ARTICLE

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Physical descriptions of experimental selectivity measurements in ion channels

Abstract Three experiments that quantify the amount of selectivity exhibited by a biological ion channel are examined with Poisson-Nernst-Planck (PNP) theory. Conductance ratios and the conductance mole fraction experiments are examined by considering a simple model ion channel for which an approximate solution to the PNP equations with Donnan boundary conditions is derived. A more general result is derived for the Goldman-Hodgkin-Katz permeability ratio. The results show that (1) the conductance ratio measures the ratio of the diffusion coefficients of the ions inside the channel, (2) the mole fraction experiment measures the difference of the excess chemical potentials of the ions inside the channel, and (3) the permeability ratio measures both diffusion coefficients and excess chemical potentials. The results are used to divide selectivity into two components: partitioning, an equilibrium measure of how well the ions enter the channel, and diffusion, a nonequilibrium measure of how well the ions move through the channel.

Keywords Poisson-Nernst-Planck · Selectivity · Conductance ratio · Permeability ratio

Abbreviations *GHK*: Goldman-Hodgkin-Katz · *MMF*: midpoint mole fraction · *PNP*: Poisson-Nernst-Planck

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Introduction

Ion channels are hollow proteins that span biological membranes and act as conduits, allowing ions to move through otherwise impermeable membranes (Hille 2001). Most electrical activity in living systems is produced by this kind of charge movement. In many circumstances it is vital that only one type of ion moves across the membrane (typically Na^+, K^+, Ca^{2+}, or Cl^-), even when other ions have a larger concentration and concentration gradient. In neurons, for example, potassium channels ensure that sodium ions do not enter the cell as potassium ions exit; vice versa, sodium channels let sodium in while keeping potassium from leaking out. Following a positive displacement in electrical potential, these two channel types open and close with different lag times to produce the transient electrical signal (the action potential) that propagates along the nerve fiber, carrying information from one end of the cell to the other.

Ion selectivity is defined operationally in experiments by several different protocols. However, one often finds that the selectivity estimated by one protocol is not the same as that estimated by another. In this paper we examine three of these experimental protocols (described below) for a model channel and show that, in fact, these protocols should *not* give the same results. Our model is analyzed with the Poisson-Nernst-Planck (PNP) theory of ion transport (Nonner and Eisenberg 1998; Chen et al. 1997a, 1997b, 1999) and is consistent with the charge/space competition model of selectivity introduced by Nonner et al. (2000, 2001). In this hypothetical channel, selectivity has contributions from various physical parameters, including the charge, diffusion coefficient, and excess chemical potential of each ion species. We find that the experimental protocols weight these contributions differently: (1) the conductance ratio measures the ratio of the diffusion coefficients of the ions inside the channel; (2) the mole fraction experiment measures the difference of the excess chemical potentials of

the ions inside the channel; (3) the permeability ratio defined by the Goldman-Hodgkin-Katz equation measures both diffusion coefficients and excess chemical potentials. This diversity of results can be used to estimate those physical parameters.

The selectivity experiments

The three selectivity experiments considered in this paper are described here using NaCl and KCl as example ion species.

Conductance ratios

Single channel current/voltage (I/V) relations are measured by placing the channel in a lipid bilayer surrounded by two baths of known ionic composition and applying a voltage across the system. If this experiment is conducted with the same concentration of NaCl (for example) in both baths, the conductance (dI/dV) measured at zero applied potential can be plotted as a function of bath concentration. Experiments often show that the conductance increases from zero at zero concentration and saturates with some maximal conductance as the concentration increases more and more (Hille 2001). If g_{Na} is this saturated conductance for Na^+ found in one experiment and if g_K is the corresponding saturated conductance for K^+ found in another experiment, then one simple measure of selectivity is the ratio of these saturated conductances:

$$\frac{g_{Na}}{g_K} \qquad (1)$$

Mole fraction experiment

In the classical conductance mole fraction experiment, the two baths are identical, containing a mixture of two salts, and the conductance at zero applied potential is measured as a function of the mole fraction of one salt. If ions were to conduct like ions in a dilute electrolyte solution, their contribution to the overall conductance would be additive; that is, the total conductance as a function of mole fraction would be linear between the two limiting conductances. For example, the total conductance would be g_{Na} when [KCl] = 0 and the total conductance would be g_K when [NaCl] = 0. In many channels the total conductance is a nonlinear function of mole fraction; if the function has a minimum or maximum, the channel is said to exhibit an anomalous mole fraction effect (AMFE).

The average of the limiting conductances g_{Na} and g_K can help define a measure of selectivity: the mole fraction of NaCl that produces the average of the limiting conductances shows the ion that is preferred by the channel. If this midpoint mole fraction (MMF) is below 1/2, then the channel prefers Na^+ over K^+ because a

smaller amount of Na^+ has the same effect as a larger amount of K^+.

Permeability ratios and the GHK equation

Introduced by Hodgkin and Katz more than 50 years ago (Hodgkin and Katz 1949), the permeability coefficient relates the reversal potential to the concentrations of the ion species in the baths through the Goldman-Hodgkin-Katz (GHK) equation (Goldman 1943; Hodgkin and Katz 1949; Hille 2001). For the specific case of NaCl and KCl in the baths, the GHK equation is:

$$V_{rev} = \frac{kT}{ze} \ln\left(\frac{P_{Na}c_{Na}(R) + P_K c_K(R) + P_{Cl}c_{Cl}(L)}{P_{Na}c_{Na}(L) + P_K c_K(L) + P_{Cl}c_{Cl}(R)}\right) \qquad (2)$$

where V_{rev} is the reversal potential (the applied potential at which no net current is measured), k is the Boltzmann constant, T is the absolute temperature, e is the elementary charge, and z is the valence of the cations and the absolute value of the valence of the anions. For ion species j, P_j is the permeability, $c_j(L)$ is the bulk bath concentration on the left (or inside or *cis*) side of the membrane, and $c_j(R)$ is the bulk bath concentration on the right (or outside or *trans*) side of the membrane. The potential is measured as the potential difference between the left side and right side. The anion current is negligible in many cation-selective channels and P_{Cl} is zero.

In the original derivations of the GHK equation, each permeability has three components (Hodgkin and Katz 1949; Hille 2001):

$$P_j = \frac{D_j \beta_j}{d} \qquad (3)$$

where D_j is the diffusion coefficient of species j inside the channel, β_j is the water/channel partition coefficient for j, and d is the length of the channel. The partition coefficient β_j relates c_j, the bath concentration of j, to C_j, the concentration just inside the channel:

$$\beta_j = \frac{C_j}{c_j} \qquad (4)$$

It is assumed that β_j is the same on each side of the channel and independent of the bath concentrations (Hodgkin and Katz 1949; Hille 2001).

Permeability ratios in the literature are nearly always measured under symmetric bi-ionic conditions (for example, 150 mM of KCl on the left side and 150 mM of NaCl on the right) and calculated from Eq. (2) with P_{Cl} set to zero. If K^+ is on the left and Na^+ on the right, then:

$$\frac{P_{Na}}{P_K} = \exp\left(\frac{ze}{kT}V_{rev}\right) \qquad (5)$$

If $\frac{P_{Na}}{P_K} > 1$, then Na^+ is more permeable than K^+.

