

ION CHANNELS - Volume 2

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CHAPTER 7

ANALYTICAL DIFFUSION MODELS FOR MEMBRANE CHANNELS

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1. INTRODUCTION

Evidence gained from such techniques as patch clamp, membrane protein reconstitution, site-directed mutagenesis, and electron microscopy yields a picture of a channel as a globular transmembrane protein containing a water-filled pore. This pore provides a low-energy pathway through which ions may pass, producing a current. This current is modulated by a process known as gating. The mechanism of gating involves some poorly understood conformational transition in the channel protein that necessarily results in a large change in the energy barrier to ion transport. Many factors in the environment of the protein affect the gating process, including transmembrane voltage, membrane distortion, and binding of specific molecules such as chemical transmitters and channel modulators. Hence, the transmembrane current of cells is determined by the number and type of channels, the probability that a channel is open (gating), and the mean open channel current (for a general reference to channel phenomenology, see Hille, 1984). This work will be specifically directed at this last factor, analyzing the current through the open channel.

Historically, this process has been modeled in two different ways: through the use of hopping models (Eyring *et al.*, 1949; Heckman, 1965a,b; Lauger, 1973) and through electrodiffusion models (Goldman, 1943; Hodgkin and Katz, 1949; Levitt, 1986). Before 1955, these two approaches

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shared a roughly equal footing with no obvious theoretical or practical reason to prefer one over the other. That situation changed with the experimental work of Hodgkin and Keynes (1955) in which they showed that ions do not move through channels independently of one another, as had been assumed in all models up to that point.

Various attempts to include nonindependent ion movement in channel transport models followed. The first truly successful models were presented by Heckmann (1965a,b, 1968, 1972). These models made use of the concept of discrete ion occupancy states and transitions between the states (see also Urban and Hladky, 1979). This approach lent itself naturally to the use of Eyring rate theory for specifying the transition rates in terms of channel structure and applied transmembrane voltage (Lauger, 1973). Thus, channel theory entered a period in which the preferred models were based on an Eyring theory version of ions hopping over barriers.

Levitt (1982) showed how to combine a diffusional model for ion movement with the concept of discrete occupancy states. This led to diffusional models that incorporated nonindependent ion movement (Levitt, 1986, 1987; Gates *et al.*, 1987). In this chapter we will review electrodiffusion models and present some further generalization and interpretation of that work as applied to the simple case of a channel that can hold at most one ion at a time. A few channel types are thought to behave this way over a wide concentration range, but any channel would behave this way in the low concentration limit. Also this is the simplest realistic channel model and so is valuable for obtaining insight into the behavior of ion channels in general. Levitt (1987) has analyzed the two-ion case using numerical techniques.

2. DERIVATION OF ONE-ION CHANNEL DIFFUSION THEORY

We begin by assuming that transport through each pore in a membrane is independent of the other pores present. This assumption is the simplest case and for many situations is the complete solution. In favorable situations the patch clamp technique allows for the direct measurement of the transport properties of a single pore in isolation. One could then correct these data for the influence on the concentration at the mouth of one channel due to the flux through adjacent channels. This aspect of the problem will not be considered here; for a discussion of these issues, consult Lauger (1976).

The essential geometry of an ion channel can be represented in three spatial domains. Two of these domains are the semi-infinite volumes on either side of the channel which provide access to the channel interior. The third domain is the channel interior itself. Many of the properties of pore transport are a consequence of the transition from an r^2 dependence of the cross-sectional area in the bath to an essentially uniform cross-sectional area in the channel itself. The physical model used here for these different domains is the classical theory of electrodiffusion (Bockris and Reddy, 1970; Sten-Knudsen, 1978).

