the membrane. The volume flux can be called Donnan osmosis. We will obtain

expressions for the electric potential (voltage) across the membrane, and for the pressure drop that drives Donnan osmosis.

4 The Augmented Nernst-Planck Equation

For the flux j_+ of + ions,

$$j_+ = -D_+ \frac{dc_+}{dx} - (D_+/k_B T) c_+ q \frac{d\phi}{dx} + c_+ J_v - (D_+/k_B T) c_+ \frac{du_+}{dx} \tag{3}$$

and for the flux j_- of − ions,

$$j_- = -D_- \frac{dc_-}{dx} + (D_-/k_B T) c_- q \frac{d\phi}{dx} + c_- J_v - (D_-/k_B T) c_- \frac{du_-}{dx} \tag{4}$$

In these equations the coordinate x marks location in the membrane and the interfaces with the reservoirs. The membrane interface with the outer reservoir, at the left hand in Figure 1, is the plane at $x = 0$. The membrane interface with the cell (right-hand) reservoir is at $x = h$, where h is then the width

of the membrane, which occupies the space $0 < x < h$ in Fig. 1. As to the sign of the fluxes, a positive flux is directed into the cell, from left to right, along with the running coordinate x. The ion concentrations are c_+ and c_-, and ϕ is the electric potential ϕ. The ionic charge of a + ion is q, the unit charge of a proton, and the charge on a − ion is $-q$. The electric field is $-d\phi/dx$, and when multiplied by $\pm q$ becomes the electric force on the charges of the ions. D_+ and D_- are the respective diffusion constants inside the membrane. The first two terms on the right-hand sides of the equations give the ordinary Nernst-Planck equations, that is, the fluxes are caused by Fick's law diffusion and by ionic mobility in an electric field. The third terms c_+J_v and c_-J_v are usually omitted in a Nernst-Planck analysis but are central to any discussion of osmotic flow. These terms account for the ions being carried along by the bulk volume flow

(but see Discussion section). Finally, the fourth terms convey the effect of the mechanical forces $-du_+/dx$ and $-du_-/dx$ between membrane and + and − ions in analogy to the electric field terms. In the fourth terms, u_+ and u_- are the respective membrane-ion potential energies generated by the work done on the ions when they move against the mechanical membrane forces acting on them, just as work is done against the electric field acting on the ionic charges.

To elaborate on the mechanical forces, we have provided Fig. 2, which shows the potential energy profiles corresponding to these forces. Here, we follow and generalize Debye, who represented the membrane by a potential energy that rises to infinity for an impermeable solute.[7] The corresponding force f_z is directed into the cell, which contains the impermeable solute. For the small permeable + and − ions, the mechanical potential

energies are not infinite, but in the usually expected case of hindered entrance to the membrane, the corresponding mechanical forces at the interfaces are directed away from the membrane.

5 Inside the Membrane

The equations simplify when applied to the interior of the membrane, where the mechanical forces $-du_+/dx$ and $-du_-/dx$ vanish, because the interior membrane environment of a + or − ion is the same on either side of the ion so that the mechanical potential energies are constant and their gradients zero (see Figure 2). Further, electroneutrality applies inside the membrane (as elsewhere), so that $c_+ = c_-$ there, and both can be designated by c_s, the salt concentration. Similarly, nonzero charge cannot build up inside the membrane, so $j_+ = j_-$ there, and both fluxes can be designated by j_s, the flux of salt. The aug-

mented Nernst-Planck equations inside the membrane can therefore be written as,

$$(q/k_BT)D_+\frac{d\phi}{dx} = -D_+\frac{d\ln c_s}{dx} + J_v - (j_s/c_s) \tag{5}$$

and,

$$(q/k_BT)D_-\frac{d\phi}{dx} = D_-\frac{d\ln c_s}{dx} - J_v + (j_s/c_s) \tag{6}$$