The GHK equation (2) is derived by a straightforward integration of a Nernst-Planck equation for each species [Eq. (9) below with μ_j^{ex} set to zero] if several assumptions are made (Hodgkin and Katz 1949; Hille 2001):

1. Ions do not interact with each other as they cross the channel.
2. Ions do not interact with the channel protein as they cross the channel.
3. The electric field inside the channel is constant. Specifically, the electric field is assumed to be spatially uniform and does not change when properties of the channel protein are changed by mutation (for example, the charge on the protein), when the partition coefficient is changed, or when bath concentrations or transmembrane potential are changed.
4. For each ion species the partition coefficient β_j defined in Eq. (4) is the same on both sides of the channel and independent of the bath composition.

Each of these assumptions, however, is not physically plausible:

1. Ions are charged particles. On very general principles, they must strongly interact through Coulomb's Law (an inverse square law) when traveling through a channel tens of angstroms in length.
2. Ignoring the interaction between the protein and the ions implies that the channel has little effect on permeation. However, biological reasoning and mutation studies suggest that the role of the channel is to control ion movement. In general, the physical location of the protein ensures that its charges interact electrostatically with ions in the pore. Chemical interactions are also quite likely because many atoms of the channel wall collide with ions. Thus channels interact with ions and the equations describing permeation should include such interactions.
3. The electric field is created by the charges in the system and the locations of the charges in the system are changed by the electric field; these quantities are strongly and nonlinearly coupled (Henisch 1984; Selberherr 1984; Jacoboni and Lugli 1989). Both of these quantities are manipulated in most experiments, changing the properties of the channel, and so it seems unwise to make an assumption about the structure of the resulting electric field.
4. If the channel has differently charged groups on each end, then the partition coefficient cannot be the same on both sides [Chen et al. (1997a) give an example of such a channel]. In any case, the partition coefficient is never independent of the surrounding bath concentrations (see below).

Despite these arguments, the permeabilities defined by the GHK equation are widely used to determine what ions a channel prefers to pass through its pore. Permeabilities have proven to be durable, if heuristic, measures of selectivity. This paper tries to clarify their physical interpretation.

Materials and methods

The PNP equations

The PNP model we will use to analyze the experiments outlined above has fit data of several channels (Kurnikova et al. 1996, 1999; Chen et al. 1997a, 1997b, 1999; Nonner and Eisenberg 1998; Cardenas et al. 2000; Im et al. 2000) and can be derived as an approximate description of the Brownian motion of a charged particle (Schuss et al. 2001).

The PNP theory describes the flux of the ions with Nernst-Planck equations:

$$-J_j = \frac{1}{kT} D_j(x) A(x) c_j(x) \frac{d\mu_j}{dx}(x) \tag{6}$$

where $A(x)$ is an area function equal to the area of the channel inside the channel [Gillespie (1999) gives a detailed explanation], J_j is the (constant) particle flux, $D_j(x)$ is the local diffusion coefficient, $c_j(x)$ is the local concentration, and $\mu_j(x)$ is the local electrochemical potential of species j. The electrochemical potential consists of an ideal component $\mu_j^{id}(x)$ and an excess component $\mu_j^{ex}(x)$:

$$\mu_j(x) = \mu_j^{id}(x) + \mu_j^{ex}(x) \tag{7}$$

with:

$$\mu_j^{id}(x) = z_j e \phi(x) + kT \ln\left[c_j(x)\right] \tag{8}$$

where $\phi(x)$ is the local electrostatic potential. With these definitions, Eq. (6) can be rewritten:

$$-J_j = D_j(x) A(x) \left(\frac{dc_j}{dx}(x) + \frac{z_j e}{kT} c_j(x) \frac{d\phi}{dx}(x) + \frac{1}{kT} c_j(x) \frac{d\mu_j^{ex}}{dx}(x) \right) \tag{9}$$

The ideal chemical potential $\mu_j^{id}(x)$ is the free energy per mole of a dilute solution of point particles that interact only through the mean electric field. This ideal solution is the ionic analog of an ideal dilute gaseous plasma. The excess chemical potential is the difference in the chemical potentials of the real solution and the ideal solution. It includes, for example, entropic effects of the finite size of the ions, electrostatic effects of interacting charged hard spheres (Nonner et al. 2000), and effects of the solvent (Nonner et al. 2001). For each ion species j, $\mu_j^{ex}(x)$ is a function of the concentrations of *all* the particles near x; that is, it depends on all the variables of the system. However, a theory to compute μ_j^{ex} in the presence of flux does not currently exist; here, we assume that each μ_j^{ex} is a given function of x.

To make the notation simpler, we will usually describe the excess chemical potential μ_j^{ex} by the activity coefficient:

$$\gamma_j = \exp\left(\frac{\mu_j^{ex}}{kT}\right) \tag{10}$$

The activity coefficient γ_j measures the ion-specific properties of a real solution which depend on the properties of all ions in the solution. In particular, the activity coefficient of Na^+ depends significantly on the concentration of K^+ in concentrated salt solutions. In contrast to the activity coefficient, the concentration c_j measures the nonspecific properties of an ideal solution. The activity of a solution:

$$\gamma_j c_j = \exp\left(\frac{\mu_j}{kT}\right) \tag{11}$$

is the effective concentration of the solution and is a measure of the free energy per mole of the ion j in the solution (Berry et al. 2000).

Lastly, the PNP theory uses the Poisson equation to compute the electrostatic potential from the charges:

$$-\left[\frac{d}{dx}\left(\epsilon(x)\frac{d\phi}{dx}(x)\right)+\epsilon(x)\frac{1}{A(x)}\frac{dA}{dx}(x)\frac{d\phi}{dx}(x)\right]$$
$$=e\sum_j z_j c_j(x)+eq(x) \tag{12}$$

where $\epsilon(x)$ is the local dielectric coefficient and $q(x)$ is the local fixed charge due to the channel protein ($q=0$ in the baths).

To calculate the transport of ions, the PNP equations are solved simultaneously (self-consistently); that is, all the Nernst-Planck equations and the Poisson equation must be satisfied at the same time. This even-handed treatment of the electric field and concentration profiles is not present in the derivation of the GHK equation [Eq. (2)], where assumptions about the electric field were made in order to simplify the equations so they could be integrated analytically. As shown in Appendix A and noted by early workers in the field (MacInnes 1939; Goldman 1943; Helfferich 1962), a simple substitution into the Poisson equation shows that a constant field is generally not consistent with the concentration profiles of the GHK equation. More quantitative analysis shows that the inconsistency has large effects on the predicted profiles of both concentrations and electrostatic potential, as well as the predicted currents (Chen et al. 1992).

Boundary conditions

Boundary conditions are needed that connect the concentration and potential at the edge of the channel to those far away in the baths, where they are measured and controlled by auxiliary systems. These systems do not maintain the concentrations and potential at the edge of the channel.

When solving the PNP equations numerically, one must either include enough of the bath in the analysis so that the concentrations and electrostatic potential are approximately equal to their bulk values (Kurnikova et al. 1996, 1999; Nonner and Eisenberg 1998; Cardenas et al. 2000; Hollerbach et al. 2001), or one must derive an analytic approximation to their values just inside the channel. In earlier numerical solutions of the PNP equations (Chen et al. 1997a, 1997b, 1999) the boundary conditions were those used to describe the contact between semiconductors and wires (Henisch 1984; Selberherr 1984; Jacoboni and Lugli 1989).