A model of the entire system can be obtained by combining the equations for transport in these three domains through the use of the steady-state assumption. This assumption allows one to equate the time-averaged fluxes through the three respective spatial domains. This in turn allows the representation of the channel current in terms of the properties of the bath where measurements can be made at a point distant from the channel. The justification of this assumption comes from a comparison of the relative time scales of the relaxation to steady state and the mean channel open time. Estimates of the relaxation times are on the order of tens of nanoseconds (Cole, 1965; Hille, 1984), while mean channel open times are on the order of milliseconds. Thus, the steady state dominates the functional properties of the channel (see Section 7.1.3).

2.1. Independence

We will first derive what will be designated the independence flux; this is the flux that would occur in a system without ion-ion interactions. In this case, occupancy states will not be needed since the rate at which ions enter the channel will be assumed independent of the occupancy state or the past history of the channel. We will then see how the derivation of the independence flux can be modified to include the effect of interactions between ions in the channel and ions in the bath. Since we are only interested in the movement of ions in the direction normal to the membrane, the problem can be reduced to one dimension by defining a coordinate x to be the position of the ion projected onto a line extending from the left opening of the pore to the right opening. The differential equation on which the model is based is the traditional Nernst-Planck equation, where the concentration $n(x)$ is the probability density per unit volume for finding an ion in the neighborhood of x (for a summary of the symbols used, see Section 9):

$$-J = D(x)A(x) \left[\frac{dn}{dx} + \frac{d\varphi}{dx} \right] \quad (1)$$

where J is the current through a single channel (ions/sec) and $\varphi(x) = U(x)/kT$. The dimensionless potential includes the ensemble average of the interaction between an ion and the channel-solvent system and other ions. We further assume that the channel is a straight-walled pipe [the cross-sectional area $A(x)$ is a constant] and that the diffusion coefficient does not depend on the position in the channel. In the steady state, Eq. (1) can be integrated from the left end of the channel ($x = 0$) to the right end of the channel ($x = \delta$) through the use of the integrating factor $e^{\varphi(x)}$ to yield

$$-J_i = \frac{DA[n(\delta)e^{\varphi(\delta)} - n(0)e^{\varphi(0)}]}{\int_0^\delta e^{\varphi(x)} dx} \quad (2)$$

where D is the diffusion coefficient in the channel and J_i represents the channel flux in the absence of ion-ion interactions. If one assumes that the rate of ion entry and exit is fast relative to the rate at which ions move through the channel, then Eq. (2), together with a model for the difference in potential between the bath and channel ends, would yield the desired independence flux. This would be equivalent to an assumption of quasi-equilibrium between the bath and the channel, and then the product of the concentrations and exponential of the potentials (i.e., the electrochemical potential) could be equated with the bath values. Applying these results to the bath on either side of the channel yields

$$-J_i = \frac{DA[n(\infty)e^{\varphi(\infty)} - n(-\infty)e^{\varphi(-\infty)}]}{\int_n^\delta e^{\varphi(x)} dx} \quad (3)$$

For illustrative purposes, consider the simplest example of the above equation. This occurs when the channel has a uniform internal structure and the applied transmembrane potential falls linearly within the channel according to

$$\varphi(x) = \Delta\varphi x/\delta + \Delta\varphi_b \quad (4)$$

where $\Delta\varphi$ is the transmembrane voltage [$\Delta\varphi = \varphi(\infty) - \varphi(-\infty)$, $\varphi(-\infty) = 0$] and $\Delta\varphi_b$ is the potential change that occurs in moving an ion from the bath into the channel. These two assumptions yield what is commonly known as the constant-field assumption. It is one of the few examples for which the integral in the denominator can be evaluated analytically. Also make the simplifying assumption that the concentrations on the two sides of the channel are the same [$n(-\infty) = n(\infty) = n$]. With these assumptions the following simple, well-known equation results:

$$-I_i = \frac{1}{\delta} zqnDA\Delta\varphi e^{-\Delta\varphi_b} \quad (5)$$

Here $I_i = zqJ_i$, z is the ion valence, and q is the electronic charge. In this case the current-voltage curves are linear and the conductance does not saturate with concentration.