When these equations are added, the fluxes cancel, and an expression for the electric field inside the membrane emerges,

$$(q/k_BT)\frac{d\phi}{dx} = -\left(\frac{D_+ - D_-}{D_+ + D_-}\right)\frac{d\ln c_s}{dx} \tag{7}$$

When the equations are subtracted, and this expression for $d\phi/dx$ substituted in the result along with some algebra, the salt flux is obtained,

$$j_s = c_sJ_v - \left(\frac{2D_+D_-}{D_+ + D_-}\right)\frac{dc_s}{dx} \tag{8}$$

Both of these equations are well-known in electrochemistry[20,21] but worth discussing here. The second term in the expression

for the salt flux is just Fick's diffusion law with the effective diffusion constant indicated. The formula for the electric potential gradient, or "diffusion potential" as it is known in electrochemistry, may be of greater interest for physiologists, because it shows that a membrane can generate a voltage with no charge separation if the diffusion constants, or mobilities, of the ions differ. In fact, the derivation shows that the electric field is a consequence of electroneutrality! If the + ions move more quickly than the − ions, they are pulled back by the charge on the latter, and at the same time, they pull the − ions forward, always maintaining electroneutrality.

In our development we will also need the integrated form of the electric potential across the interior of the membrane. It follows easily from its derivative,

$$(q/k_B T)(\phi'' - \phi') = -\left(\frac{D_+ - D_-}{D_+ + D_-}\right) \ln \frac{c_s''}{c_s'} \tag{9}$$

where the meaning of the primed symbols is clear from Figure 1.

6 The Interfaces

There are discontinuities at the membrane-reservoir interfaces. The most obvious one is the discontinuity in concentration of the impermeable anion, which rises (left to right in Fig.1) from zero just inside the membrane at the membrane-cell interface to the nonzero value c_z of its concentration in the cell. Another example is from the analysis of van't Hoff osmosis,[1] where the osmotic flow of water into the reservoir containing the impermeable neutral solute is caused by the abrupt drop of pressure at the membrane-solution interface from just outside the membrane to just inside.

We will have to integrate across the discontinuity. In doing

so, we will encounter two types of terms. Terms of the first type involve a function of x evaluated at the plane of discontinuity $x = 0$ or $x = h$. The interfaces are idealized as planes of zero width. The integral of the function across the interface at $x = 0$ is then equal to the function evaluated at $x = 0$ times the zero width of the interface. Therefore the integral across the interface at $x = 0$ is equal to zero. The integral of such a term across the interface $x = h$ is equal to the value of the function at $x = h$ times the zero width of the interface, and thus also equals zero. Terms of the second type to be encountered involve the derivative with respect to x of some function of x, such as the electric or mechanical potentials or the concentration of some solute. The integral of such a term across either of the interfaces is the integral of a derivative of a function, and equals the difference in values of the function itself across the interface.

With this explanation we can integrate Eq. 3 term by term from left to right across the interface at $x = 0$. With some rearrangement, attention to electroneutrality, and cancellation of the diffusion constant D_+, we obtain,

$$(q/k_B T)(\phi' - \phi_{\text{out}}) = \ln \frac{c_s'}{c_s^{\text{out}}} + u_+ \tag{10}$$

The argument of the logarithm is the ratio of salt concentrations just inside the membrane at $x = 0$ and just outside. The mechanical potential energy u_+ is the potential energy difference between its values just inside and just outside (where it is zero). Integration of Eq. 4 in exactly the same way yields,

$$(q/k_B T)(\phi' - \phi_{\text{out}}) = \ln \frac{c_s'}{c_s^{\text{out}}} + u_- \tag{11}$$

If these two equations are subtracted, we find that the salt concentration c_s' just inside the membrane at $x = 0$ is related to the salt concentration just outside,

$$c_s' = \beta c_s^{\text{out}} \tag{12}$$

where,

$$\beta = e^{-\frac{u_+ + u_-}{2k_B T}} \tag{13}$$

thus identifying the partition coefficient β for the permeable salt.