These "built-in" boundary conditions give the Donnan potential of classical physiology (MacInnes 1939; Helfferich 1962). Because the channel has a high fixed charge density, a potential difference exists between the bulk bath and the end of the channel; this is the Donnan potential (Gillespie and Eisenberg 2001). If the channel is located in the interval $(0,d)$, the approximation of the potential $\phi(0)$ and concentration $c_j(0)$ at the channel's left edge are given by:

$$\phi(0)=V+\Psi_L \tag{13}$$

$$c_j(0)=\frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}}c_j(L)\exp\left(-\frac{z_j e}{kT}\Psi_L\right) \tag{14}$$

where the classic Donnan potential Ψ_L on the left side of the channel is given by solving:

$$0=\sum_j z_j\frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}}c_j(L)Y_L^{-z_j}+q_L \tag{15}$$

for:

$$Y_L=\exp\left(\frac{e}{kT}\Psi_L\right) \tag{16}$$

Here $\gamma_j^{bath}(L)$ is the activity coefficient of species j in the left bath and γ_j^{chan} its activity coefficient just inside the channel. q_L is the fixed charge just inside the left side of the channel. At the right edge [with an equation similar to Eq. (15)]:

$$\phi(d)=\Psi_R \tag{17}$$

$$c_j(d)=\frac{\gamma_j^{bath}(R)}{\gamma_j^{chan}}c_j(R)\exp\left(-\frac{z_j e}{kT}\Psi_R\right) \tag{18}$$

where V is the applied voltage and we assume the potential at the right electrode is 0.

Corrections to the Donnan boundary conditions that give more accurate approximations to the potentials and concentrations at the channel edges have recently been derived (Gillespie and Eisenberg 2001). That paper also shows that the classic Donnan boundary conditions [Eqs. (13, 14, 15, 16, 17, 18)] give the same current/voltage relations as the modified Donnan potentials (with the assumptions listed in the "Assumptions" section below). Thus, when deriving current/voltage relations, classic Donnan potentials can be used, but the modified Donnan potentials must be used if accurate values of the potentials and concentrations at the edges of the channel are required.

In this paper, our choice of boundary conditions is guided by this distinction. Specifically, the conductance and mole fraction experiments (see the "Conductance ratios" section, "Mole fraction experiment" section, and Appendix A) involve finding current/voltage relations and therefore we will use the classic Donnan boundary conditions for those cases. The GHK permeability result (see "Permeability ratios" section) and the analysis in the Discussion, on the other hand, require accurate values of the potentials and concentrations, necessitating the use of modified Donnan boundary conditions.

Assumptions

In order to derive analytic results we make several assumptions. For the GHK permeability result we assume:

1. Inside the channel, the diffusion coefficients D_j and the activity coefficients γ_j are scaled by a multiplicative constant and they have the same functional form:

$$D_j(x)=D_j^{chan}D(x) \tag{19}$$

and:

$$\gamma_j(x)=\gamma_j^{chan}\gamma(x) \tag{20}$$

for all x inside the channel, where D_j^{chan} and γ_j^{chan} are species-dependent constants and $D(x)$ and $\gamma(x)$ are species-independent functions of x. Furthermore, γ_j^{chan} can be different for each experimental protocol; that is, γ_j^{chan} depends on the applied voltage and the bath concentrations:

$$\gamma_j^{chan}=\gamma_j^{chan}(V,c_1(L),c_2(L),...,c_1(R),c_2(R),...) \tag{21}$$

2. The area A is constant inside the channel.
3. The dielectric coefficient ϵ is constant throughout the system. The details of a continuous $\epsilon(x)$ have only a small impact on the solution of the PNP equations (Gillespie 1999; Gillespie and Eisenberg 2001). The discontinuous case can be solved by using an auxiliary Poisson equation (Schuss et al. 2001).
4. The relative resistance of the channel is so great compared to the resistance of the bath that the current does not significantly change the concentrations or electrostatic potential across the baths. Gillespie (1999) shows that this assumption is satisfied if the bulk concentrations are sufficiently large ($\gtrsim 50$ mM) and if the diffusion coefficients in the baths are much larger than those in the channel.
5. The anion current and concentration inside the channel are negligible.
6. All cations have the same valence z_c.

When deriving the conductance and mole fraction results, we also use the following assumptions:

1. The channel is assumed to have a spatially uniform fixed charge density $q < 0$ due to the permanent charges of the channel protein. This is the simplest charge structure that can be imposed on the PNP system to produce a cation-selective channel (Chen et al. 1997b, 1999; Nonner and Eisenberg 1998). More complex charge structures are of considerable biological importance (Chen 1997a), but need to be analyzed separately.
2. The diffusion coefficients D_j and the activity coefficients γ_j (excess chemical potentials μ_j^{ex}) are piecewise constant, taking values $D_j^{bath}(L)$, $\gamma_j^{bath}(L)$, $D_j^{bath}(R)$, $\gamma_j^{bath}(R)$ in the left and right baths and D_j^{chan} and γ_j^{chan} inside the channel. Eq. (21) is assumed to hold.
3. The potential at the edges of the channel are given by the Donnan boundary conditions. Gillespie and Eisenberg (2001) discuss the consequences of this assumption.
4. The anions have valence –1.

Results

An approximate analytic solution to the PNP equations is derived in Appendix A, with Eq. (99) giving an equation for the flux of species j. Equations (13, 14, 15, 16, 17, 18) allow the results to be rewritten as:

$$J_j = z_c \frac{A}{d} \frac{D_j^{chan}}{\gamma_j^{chan}} \left[\frac{e}{kT} V - \ln\left(\frac{Y_R}{Y_L} \right) \right]$$

$$\times \frac{\gamma_j^{bath}(R)c_j(R) - \gamma_j^{bath}(L)c_j(L) \exp\left(\frac{z_c e}{kT} V\right)}{Y_R^{z_c} - Y_L^{z_c} \exp\left(\frac{z_c e}{kT} V\right)} \qquad (22)$$

where z_c is the valence of all cations, and the left and right side Donnan potentials Ψ_L and Ψ_R, respectively, define Y_L and Y_R by:

$$Y_L = \exp\left(\frac{e}{kT} \Psi_L \right) \qquad (23)$$

$$Y_R = \exp\left(\frac{e}{kT} \Psi_R \right) \qquad (24)$$

A further simplification can be made if the bath concentrations are small compared to the fixed charge concentration $|q|$:

$$\frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}} c_j(L) \ll |q| \qquad (25)$$

and:

$$\frac{\gamma_j^{bath}(R)}{\gamma_j^{chan}} c_j(R) \ll |q| \qquad (26)$$

In that case, in Appendix B we show that:

$$Y_L^{z_c} \approx \frac{z_c}{|q|} \sum_{z_j = z_c} \frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}} c_j(L) \qquad (27)$$

and similarly:

$$Y_R^{z_c} \approx \frac{z_c}{|q|} \sum_{z_j = z_c} \frac{\gamma_j^{bath}(R)}{\gamma_j^{chan}} c_j(R) \qquad (28)$$