In general, we would not expect the exchange of ions between the channel and bath to be much faster than the rate at which ions move through the channel. In particular, the rate at which ions move through the channel would depend on the applied electrical potential difference; hence, at large potential differences the quasi-equilibrium assumption would break down even if it were valid at small potentials. We therefore need a model for the rate at which ions enter the channel. For this process we use the radial Nernst-Planck equation:

$$-J = 2\pi r^2 D_b \left[\frac{dn}{dr} + n \frac{d\varphi}{dr} \right] \quad (6)$$

Here we have assumed that the diffusion coefficient in the bath, D_b , is constant. At this point we will further assume that the flux in the bath is in the steady state. Equation (6) can be integrated with respect to the radial coordinate through the use of the integrating factor $e^{\varphi(r)}$ to obtain

$$-J_i = \frac{D_b [n(b)e^{\varphi(b)} - n(a)e^{\varphi(a)}]}{\frac{1}{2\pi} \int_a^b r^{-2} e^{\varphi(r)} dr} \quad (7)$$

We have specified the value of the concentration and potential at the boundaries $r = a$ and $r = b$. For bounded φ , the limit of Eq. (7) can be taken as b goes to infinity. We can now use Eq. (7) for the rate at which ions enter the channel by defining a capture radius ρ (Lauger, 1976) within which ions are considered to have entered the channel. Equation (7) can then be integrated from the capture radius ($r = \rho$) through the bath ($r \rightarrow \infty$) and solved for the electrochemical potential at the capture radius to yield

$$n(\rho)e^{\varphi(\rho)} = n(\infty)e^{\varphi(\infty)} + (J/k_f)e^{\Delta\varphi} \quad (8)$$

$$\frac{1}{k_f} e^{\Delta\varphi} = \frac{1}{2\pi D_b} \int_{\rho}^{\infty} r^{-2} e^{\varphi(r)} dr \quad (9)$$

We have assumed in Eq. (9) that the potential in the bath on the right end of the channel can be written as $\varphi(r) = \varphi^{\sim}(r) + \Delta\varphi$ with φ^{\sim} the potential when $\Delta\varphi = 0$. This is equivalent to assuming that the membrane capacitive charge has infinitesimal thickness. By convention we take the bath on the right end of the channel to be at positive radial coordinates; hence, Eq. (8) yields the concentration at the right end of the channel. The concentration at the left end of the channel is obtained by integrating Eq. (7) from negative infinity to the capture radius at the left end of the channel and again solving for the electrochemical potential:

$$n(-\rho)e^{\varphi(-\rho)} = n(-\infty) - (J/k_f) \quad (10)$$

Here we have assumed k_f to be the same in both baths.

Now we seek an expression for the flux through a channel connected to the bath. We equate the concentrations at the capture radii with the concentrations in the channel at $x = 0$ and $x = \delta$. Equations (3), (8), and (10) can then be combined and solved for the flux to yield

$$-J_i = \frac{n(\infty)e^{\Delta\varphi} - n(-\infty)}{\frac{1}{k_f} (e^{\Delta\varphi} + 1) + \frac{1}{AD} \int_0^{\delta} e^{\varphi(x)} dx} \quad (11)$$

This equation then generalizes the independence flux to include the effects of the bath.