The analogous integrations left to right of Eqs. 3 and 4 across $x = h$ gives the partitioning of salt at the other interface,

$$c_s'' = \beta c_s^{\text{cell}} \sqrt{1 + \frac{z c_z}{c_s^{\text{cell}}}} \tag{14}$$

If the two equations are added, we can solve for the discontinuity of electric potential at $x = 0$,

$$2q(\phi' - \phi_{\text{out}}) = -(u_+ - u_-) \tag{15}$$

The analogous discontinuity at $x = h$ is determined in the same way,

$$2q(\phi_{\text{cell}} - \phi'') = -k_B T \ln\left(1 + \frac{z c_z}{c_s^{\text{cell}}}\right) + u_+ - u_- \tag{16}$$

7 The Donnan Nonequilibrium Electric Potential

We have everything in place now to calculate the Donnan electric potential difference, or voltage, $\Delta\phi_D = \phi_{\text{cell}} - \phi_{\text{out}}$, between the cell and outer reservoirs in a nonequilibrium state, since $\Delta\phi_D = (\phi_{\text{cell}} - \phi'') + (\phi'' - \phi') + (\phi' - \phi_{\text{out}})$, and all three of these terms have been calculated above,

$$(q/k_B T)\Delta\phi_D = -\frac{D_+}{D_+ + D_-}\ln\left(1 + \frac{z c_z}{c_s^{\text{cell}}}\right) - \left(\frac{D_+ - D_-}{D_+ + D_-}\right)\ln\frac{c_s^{\text{cell}}}{c_s^{\text{out}}} \tag{17}$$

Notice that the effect of partitioning of salt into the membrane has canceled out (mathematically from the equations, but also physically because a test charge moving from the outer reservoir into the cell first passes into the membrane at $x = 0$, then passes back out at $x = h$).

8 Donnan Osmotic Flow

We write the hydrodynamic equation for the volume flux, which for dilute solutions can be regarded as the flux of water,

$$J_v = -hL_p \left(\frac{dP}{dx} - c_z f_z - c_+ f_+ - c_- f_- \right) \tag{18}$$

The coefficient L_p is the same hydraulic permeability as in the second of the Kedem-Kachalsky equations. The collection of terms in parentheses is the total force acting on a cross-sectional slab of width dx located at x. The pressure derivative is the net force exerted per unit area on the two planar surfaces of the slab. The second term is the mechanical force from the membrane material exerted on molecules of the impermeable anion located within the slab. Of course the concentration c_z of the impermeable anion is zero inside the membrane, and the repelling force f_z acts only at the membrane interface with the reservoir containing it ($x = h$ in Fig. 1). The third and fourth

terms are the mechanical forces on the + and − ions located within the slab, and these net forces also are nonzero only at the interfaces. The forces made explicit in this equation are the mechanical ion-membrane forces. There are also electrical forces on all of the ionic charges due to the electric field $-d\phi/dx$. But the slab is net electrically neutral, as the total charge of all the ions inside it sum to zero, so the total electric force on the slab is zero. In this section we use forces instead of negative potential energy gradients, the two representations being mathematically and physically equivalent, as perhaps an aid to intuition.

For $c_z f_z$, set the impermeable anion flux equal to zero,

$$0 = j_z = -D_z \frac{dc_z}{dx} + (D_z/k_B T) c_z f_z + (D_z/k_B T) z q c_z \frac{d\phi}{dx} \quad (19)$$

solve for $c_z f_z$, and substitute into the equation for J_v to get,

$$J_v = -hL_p \left[\frac{dP}{dx} - k_B T \frac{dc_z}{dx} + (c_+ - c_-) q \frac{d\phi}{dx} - c_+ f_+ - c_- f_- \right] \quad (20)$$

where we have also used electroneutrality, $zc_z = c_+ - c_-$.