With this simplification, Eq. (22) becomes an explicit equation for the flux:

$$J_j = \frac{A}{d} |q| D_j^{chan} \left[\frac{e}{kT} V - \ln\left(\frac{\sum_{z_j = z_c} \lambda_j(R)c_j(R)}{\sum_{z_j = z_c} \lambda_j(L)c_j(L)} \right) \right]$$

$$\times \frac{\lambda_j(R)c_j(R) - \lambda_j(L)c_j(L) \exp\left(\frac{z_c e}{kT} V\right)}{\sum_{z_j = z_c} \lambda_j(R)c_j(R) - \left(\sum_{z_j = z_c} \lambda_j(L)c_j(L)\right) \exp\left(\frac{z_c e}{kT} V\right)} \qquad (29)$$

where:

$$\lambda_j(L) = \frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}} \qquad (30)$$

$$= \exp\left[\frac{1}{kT} \left(\mu_j^{ex}(L \text{ bath}) - \mu_j^{ex}(\text{channel}) \right) \right] \qquad (31)$$

and:

$$\lambda_j(R) = \frac{\gamma_j^{bath}(R)}{\gamma_j^{chan}} \qquad (32)$$

$$= \exp\left[\frac{1}{kT} \left(\mu_j^{ex}(R \text{ bath}) - \mu_j^{ex}(\text{channel}) \right) \right] \qquad (33)$$

Conductance ratios

Conductance ratio experiments are usually done with the same salt on both sides of the channel. In that case, Eq. (29) simplifies to:

$$I = z_c \frac{e^2}{kT} \frac{A}{d} |q| D_j^{chan} V \qquad (34)$$

where, because the anion current is negligible, the total current I is:

$$I = e z_c J_j \qquad (35)$$

Thus in this experiment the uniform fixed charge channel (see "Assumptions" section) has a linear current/voltage relation with conductance:

$$g_j = \frac{dI}{dV} = z_c \frac{e^2}{kT} \frac{A}{d} |q| D_j^{chan} \qquad (36)$$

(Obviously this result is not true in general; the conductance is independent of the bath concentrations only in special cases. For example, this approximation is useful if the bath concentrations are high enough to make g_j a "saturated" conductance. Furthermore, conditions (25) and (26) must hold; that is, the bath concentrations must be much less than the fixed charge density of the channel. In the data fitting of channels

(Chen et al. 1997b, 1999; Nonner and Eisenberg 1998), q is 4–30 M and therefore the latter condition is generally not a constraint.)

Applying Eq. (36) to two separate experiments (one with cation species 1 on both sides of the channel and the other with cation species 2 on both sides) shows that the ratio of the conductances is an estimate of the ratio of the diffusion coefficients inside the channel:

$$\frac{g_2}{g_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} \tag{37}$$

Mole fraction experiment

In a mole fraction experiment, if cation species 1 has mole fraction r, then:

$$c_1(\text{L}) = c_1(\text{R}) = rc \tag{38}$$

and:

$$c_2(\text{L}) = c_2(\text{R}) = (1-r)c \tag{39}$$

where c is the total bath concentration. Because the ionic strength is maintained under the experimental conditions, it is plausible to assume that the activity coefficients of the two cations are approximately constant for all mole fractions; that is, for all mole fractions r, the activity coefficient of species 1 (species 2) is equal to its activity coefficient at mole fraction $r=1$ ($r=0$), when species 1 (species 2) is the only cation. Calculations using the mean spherical approximation (Simonin 1996; Barthel et al. 1998; Durand-Vidal et al. 2000) support this approximation and therefore we use it.

Equation (29) then gives:

$$\frac{dJ_1}{dV} = \frac{e}{kT}\frac{A}{d}D_1^{\text{chan}}|q|\frac{\lambda_1 r}{\lambda_1 r + \lambda_2(1-r)} \tag{40}$$

$$\frac{dJ_2}{dV} = \frac{e}{kT}\frac{A}{d}D_2^{\text{chan}}|q|\frac{\lambda_2(1-r)}{\lambda_1 r + \lambda_2(1-r)} \tag{41}$$

where:

$$\lambda_j = \frac{\gamma_j^{\text{bath}}}{\gamma_j^{\text{chan}}} \tag{42}$$

Even if γ_j^{bath} is independent of the mole fraction, by Eq. (21) each λ_j is a function of the mole fraction r; that is:

$$\lambda_j = \lambda_j(r) \tag{43}$$

Then the total conductance measured in the experiment is:

$$g(r) = z_c\frac{e^2}{kT}\frac{A}{d}|q|\frac{D_1^{\text{chan}}r\lambda_1(r) + D_2^{\text{chan}}(1-r)\lambda_2(r)}{r\lambda_1(r) + (1-r)\lambda_2(r)} \tag{44}$$

Since the mole fraction is between 0 and 1, there are two limiting conductances, when the solutions contain only one type of cation. These are:

$$g_1 = g(r=1) = z_c\frac{e^2}{kT}\frac{A}{d}|q|D_1^{\text{chan}} \tag{45}$$

when the solution contains only cation species 1, and:

$$g_2 = g(r=0) = z_c\frac{e^2}{kT}\frac{A}{d}|q|D_2^{\text{chan}} \tag{46}$$

when the solution contains only cation species 2. Equation (44) then becomes:

$$g(r) = \frac{g_1 r\lambda_1(r) + g_2(1-r)\lambda_2(r)}{r\lambda_1(r) + (1-r)\lambda_2(r)} \tag{47}$$

The midpoint mole fraction (MMF) (denoted r_{MMF}) defined by:

$$g(r_{\text{MMF}}) = \frac{1}{2}(g_1 + g_2) \tag{48}$$

can be determined by solving the implicit equation:

$$r_{\text{MMF}} = \frac{\lambda_2(r_{\text{MMF}})}{\lambda_1(r_{\text{MMF}}) + \lambda_2(r_{\text{MMF}})} \tag{49}$$

If both λ_j are independent of the mole fraction (Nonner and Eisenberg 1998; Chen et al. 1999), then Eq. (49) is explicit. In any case, Eq. (49) together with Eq. (42) shows that *the MMF is determined by the activity coefficients of the ions inside the channel*.

Figure 1 shows a mole fraction experiment [Eq. (47)] computed assuming the λ_j are independent of the mole fraction. In that case, our model channel does not exhibit an anomalous mole fraction effect; it does not have an extremum. However, the conductance versus mole fraction curve is nonlinear whenever:

$$\lambda_1 = \frac{\gamma_1^{\text{bath}}}{\gamma_1^{\text{chan}}} \neq \lambda_2 = \frac{\gamma_2^{\text{bath}}}{\gamma_2^{\text{chan}}} \tag{50}$$

or, equivalently:

$$\mu_1^{\text{ex}}(\text{bath}) - \mu_1^{\text{ex}}(\text{channel}) \neq \mu_2^{\text{ex}}(\text{bath}) - \mu_2^{\text{ex}}(\text{channel}) \tag{51}$$

Permeability ratios

We now turn to the most commonly used selectivity experiment, the measurement of the reversal potential to estimate the permeability of the channel to ions. This situation can be studied without the analytic approximation to the PNP equations derived in Appendix A. In particular, the analysis of this section does *not* require a channel with uniform fixed charge (see "Assumptions" section).