In order to show the consequences of this generalization we need to specify $\varphi(r)$. We assume that $\varphi(r)$ is a $1 k_b T$ barrier to entrance that represents the effects of partial dehydration of the ion as it enters the channel. Our constant-field expression then becomes

$$-I_i = nk_f \frac{zq(e^{\Delta\varphi} - 1)}{e^{\Delta\varphi} + 1 + (k_f \delta e^{\Delta\varphi b} / AD\Delta\varphi)(e^{\Delta\varphi} - 1)} \quad (12)$$

Figure 1 shows the potential function we have assumed for the system. The effects this has on the current–voltage relation are seen in Fig. 2. Note that the main effect is to decrease the current at large voltages. This occurs because eventually the current through the channel becomes limited by the rate at which the baths can supply ions to the channel mouth. Since ion movement in the bath is voltage independent, the current saturates at a fixed value given by

$$|I_i| = zqnk_f \quad (13)$$

2.2. Nonindependence

We now relax the assumption of independence. The channel environment involves chemical groups that are constrained by the structure of the protein and may therefore stabilize positive charge over negative or vice versa. This fact has been experimentally verified repeatedly in biological channels (see Hille, 1984). This gives rise to an asymmetry in the interaction between ions in the bath and ions in the channel since ions of like charge in the bath are shielded by counterions. We therefore assume that an ion in the channel strongly influences other ions in the channel and in the bath access regions while ions in the bath weakly influence the concentration of ions in the channel. We will consider the simplest consequence of this asymmetry, where at most one ion may enter the channel at a time (see Section 7.1.2).

One-Ion Channel

We proceed by considering that the channel can be characterized by two distinct states. The first state is associated with the channel being occupied by one ion and the second state is associated with the channel being empty. In defining these states we will generate a means by which boundary conditions can be obtained for a flux equation in the channel. The procedure will involve writing flux equations for each of the three regions of the channel system; the bath access regions and channel itself. We will then make the steady-state assumption and match the boundaries through the aid of the