Now go back to the Nernst-Planck equations 3 and 4, where many of these terms also appear. In these equations, use f_+ and f_- instead of the corresponding negative potential energy derivatives. Solve eq. 3 for $c_+q(d\phi/dx)$ and eq. 4 for $c_-q(d\phi/dx)$, and substitute into the previous equation for J_v, which becomes,

$$J_v = -hL_p\left[\frac{dP}{dx} - k_BT\frac{dc_z}{dx} - 2k_BT\frac{dc_s}{dx} + k_BT\frac{D_+ + D_-}{D_+D_-}(c_sJ_v - j_s)\right] \tag{21}$$

where both c_+ and c_- have been replaced with c_s by electroneutrality, and the ionic fluxes replaced with j_s since no charge buildup is allowed (same as electroneutrality).

Although a quick glance might suggest that we have been going around in circles, we are actually positioned at this point to see how the pressure discontinuities arise at the membrane interfaces. Referring if need be back to the explanation of how to

integrate across the interfaces, we first integrate this equation, term by term, from just outside the membrane at $x = 0$ to just inside. The terms involving J_v and j_s integrate to zero, while the terms involving derivatives integrate to the differences between values just inside and just outside. Referring to Fig. 1 to be sure of awareness of the quantities involved, a reader will find

$$P' - P_0 = 2k_B T(c_s' - c_s^{\text{out}}) \tag{22}$$

Next, integrate the same equation term by term from just inside the membrane at $x = h$ to just outside,

$$P_0 - P'' = k_B T(z + 1)c_z + 2k_B T(c_s^{\text{cell}} - c_s'') \tag{23}$$

We are still not quite finished because the primed salt concentrations are inside the membrane at the interfaces, while we want the given concentrations in the reservoirs (the extracellular fluid and the cytoplasm in the cell). But we have already found the relations between them in Eqs. 12 and 14. On the

other hand, the primed pressures are exactly what we do want, because it is the difference between the pressure jumps from the common outside pressures P_0 that are responsible for the pressure gradient inside the membrane that drives Donnan osmotic flow, and $P' - P'' = (P' - P_0) + (P_0 - P'')$,

$$P' - P'' = k_B T(z+1)c_z + 2k_B T c_s^{\text{cell}} \left(1 - \beta\sqrt{1 + \frac{z c_z}{c_s^{\text{cell}}}}\right) - 2(1-\beta)k_B T c_s^{\text{out}} \tag{24}$$

To gain some feeling for this result, a reader might want to check some special cases, such as $\beta = 0$ (impermeable salt ions), $z = 0$ together with $\beta = 1$ (van't Hoff osmosis), and $\beta = 1$ (freely permeable salt ions).

Figure 3 shows the Donnan osmotic driving pressure for $z = 1$ and concentrations all equal to 0.1 M for salt partition coefficients $0 \leq \beta \leq 1$. The positive values mean that $P' > P''$, so water flows into the cell from high to low pressure inside the

membrane.

9 A Salt Pump

If water continuously flows from the extracellular fluid into a cell, as driven by Donnan pressure, the cell will ultimately burst. Plant cell membranes are strengthened against bursting by their surrounding rigid walls, but the cell membranes of higher organisms are not. Some mechanism must have been hit upon by evolutionary processes to maintain cellular volume stability. This mechanism is the sodium pump, a protein that penetrates the cell membrane from inside to outside, and, fueled by energy from the hydrolysis of ATP, pumps sodium ions out of the cell as soon as they enter (actually, each pump cycle removes three sodium ions from the cell and replaces them with two potassium ions from the outside fluid). Effectively, the sodium ions outside the

cell are made impermeable to balance the impermeability of the proteins inside the cell, abolishing the Donnan osmotic flow.[8]