We start by writing the Nernst-Planck equation [Eq. (9)] in integral form (with no approximations) by integrating over the channel which lies in $(0,d)$:
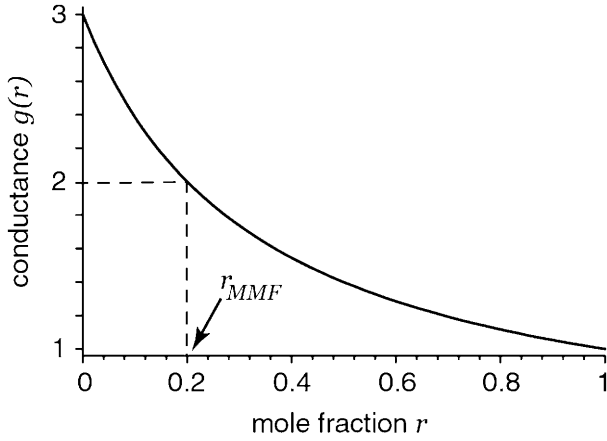
Fig. 1. Graph of Eq. (47) with $g_1 = 1$, $g_2 = 3$, and $\lambda_1/\lambda_2 = 4$. While this channel does not exhibit an anomalous mole fraction effect (it does not have an extremum), it is nonlinear. The curve has a midpoint mole fraction of $r_{MMF} = 1/(1 + \lambda_1/\lambda_2) = 0.2$

$$-J_j \int_0^d \frac{\gamma_j(s)}{D_j(s)A(s)} \exp\left(\frac{z_j e}{kT}\phi(s)\right) ds$$
$$= \gamma_j(d)c_j(d)\exp\left(\frac{z_j e}{kT}\phi(d)\right) - \gamma_j(0)c_j(0)\exp\left(\frac{z_j e}{kT}\phi(0)\right)$$
(52)

As discussed in the "Boundary conditions" section, we use the results of Gillespie and Eisenberg (2001) to evaluate the right-hand side of Eq. (52). Specifically, Eq. (70) of that paper shows that:

$$\gamma_j(0)c_j(0)\exp\left(\frac{z_j e}{kT}\phi(0)\right) \approx \gamma_j^{\text{bath}}(L)c_j(L)\exp\left(\frac{z_j e}{kT}V\right)$$
(53)

$$\gamma_j(d)c_j(d)\exp\left(\frac{z_j e}{kT}\phi(d)\right) \approx \gamma_j^{\text{bath}}(R)c_j(R)$$
(54)

and so Eq. (52) becomes:

$$-J_j \int_0^d \frac{\gamma_j(s)}{D_j(s)A(s)}\exp\left(\frac{z_j e}{kT}\phi(s)\right) ds$$
$$= \gamma_j^{\text{bath}}(R)c_j(R) - \gamma_j^{\text{bath}}(L)c_j(L)\exp\left(\frac{z_j e}{kT}V\right)$$
(55)

Therefore, for a channel with the GHK assumptions listed previously, we have:

$$-J_j \frac{\gamma_j^{\text{chan}}}{D_j^{\text{chan}}} \int_0^d \frac{\gamma(s)}{A(s)D(s)}\exp\left(\frac{z_c e}{kT}\phi(s)\right) ds$$
$$= \gamma_j^{\text{bath}}(R)c_j(R) - \gamma_j^{\text{bath}}(L)c_j(L)\exp\left(\frac{z_c e}{kT}V\right)$$
(56)

for cation species j. If there are two cation species 1 and 2 and the anion current is negligible, then at the reversal potential V_{rev}:

$$J_2 = -J_1$$
(57)

and:

$$-1 = \frac{J_2}{J_1}$$
$$= \frac{D_2^{\text{chan}}/D_1^{\text{chan}}}{\gamma_2^{\text{chan}}/\gamma_1^{\text{chan}}} \frac{\gamma_2^{\text{bath}}(R)c_2(R) - \gamma_2^{\text{bath}}(L)c_2(L)\exp\left(\frac{z_c e}{kT}V_{\text{rev}}\right)}{\gamma_1^{\text{bath}}(R)c_1(R) - \gamma_1^{\text{bath}}(L)c_1(L)\exp\left(\frac{z_c e}{kT}V_{\text{rev}}\right)}$$
(58)

Under the bi-ionic conditions of the permeability experiment, with only species 1 in the left bath ($c_2(L) = 0$) and only species 2 in the right ($c_1(R) = 0$), by Eq. (5) we have:

$$\frac{P_2}{P_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} \frac{\lambda_2}{\lambda_1}$$
(59)

where, for bath concentration c, we define:

$$\lambda_j = \frac{\gamma_j^{\text{bath}}(c)}{\gamma_j^{\text{chan}}}$$
(60)

This derivation of the GHK equation is mathematically similar to the one by Hille (2001), but includes more physics.

Determining model parameters

The results of the previous sections can be used to estimate the physical and chemical properties of ions in channels, given certain assumptions. Specifically, for each ion species j, the diffusion coefficient D_j^{chan} and the activity coefficient γ_j^{chan} can be determined from experimental measurements (assuming our model of the channel). Bath values of these parameters are known (Robinson and Stokes 1965; Conway 1969; Zematis et al. 1986) or can be calculated from separate theories (Simonin 1996; Barthel et al. 1998; Durand-Vidal et al. 2000).

In order to find the ion parameters, one must know the dimensions and fixed charge profile of the channel. For many channels the dimensions such as the radius and length of the channel (or the selectivity filter) are approximately known from experiments. The tertiary structure of the channel shows which amino acid side chains face the conduction pathway, when the structure is known. Genetic manipulations gives this information in other cases. The identity of the amino acid residues allows good estimates of the permanent charge q (perhaps depending on protonation state). When we assume uniformly spread fixed charge, q is given by the sum of the charges of the amino acid residues divided by the volume of the pore determined from the estimates of A and d.

It is important to note that if the channel contains both positively and negatively charged amino acid residues (voltage-gated sodium channels, for example), this approach should not be used; the spatial distribution of permanent charge in the channel has profound effects on

the qualitative and quantitative properties of the channel, including its current/voltage relations. This result is well known from the analogous analysis of semiconductors (Henisch 1984; Selberherr 1984; Jacoboni and Lugli 1989).

If the charge, length, and area of the channel (q, d, and A) are known, diffusion and chemical parameters can be determined from the conductance and mole fraction experiments. Equation (36) can be used to estimate the diffusion coefficient of species j from its saturated conductance g_j:

$$D_j^{\text{chan}} = \frac{kT}{e^2}\frac{d}{A}\frac{g_j}{z_j|q|} \tag{61}$$

Determining the channel activity coefficients (that is, excess chemical potentials or free energies) first requires assuming that these activity coefficients inside the channel are the same for all experiments; that is, we must assume that Eq. (21) is *not* true. In that case, the measurement of the midpoint mole fraction r_{MMF} allows an estimate of the relative channel activity coefficients through Eq. (49):

$$\frac{\gamma_2^{\text{chan}}}{\gamma_1^{\text{chan}}} = \frac{\gamma_2^{\text{bath}}}{\gamma_1^{\text{bath}}}\left(\frac{1}{r_{\text{MMF}}} - 1\right) \tag{62}$$

By using Eq. (62), the absolute channel activity coefficients of all the experimental ions can be determined if one knows the absolute activity coefficient of one ion. Nonner and Eisenberg (1998) do this by assuming the activity coefficient of protons is 1.