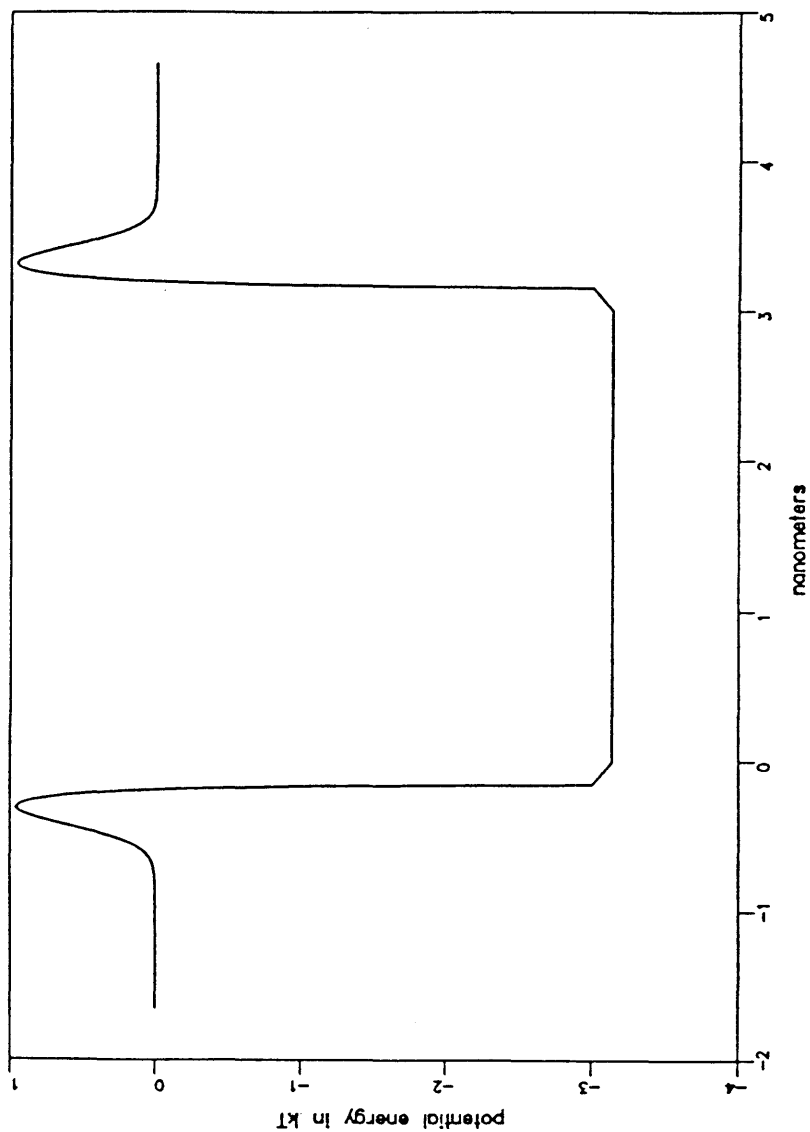


FIGURE 1. The potential profile in a 3-nm channel under the assumption of a constant field. The profile includes a 1k_BT barrier in the bath to model the effects of the partial dehydration of the permeant ion on entering the channel. The offset of the potential is necessary to give a 340 mM half saturation in the small signal conductance.