As we have seen, Donnan pressure osmosis continuously drives water into the cell and must be countered if uncontrolled swelling with ultimate rupture is to be contained. We can bring J_v to zero in our model by adding an ion "pump" in the following way. Let us locate this pump at the cell-membrane interface at $x = h$, and give it the property of forcing a + ion back into the membrane as it tries to exit the membrane into the cell. Additional work must then be done against this repelling pump force if the ion is to exit. When the ion does succeed in exiting, it is only by acquiring a positive amount of potential energy u_+^{pump}. The potential energy profile of a + ion in the presence of the pump then looks as drawn in Figure 4, which should be compared with Figure 2 in the absence of a pump. Alternatively,

the pump could be placed at $x = 0$, where it would repel a + ion back into the outer fluid, preventing it from entering the membrane. Then the potential energy of the ion in the outer reservoir would be *lowered*, while the shape of its profile would be the same as in Figure 4.

Repetition of the entire derivation with the energy profile of Figure 4 replacing that of Figure 2 reveals the influence of the pump energy on the pressure gradient,

$$P' - P'' = k_B T(z+1)c_z + 2k_B T c_s^{\text{cell}} \left[1 - \beta e^{\frac{u_+^{\text{pump}}}{2k_B T}} \sqrt{1 + \frac{z c_z}{c_s^{\text{cell}}}} \right] - 2(1-\beta) k_B T c_s^{\text{out}} \tag{25}$$

This expression for the driving pressure gradient is similar to Eq. 24 without the pump, but the additional Boltzmann factor in the bracketed term clearly makes the gradient smaller. If $P' - P''$ is set equal to zero, corresponding to zero volume flow J_v, with the same parameters as in Figure 3, we find a pump

energy $u_+^{\text{pump}} = 2.8k_BT$ for a partition coefficient $\beta = 1$ (freely permeable salt ions). This energy, 7.2 kJ mol^{-1} at physiological temperature, is the work that the pump must expend on a mole of + ions to force them out of the cell if Donnan osmosis is to be stopped. If $\beta = 0.1$, corresponding to nearly impermeable salt ions, the pumping work on a + ion required to stop osmosis would be $16k_BT$.

There are quantities other than the pressure drop that are affected by the presence of the pump. The salt concentration c_s'' just inside the membrane at its interface with the cell, given by Eq. 14 in the absence of the pump, is increased by the same factor $e^{\frac{u_+^{\text{pump}}}{2k_BT}}$ in its presence. In other words the outward flow of permeable salt is increased when + ions are pumped out of the cell. The Donnan voltage, given by Eq. 17 in the absence of the

pump, is modified by its presence,

$$(q/k_BT)\Delta\phi_D = -\frac{D_+}{D_+ + D_-}\ln\left(1 + \frac{zc_z}{c_s^{\text{cell}}}\right) - \left(\frac{D_+ - D_-}{D_+ + D_-}\right)\ln\left(\frac{c_s^{\text{cell}}}{c_s^{\text{out}}}e^{\frac{u_+^{\text{pump}}}{2k_BT}}\right) \tag{26}$$

If the diffusion of + ions is faster than "−" ions, then pumping + ions out of the cell decreases the cell voltage (makes it more negative if already negative without the pump).

10 Discussion

A tacit assumption in our analysis is that water exists in something like its bulk form in the interior of the membrane, and that the small ions are dissolved in it. This picture of the membrane is consistent with most synthetic membranes used for water or protein purification and in batteries and fuel cells. In biology, the smallest pores crossing capillary walls are about 5 nm wide,[22] much larger than a water molecule (0.3 nm), so this model might

be realistic for biological microvessels as well. But more typical membranes surrounding the cells of higher organisms require discussion.

Most membranes containing the cells of higher organisms, for example red blood cells, are composed of a lipid bilayer punctuated by protein channels for small ions. Water molecules have significant permeability as discrete molecules through the lipid and also cross through channels selective for water. For these membranes the small ions are not swept along by bulk water flow, and the term $c_s \boldsymbol{J}_v$ in Eq. 8 for the salt flux is absent. On the other hand, the Donnan pressure drop that drives osmosis, embodied in Eq. 24, is not affected by this consideration. See Ref. 1, Appendix 4, for a quantitative description of the movement of individual water molecules down a pressure gradient.