Discussion

Summary

We have examined three selectivity experiments (see above) in terms of the PNP electrodiffusion model. The GHK permeability ratio was examined for a fairly general class of channels (see "Assumptions" section above). The conductance ratio and conductance mole fraction experiments were treated with a simple model channel with a constant fixed charge density in the channel. For that model, we derived an approximate analytical solution to the PNP equations with Donnan boundary conditions (see "Boundary conditions" section).

The PNP model we consider [Eqs. (9) and (12)] contains two ion parameters: the diffusion coefficient and the excess chemical potential. Both take into account the size of the ions, but measure different physical properties; the diffusion coefficient measures the resistance a moving ion encounters, while the excess chemical potential measures the effects of the ions' packing and the electrostatic effects of such packing (Nonner et al. 2000), as well as the solvation energy (Nonner et al. 2001).

In the case of the model channel, the conductance ratio and mole fraction experiments each directly measure one of these two properties. Specifically, if g_j is the saturated conductance of cation j, then, for two cations, the ratio of these conductances is the ratio of the diffusion coefficients:

$$\frac{g_2}{g_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} \tag{63}$$

Furthermore, in the mole fraction experiment the midpoint mole fraction r_{MMF} (where the total conductance is half way between the limiting conductances) is determined by the ratio of the activity coefficients of the ions inside the channel:

$$r_{\text{MMF}} = \frac{1}{1 + \lambda_1/\lambda_2} \tag{64}$$

where:

$$\lambda_j = \frac{\gamma_j^{\text{bath}}}{\gamma_j^{\text{chan}}} \tag{65}$$

and the activity coefficient is linked to the excess chemical potential by Eq. (10). Lastly, the GHK permeability ratio is a combination of the diffusion and activity coefficients inside the channel:

$$\frac{P_2}{P_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}}\frac{\lambda_2}{\lambda_1} \tag{66}$$

Note that by Eq. (21) it is possible that the λ_j are functions of the concentration and in that case Eq. (64) is an implicit equation and the permeability ratio in Eq. (66) is concentration dependent. Even when the γ_j^{chan} are *not* functions of the mole fraction, the permeability ratio given by Eq. (66) is concentration dependent because the bath activity coefficients will vary with bath concentrations. For LiCl and KCl solutions this effect alone can give a permeability ratio at 2 M that is 60% larger than that at 100 mM (Robinson and Stokes 1965; Conway 1969; Zematis et al. 1986).

Although we invert these equations to give ion parameters (see "Determining model parameters" section), it must be understood that the equations depend on many assumptions designed to make this model analytically simple – and the inversion requires even more assumptions. For example, the inversion requires the unrealistic assumption that the excess chemical potentials are the same under all experimental conditions.

To do the inversion correctly, one needs a correct general theory and then a procedure based on that theory to measure the parameters of the channel. Solving this kind of "inverse" or "reverse engineering" problem sometimes yields formulas, as in this paper. Generally, however, parameters must be determined by the best fit between theory and experiment (Chen et al. 1997a, 1997b, 1999). Theories of how to calculate the excess chemical potentials of an electrolyte solution confined in complex geometries are only now being developed (Rosenfeld 1993; Rosenfeld et al. 1997) and do not yet

include flux. In this paper we use our simplified channel to try to make theoretical inroads into the physics and chemistry of selectivity.

Thermodynamics of selectivity

A closer examination of the derivation of the conductance ratio result [Eq. (36)] shows that each individual cation species conductance is proportional to its own diffusion coefficient when it is the only cation species in both baths:

$$g_j = z_j \frac{e^2}{kT} \frac{A}{d} |q| D_j^{\text{chan}} \tag{67}$$

This is true with either symmetric or asymmetric bath concentrations.

It is interesting to note that the *conductance does not depend on the activity coefficient of the cation*. The reason is that the inside of the channel is (nearly) charge neutral [see Eq. (96)] [Gillespie and Eisenberg (2001) give a detailed discussion]. Because the channel protein is negatively charged with charge density q, it attracts sufficient cations to neutralize (that is, screen) its own charge. Since charge neutrality is the end result, the specifics of the cations' packing and their dehydration energy (which are measured by the activity coefficient) are unimportant. In this case, the only difference between cations in two experiments is the resistance they experience as they move through the channel. That is measured by the diffusion coefficient alone.

When there are several types of cations to screen the fixed charge of the channel protein, then, for each ion species, the difference between its electrochemical potentials in the baths and inside the channel determines how well the ion partitions into the channel (Nonner et al. 2000, 2001). In general, the more negative the electrochemical potential of an ion is (either in the bath or in the channel), the more energy it takes to remove it and any difference in the electrochemical potential between two compartments is a driving force on the ion.

As discussed in "The PNP equations" section, the local electrochemical potential of ion species j has two components, ideal (id) and excess (ex) (Nonner et al. 2000):

$$\mu_j(x) = \mu_j^{\text{id}}(x) + \mu_j^{\text{ex}}(x) \tag{68}$$

where:

$$\mu_j^{\text{id}}(x) = z_j e\phi(x) + kT\ln\left[c_j(x)\right] \tag{69}$$

Here $\phi(x)$ is the electrostatic potential and $c_j(x)$ is the concentration of species j at position x. With these definitions the electrochemical potential difference between two points x_1 and x_2 can be split into two parts:

$$\Delta\mu_j(x_1, x_2) = \Delta\mu_j^{\text{id}}(x_1, x_2) + \Delta\mu_j^{\text{ex}}(x_1, x_2) \tag{70}$$

where:

$$\Delta\mu_j^{\text{id}}(x_1, x_2) = \mu_j^{\text{ex}}(x_1) - \mu_j^{\text{id}}(x_2) \tag{71}$$

$$= z_j e[\phi(x_1) - \phi(x_2)] + kT\ln\left(\frac{c_j(x_1)}{c_j(x_2)}\right) \tag{72}$$

and:

$$\Delta\mu_j^{\text{ex}}(x_1, x_2) = \mu_j^{\text{id}}(x_1) - \mu_j^{\text{ex}}(x_2) = kT\ln\left(\frac{\gamma_j(x_1)}{\gamma_j(x_2)}\right) \tag{73}$$

Now suppose that we take x_1 to be in the left bath and $x_2 = 0$ (the left edge of the channel) to give:

$$\Delta\mu_j(\text{L}, 0) = z_j e[V - \phi(0)] + kT\ln\left(\frac{c_j(\text{L})}{c_j(0)}\right) + kT\ln\left[\lambda_j(\text{L})\right] \tag{74}$$

By Eq. (70) of Gillespie and Eisenberg (2001), we have:

$$z_j e[V - \phi(0)] + kT\ln\left(\frac{c_j(\text{L})}{c_j(0)}\right) \approx kT\ln\left(\frac{\gamma_j^{\text{chan}}(0)}{\gamma_j^{\text{bath}}(\text{L})}\right)$$
$$= -kT\ln\left[\lambda_j(\text{L})\right] \tag{75}$$

where $\gamma_j^{\text{chan}}(0)$ is the activity coefficient just inside the left side of the channel. Therefore:

$$\Delta\mu_j(\text{L}, 0) \approx 0 \tag{76}$$

that is, the (left) bath and the channel are close to equilibrium. A similar results holds for the right bath if we take x_1 in the right bath and $x_2 = d$ (the right edge of the channel):

$$\Delta\mu_j(\text{R}, d) \approx 0 \tag{77}$$

This near-equilibrium situation then allows us to determine the free energy of partitioning for each ion species from the left and right baths into the channel:

$$s_j(\text{L}) = \ln\left(\frac{c_j(0)}{c_j(\text{L})}\right) \approx \frac{z_j e}{kT}[V - \phi(0)] + \ln\left[\lambda_j(\text{L})\right] \tag{78}$$

$$s_j(\text{R}) = \ln\left(\frac{c_j(d)}{c_j(\text{R})}\right) \approx -\frac{z_j e}{kT}\phi(d) + \ln\left[\lambda_j(\text{R})\right] \tag{79}$$

Each s_j is then in energy units of kT. If $s_j(\text{L}) > 0$, then species j partitions into the channel from the left bath and similarly for the right bath.