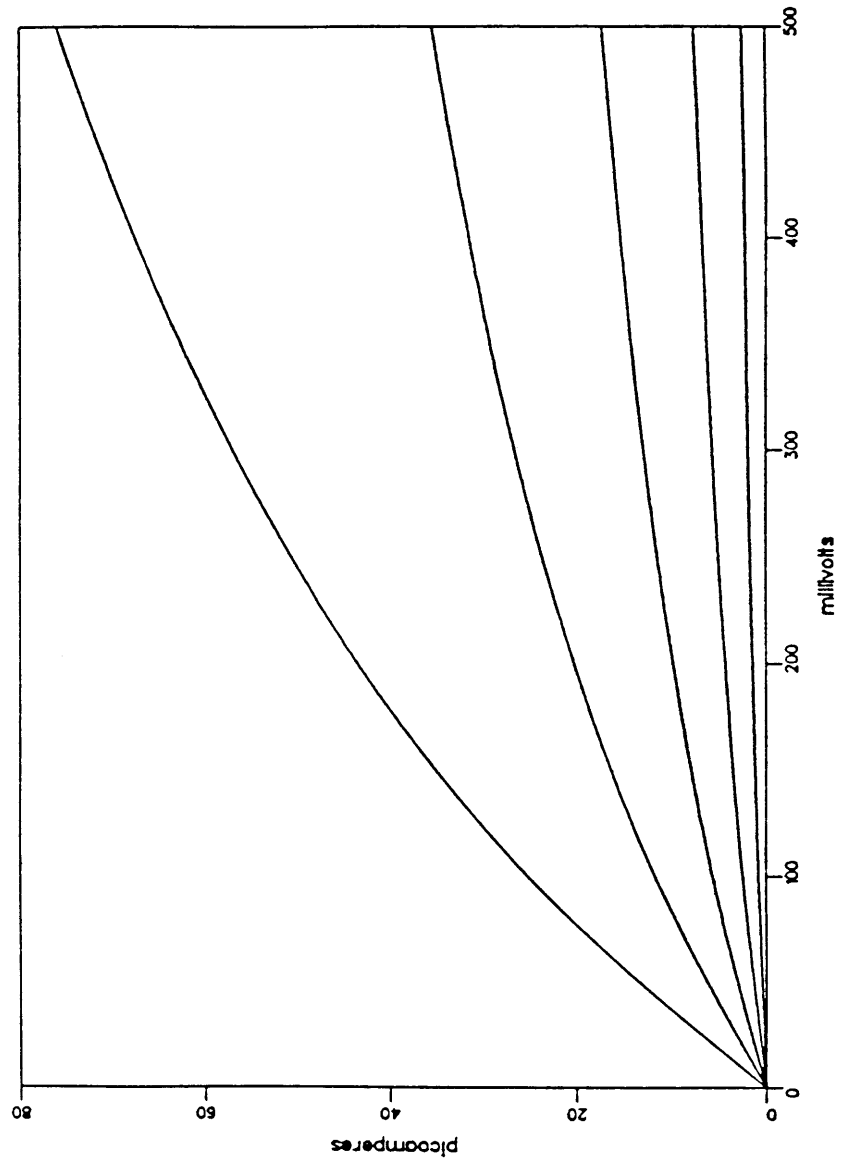


FIGURE 2. The independence current through the constant-field channel when bath access effects are included. The bath diffusion constant was assumed to be approximately that of sodium in solution (1.5×10^{-9} m²/sec). The channel diffusion constant was taken to be 0.1×10^{-10} m²/sec. The concentrations are symmetric and from top to bottom are 1.5, 0.7, 0.34, 0.15, and 0.05 M.

channel occupancy states. Strictly speaking, this procedure is not rigorous. In fact, the more rigorous procedure would be to obtain the boundary conditions of the time-dependent Nernst–Planck equation and then average to obtain the steady state. This is a more difficult problem and will not be considered here. The matching of the boundaries after having made the steady-state assumption is equivalent to assuming that the flux in the bath instantaneously reaches the steady state after an ion leaves the channel. A more detailed treatment of this last assumption is given in Section 7.1.3.

In order to construct the steady state in the bath we note that the bath flux is given by the product of the flux in the the bath when the channel is empty (J_0) times the probability the channel is empty (P_0) plus the flux in the bath when the channel is occupied (J_1) times the probability the channel is occupied ($1 - P_0$); thus, we have

$$J = J_0P_0 + J_1(1 - P_0) \quad (14)$$

(A more detailed argument is given in Gates, 1988.) The assumption that the channel will admit at most one ion implies that no additional ion can enter when the channel is occupied, thus $J_1 = 0$. Thus, the flux in the bath averaged over all time is given by

$$-J = -J_0P_0 = P_0 \frac{n(\infty)e^{\Delta\phi} - n_0(\rho)e^{\psi(\rho)}}{\frac{1}{2\pi D_b} \int_{\rho}^{\infty} r^{-2} e^{\psi(r)} dr} \quad (15)$$

where $n_0(\rho)$ is the concentration at the capture radius $r = \rho$, on the right end of the channel averaged over those times when the channel is empty. Notice that J_0 is exactly the previously defined independence flux. At this point we must link these results to the flux expression in the channel. We assert that the electrochemical potential must be continuous at ρ :

$$n(\rho)e^{\psi(\rho)} = n(\delta)e^{\psi(\delta)} \quad (16)$$

This is a consequence of the fact that a gradient in the electrochemical potential is the driving force for flux. Thus, a discontinuity in this potential would give rise to an infinite flux.

As with the flux, the above concentrations can be represented in terms of a weighted average of the concentrations during those times the channel is empty (n_0) and occupied (n_1) yielding

$$n(\rho) = n_0(\rho)P_0 + n_1(\rho)(1 - P_0) \quad (17)$$

Consider the case when the channel is already occupied by an ion. Under the one-ion assumption, there is some potential of interaction between an ion in the channel and ions in the bath such that additional ions are not allowed to

enter. If we assume that the concentration is continuous, then this implies that the concentration just outside the channel, i.e., $n_1(\rho)$, is zero, thus

$$n(\rho) = n_0(\rho)P_0 \quad (18)$$

Substitute this into Eq. (15) to obtain

$$-J = \frac{n(\infty)P_0e^{\Delta\varphi} - n(\rho)e^{\varphi(\rho)}}{\frac{1}{2\pi D_b} \int_{\rho}^{\infty} r^{-2} e^{\varphi(r)} dr} \quad (19)$$

where $n(\rho)$ is now the concentration averaged over all time. We can now make use of Eq. (16) together with Eq. (19) to obtain a boundary condition for the right end of the channel:

$$n(\delta)e^{\varphi(\delta)} = n(\infty)P_0e^{\Delta\varphi} + (J/k_f)e^{\Delta\varphi} \quad (20)$$

The analogous expression for the left end of the channel is

$$n(0)e^{\varphi(0)} = n(-\infty)P_0 - (J/k_f) \quad (21)$$

where, as with the right end of the channel, we take $n(0)e^{\varphi(0)} = n(-\rho)e^{\varphi(-\rho)}$ and use the negative radial coordinate to indicate the left end of the channel.