The postulate of electroneutrality in the interior of the mem-

brane is a key feature of the entire treatment. Electroneutrality inside macroscopic synthetic membranes is a safe assumption and has even been verified by direct measurement.[23] But inside a biological membrane, where the small ions cross through single-ion selective channels, while water molecules cross either through separate water-selective channels, or through the lipid bilayer, electroneutrality is not obvious. The lipid bilayer is fluid in the plane of the membrane, and protein channels are not necessarily fixed in place. Protein molecules containing potassium or sodium ions, for example, carry the positive charge of these ions, and a protein channel selective for chloride ions carries their negative charge. It is accepted in physiology texts that positive ions do not cross the cell membrane unaccompanied by negative ions,[8] and if this is true it is difficult to understand how the interior of the membrane could not be electroneutral.

We take the view here that the biological membrane interior is akin to a thin layer of electroneutral solution with positively and negatively charged protein channels as solutes mobile in the plane of the membrane. Coulomb forces are very strong, and excess charge in the interior of the membrane would disrupt the membrane or be quickly dissipated by lateral movement driven by Coulomb repulsion.

Electrical capacitance is an important feature of biological membranes because the lipid interior is a good insulator. Capacitance is central to an understanding of the time dependence of cross membrane currents and voltages. The present paper stops short of consideration of current-voltage relationships. The electrical current per unit area would be the product of charge q and the difference $j_+ - j_-$ between the fluxes of $+$ and $-$ ions. Since in this paper $j_+ = j_- = j_s$, our discussion is limited to zero

current. However, a relevant point can be made. A resting membrane potential, or electric voltage, need not arise solely in the context of charge separation. There is no charge separation anywhere in our analysis, yet there is an electric potential. There are three intertwined Donnan effects, both in equilibrium and nonequilibrium states, salt redistribution, pressure, and voltage, none of them involving charge separation across the membrane.

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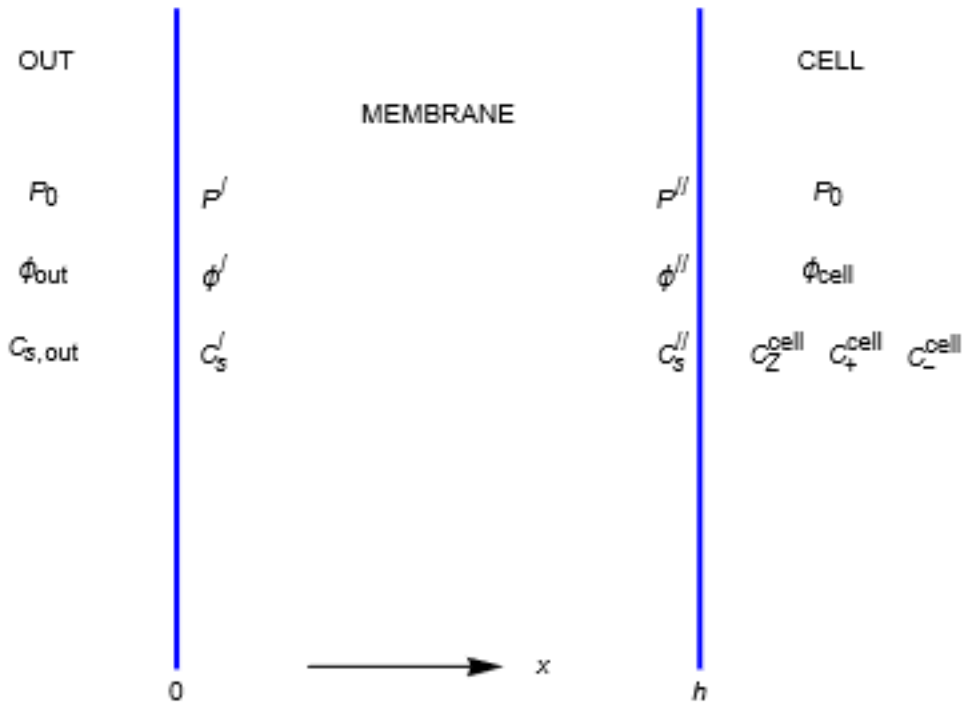