Therefore, one quantitative measure of selectivity of species 1 and 2 is:

$$\Lambda_{1,2} = \exp\left(\frac{1}{2}\{s_1(\text{L}) + s_1(\text{R}) - [s_2(\text{L}) + s_2(\text{R})]\}\right) \tag{80}$$

$$\approx \exp\left(\frac{e}{kT}\frac{z_1 - z_2}{2}[V - \phi(0) - \phi(d)]\right)\left(\frac{\lambda_1(\text{L})\lambda_1(\text{R})}{\lambda_2(\text{L})\lambda_2(\text{R})}\right)^{1/2} \tag{81}$$

In the case that $z_1 \neq z_2$, the terms $V - \phi(0)$ and $\phi(d)$ may be calculated from Eq. (68) of Gillespie and Eisenberg (2001) or can be approximated more coarsely as Donnan potentials (Nonner et al. 2000, 2001). If $\Lambda_{1,2} > 1$, then one can infer that species 1 partitions more easily into the channel compared to species 2.

In the specific case considered in this paper, we have $z_1 = z_2$ and:

$$\frac{\gamma_j^{\text{chan}}(0)}{\gamma_j^{\text{chan}}(d)} = 1 \tag{82}$$

For the mole fraction (mf) experiment:

$$\gamma_j^{\text{bath}}(\text{L}) = \gamma_j^{\text{bath}}(\text{R}) \equiv \gamma_j^{\text{bath}} \tag{83}$$

and therefore:

$$\Lambda_{1,2}^{\text{mf}} = \frac{\lambda_1}{\lambda_2} \tag{84}$$

For the bi-ionic reversal potential experiment, on the other hand:

$$\gamma_2^{\text{bath}}(\text{L}) = \gamma_1^{\text{bath}}(\text{R}) = 1 \tag{85}$$

and so:

$$\Lambda_{1,2}^{\text{GHK}} = \left(\frac{\gamma_2^{\text{chan}}}{\gamma_1^{\text{chan}}} \frac{\lambda_1}{\lambda_2} \right)^{1/2} = \frac{\lambda_1}{\lambda_2} \left(\frac{\gamma_2^{\text{bath}}(\text{R})}{\gamma_1^{\text{bath}}(\text{L})} \right)^{1/2} \tag{86}$$

The partitioning selectivity in the mole fraction experiment [Eq. (84)] is different from the partitioning selectivity in the reversal potential experiment [Eq. (86)] because the measurements are made under different conditions. Under bi-ionic conditions, species 1 can only partition in from the left bath and species 2 only from the right bath, while in the mole fraction experiment, both species partition from both baths. However, the term:

$$\left(\frac{\gamma_2^{\text{bath}}}{\gamma_1^{\text{bath}}} \right)^{1/2} \tag{87}$$

is usually close to 1 if the two baths are at the same concentration; for the rather extreme case of 2 M KCl and 2 M LiCl, it less than 1.3 (Robinson and Stokes 1965; Conway 1969; Zematis et al. 1986). Therefore, the partitioning selectivities are approximately the same in the mole fraction and reversal potential experiments [with the value given by Eq. (84)] if the channel activity coefficients between the two experiments are not too different [Eq. (21)].

This difference in the partitioning selectivity $\Lambda_{1,2}$ between the two experiments is one example of the variability of the partitioning selectivity; the partitioning selectivity $\Lambda_{1,2}$ is *not* a constant for the two species 1 and 2. $\Lambda_{1,2}$ not only contains the bath activity coefficients of both ions that vary with bath concentration, but it also contains the channel activity coefficients. The channel activity coefficients γ_j^{chan} have several components with different physical and chemical origins. First, there are terms that measure the excluded volume (entropic) interactions arising because two ions cannot occupy the same space. Intuitively one might think that the smaller ion would always be favored because it is seemingly easier to pack into an already crowded volume. However, density functional theory studies of uncharged systems have shown that in various confined geometries *larger spheres can be selected solely on the basis of excluded volume* (Goulding et al. 2000, 2001). γ_j^{chan} also depends on electrostatic effects that can be different even for ions of the same valence (Nonner et al. 2000) and solvation effects that measure the difficulty of removing an ion from the bath (Nonner et al. 2001). Each of these components can change for each ion species, depending on the exact experimental conditions.

Although the partitioning selectivity $\Lambda_{1,2}$ is not a constant for the two cation species, we can use it to divide selectivity into equilibrium and nonequilibrium components. Specifically, because each side of the channel is in near equilibrium with the bath on that side of the channel [Eqs. (76) and (77)], the partitioning selectivity $\Lambda_{1,2}$ as defined in Eq. (80) is an (approximately) equilibrium quantity. However, as the conductance ratio and permeability results suggest, there is a nonequilibrium component to selectivity as well, namely the ratio of the diffusion coefficients of the cations inside the channel. We will call this the diffusion selectivity (it is possible, of course, that that the formula for the diffusion selectivity is more complex in channels for which our assumptions do not hold). Both of the equilibrium and nonequilibrium components are necessary to determine the function of the channel; the partitioning selectivity measures the energetic differences that determine the contents of the channel, while the diffusion selectivity measures the relative resistance encountered by the ions moving through the confined geometry of the channel.

An interesting consequence of separating selectivity into equilibrium (partitioning) and nonequilibrium (diffusion) components is the possibility that these two components can have opposite, perhaps balancing, effects. For example, ion species 1 might partition more easily into the channel (compared to ion species 2), but might have a lower diffusion coefficient in the channel. In that case:

$$\Lambda_{1,2} > 1 \quad (\text{partitioning favors 1}) \tag{88}$$

while:

$$\frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} > 1 \quad (\text{diffusion favors 2}) \tag{89}$$

An example of such a case is the ryanodine receptor with Li^+ and K^+ as species 1 and 2, respectively (Chen et al. 1999).

For our model channel, both the conductance and mole fraction experiments give clear measures of the diffusion and partitioning selectivities, respectively, if the activity coefficients inside the channel are assumed constant:

$$\frac{g_2}{g_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} > 1 \qquad (90)$$

$$r_{\text{MMF}} = \frac{1}{1 + \Lambda_{1,2}} < \frac{1}{2} \qquad (91)$$

The GHK permeability ratio, however, gives a composite and thus ambiguous measure of the diffusion and partitioning selectivities:

$$\frac{P_2}{P_1} = \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} \frac{\lambda_2}{\lambda_1} \approx \frac{D_2^{\text{chan}}}{D_1^{\text{chan}}} \frac{1}{\Lambda_{1,2}} \qquad (92)$$

and it may be less than or greater than 1. This ambiguity shows that the GHK permeability ratio is not an ideal indicator of which ion is preferred by the channel. The GHK permeability ratio does, however, measure the relative size of the partitioning and diffusion selectivities.