Figure 1. The Donnan system, showing the relevant quantities pressure, electric potential, and salt concentration in the outer compartment x<0, and the impermeable anion concentration and individual small ion concentrations in the cell, x>h. These quantities are discontinuous at the membrane boundaries x=0 and x=h, and their values just inside the membrane are designated by primes and double primes.

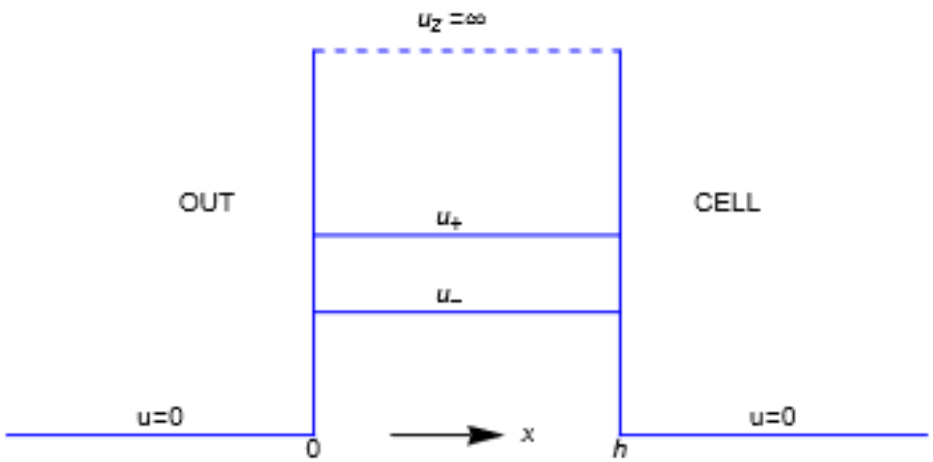

Figure 2. Profiles of the Debye potential energies representing the mechanical interactions of the membrane with the three types of ion considered in this paper. The potential energy for the + ion is drawn as higher than for the - ion, but this ordering does not matter.