Conclusion

The work in this paper is a first extrapolation of the selectivity theory introduced by Nonner et al. (2000, 2001). There the authors considered only an equilibrium situation, but calculated the excess chemical potentials from the concentrations of all the ions to show how both charge interactions and the space available to the ions affect selectivity. Here, we showed that even the simplest implementation of the charge/space competition idea in a flux model offers new ways to measure selectivity of a channel using classical experimental protocols. Consideration of the detailed properties of charge/space competition is likely to lead to other methods of measuring the physical properties of open ionic channels and more insight into the physics and chemistry of selectivity.

Appendix

Appendix A: Approximate solution to the PNP equations

Here we derive the approximate analytic solution to the PNP equations using all the assumptions mentioned previously (see above). We start by rewriting the PNP equations (12) and (9) for the inside of the channel with these assumptions:

$$-\epsilon \frac{\mathrm{d}^2\phi}{\mathrm{d}x^2} = \sum_j z_j c_j + q \qquad (93)$$

$$-J_j = D_j A \left(\frac{\mathrm{d}c_j}{\mathrm{d}x} + \frac{z_j e}{kT} c_j \frac{\mathrm{d}\phi}{\mathrm{d}x} \right) \qquad (94)$$

with the channel located on the interval $(0, d)$.

For boundary conditions we will use the classic Donnan boundary conditions of Eqs. (13, 14, 15, 16, 17, 18). In general we do not require all of the specific details of these boundary conditions, but only the fact that due to Eq. (15) the channel is charge neutral at the edges; that is:

$$0 = \sum_j z_j c_j(0) + q(0) = \sum_j z_j c_j(d) + q(d) \qquad (95)$$

In this analysis we could use any boundary conditions which satisfy this condition. However, for channels the Donnan boundary conditions are the only boundary conditions we are aware of that give charge neutrality at the edges.

We will show that when we use boundary conditions for the concentrations that include charge neutrality and when the anion concentration is negligible, the exact solution of the PNP equations follows by requiring charge neutrality everywhere inside the channel; that is:

$$0 = \sum_j z_j c_j + q \qquad (96)$$

or, equivalently by the Poisson equation (93):

$$\frac{\mathrm{d}^2\phi}{\mathrm{d}x^2} = 0 \qquad (97)$$

throughout the channel. We show this by deriving a general condition that must be satisfied whenever condition (97) is assumed.

For the moment we do not assume that q is constant, but some function of x. By Eq. (97), ϕ is linear and necessarily:

$$\phi(x) = [\phi(d) - \phi(0)] \frac{x}{d} + \phi(0) \qquad (98)$$

Integrating the Nernst-Planck equation (94) gives:

$$J_j = -z_j \frac{A}{d} D_j \psi \frac{c_j(d) - c_j(0) \exp(-z_j \psi)}{1 - \exp(-z_j \psi)} \qquad (99)$$

where:

$$\psi = \frac{e}{kT} [\phi(d) - \phi(0)] \qquad (100)$$

Substituting Eq. (99) into the Nernst-Planck equation (94) gives:

$$z_j \frac{\psi}{d} \frac{c_j(d) - c_j(0) \exp(-z_j \psi)}{1 - \exp(-z_j \psi)} = \frac{\mathrm{d}c_j}{\mathrm{d}x} + z_j c_j \frac{\psi}{d} \qquad (101)$$

whose solution is:

$$c_j(x)$$
$$= \frac{c_j(d) - c_j(0) \exp(-z_j \psi)}{1 - \exp(-z_j \psi)} - \frac{c_j(d) - c_j(0)}{1 - \exp(-z_j \psi)} \exp\left(-z_j \psi \frac{x}{d}\right) \qquad (102)$$

Equation (102) is a solution for the concentrations if it satisfies the Poisson equation (93). Therefore, substituting Eq. (102) into Eq. (96) shows that:

$$0 = q(x) + \sum_j z_j \left(\frac{c_j(d) - c_j(0)\exp(-z_j\psi)}{1 - \exp(-z_j\psi)} + \frac{c_j(0) - c_j(d)}{1 - \exp(-z_j\psi)}\exp\left(-z_j\psi\frac{x}{d}\right) \right) \quad (103)$$

Therefore any time one assumes that ϕ is linear, the measure of how good is this approximation is how close the right-hand side of Eq. (103) is to zero for all x (in an absolute sense if all concentrations are measured in molar). If the right-hand side is not small for some region of x, then there is a region of space charge. In that case, the original assumption of charge neutrality is violated and, by the Poisson equation (93), the potential ϕ is no longer linear. This, in turn, will invalidate the predicted concentration profiles and current.

In the case that we consider where q is constant inside the channel, the co-ions have negligible concentration inside the channel, and all the counter-ions are of the same valence, then Eq. (103) is zero because we assumed that the channel was charge neutral at the edges. Therefore, up to these approximations, Eqs. (98) and (102) are an exact solution to the PNP equations.

Appendix B: Low concentration approximation of Donnan potentials

If the cations all have valence z_c and the anions have valence -1, we will show that:

$$\exp\left(\frac{z_c e}{kT}\Psi_L\right) \approx \frac{z_c}{|q|}\sum_{z_j = z_c} \frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}}c_j(L) \quad (104)$$

To start, by Eq. (15) the Donnan potential Ψ_L must satisfy:

$$0 = NY^{z_c+1} - qY^{z_c} - z_c P \quad (105)$$

where:

$$P = \sum_{z_j = z_c} \frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}}c_j(L) \quad (106)$$

$$N = \sum_{z_j = -1} \frac{\gamma_j^{bath}(L)}{\gamma_j^{chan}}c_j(L) \quad (107)$$

$$Y = \exp\left(\frac{e}{kT}\psi_L\right) \quad (108)$$

(we show the work on the left side of the channel. Similar formulas are valid for the right side of the channel by substituting R for L). For cation channels, $q < 0$, and so we have:

$$0 = \alpha\varepsilon Y^{z_c+1} + Y^{z_c} - z_c\varepsilon \quad (109)$$

where we have dropped the L subscript:

$$\alpha = \frac{N}{P} \quad (110)$$

and:

$$\varepsilon = \frac{P}{|q|} \ll 1 \quad (111)$$

Rewriting Eq. (109) as:

$$Y^{z_c} = z_c\varepsilon - \alpha\varepsilon Y^{z_c+1} \quad (112)$$

we find that the right-hand side is of order ε and so $Y^{z_c} = O(\varepsilon)$ (Kevorkian and Cole 1996). In that case:

$$\alpha\varepsilon Y^{z_c+1} = O\left(\varepsilon^{2+1/z_c}\right) \quad (113)$$

and therefore:

$$Y^{z_c} = z_c\varepsilon + O\left(\varepsilon^{2+1/z_c}\right) \quad (114)$$

which is equivalent to Eq. (104).

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