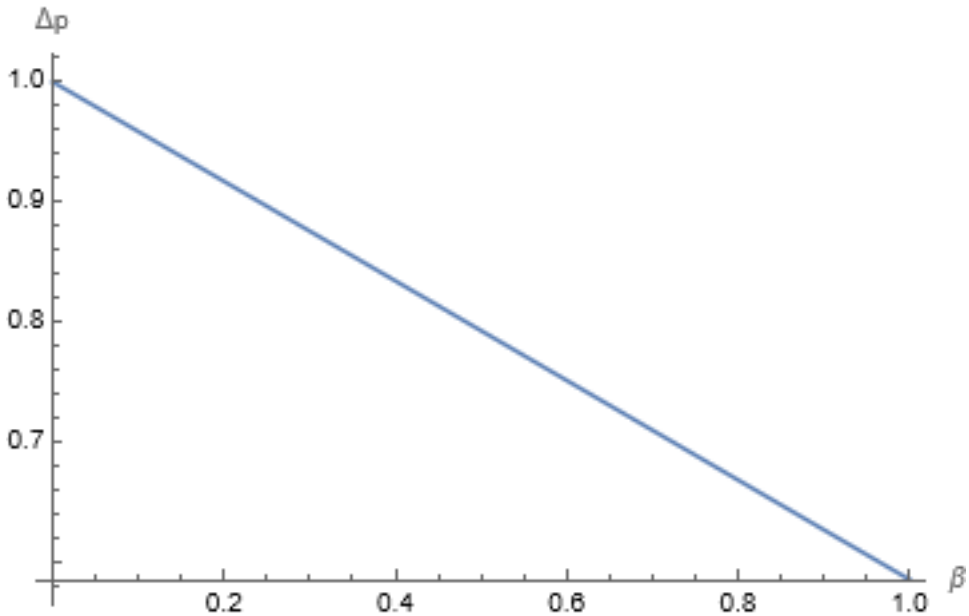

Figure 3. A plot of normalized Donnan pressure drop $\Delta P/k_BT(z+1)c_z$ vs. salt partition coefficient β. Note that all values of the pressure drop are positive, indicating water flow into the cell.

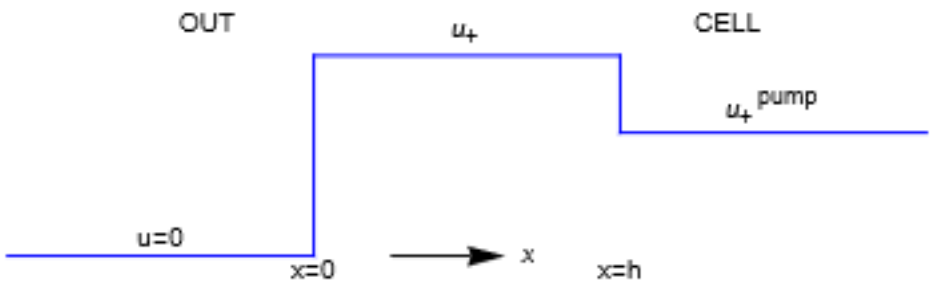

Figure 4. The membrane potential energy profile when a salt pump is present.

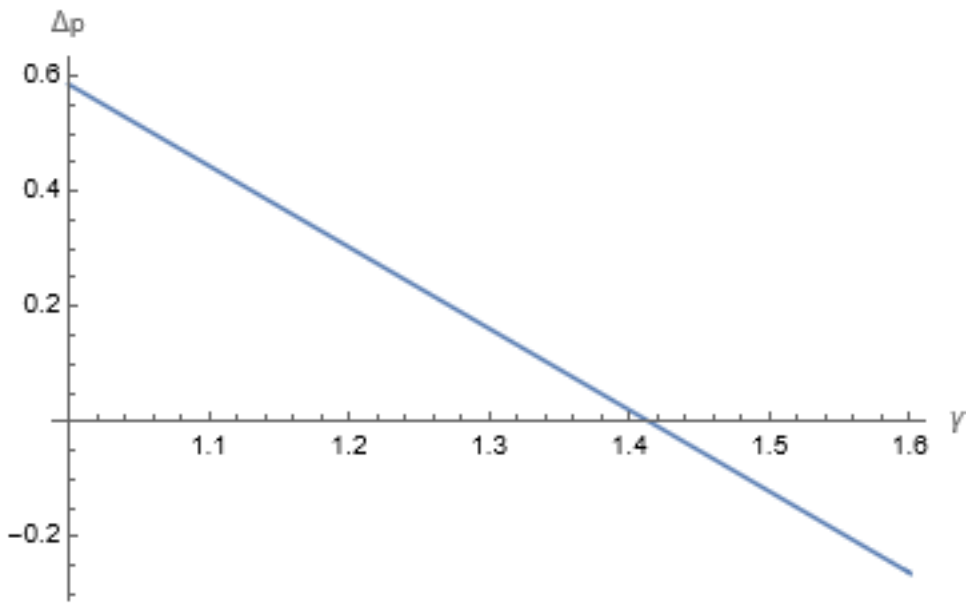

Figure 5. A plot of normalized Donnan pressure drop $\Delta P/k_B T(z+1)c_z$ vs. pump energy factor γ for $\beta=1$ (freely permeable salt). Note that $\Delta p=0$ when $\Upsilon=1.41$, indicating that water flow into the cell has been abolished.