We now replace Eqs. (8) and (10) with Eqs. (20) and (21), again equate the channel and bath fluxes, and solve for the flux to yield

$$J = \frac{P_0[n(-\infty) - n(\infty)e^{\Delta\varphi}]}{\frac{1}{k_f}(e^{\Delta\varphi} + 1) + \frac{1}{AD} \int_0^{\delta} e^{\varphi(x)} dx} = P_0J_i \quad (22)$$

In order to completely specify the flux under the one-ion assumption, we must now obtain an expression for the probability P_0 that the channel is empty. This is done by integrating Eq. (1) from zero to an arbitrary position x in the channel and then solving for the concentration at x :

$$n(x) = \left[n(0)e^{\varphi(0)} - \frac{J}{DA} \int_0^x e^{\varphi(s)} ds \right] e^{-\varphi(x)} \quad (23)$$

We can now combine Eqs. (21) and (23), multiply the result by the channel cross-sectional area, and integrate from $x = 0$ to $x = \delta$ to yield the probability the channel is occupied by one ion. Since we have assumed that the channel is either occupied by one ion or is empty, we have that the empty probability is one minus the above integral:

$$P_0 = 1 - n(-\infty)AP_0 \int_0^{\delta} e^{-\varphi(x)} dx + JB \quad (24)$$

where

$$B = \left[\frac{A}{k_f} \int_0^\delta e^{-\varphi(x)} dx + \frac{1}{D} \int_0^\delta e^{-\varphi(x)} \int_0^x e^{\varphi(s)} ds dx \right] \quad (25)$$

Using Eq. (22) to eliminate the flux from Eq. (24) and solving for P_0 yields

$$P_0 = \left[1 + n(-\infty)A \int_0^\delta e^{-\varphi(x)} dx - J_i B \right]^{-1} \quad (26)$$

Combining Eqs. (22) and (26) yields an expression for the flux:

$$J = \frac{J_i}{1 + n(-\infty)A \int_0^\delta e^{-\varphi(x)} dx - J_i B} \quad (27)$$

Equation (27) reduces to Levitt's (1986) result [Eq. (A4)] when Eq. (9) is negligible compared to the denominator of Eq. (3) divided by AD .

We now can see how ion-ion interaction modifies the independence flux. It does so by scaling the independence flux by an amount that is a function of concentration and applied transmembrane voltage. The effects this has on the current-voltage relation under the constant-field assumption can be seen in Fig. 3. One effect is to linearize the curve. Another effect is to give rise to a saturating relation between conductances and concentration. Note that we obtained this result without having a specific binding site in the channel. In our constant-field case the entire channel acts like a binding site. The equation for the affinity constant of this binding site (in the near-equilibrium case) is

$$K_a = A\delta e^{-\Delta\varphi} \quad (28)$$

This is the well-known result that the equilibrium affinity constant is the effective volume of the binding site times the Boltzmann factor. Near equilibrium we define a small signal conductance and plot it against concentration in Fig. 4:

$$G_{SS} = n/[k_b T(2/k_f + \delta^2/DK_a)(1 + nK_a)] \quad (29)$$

One is still left wondering about possible ways to interpret the various integrals in Eq. (27). In particular, note that the quantity labeled B has units of time. One of the goals of this chapter is to develop a detailed understanding of this time (see Section 5). Also in the above derivation we made use of two states, the probability the channel was occupied and the probability the channel was empty. Such state probabilities are reminiscent of the occupancies of states in chemical kinetic schemes more traditionally used in modeling ion permeation in channels. We would like to organize the derivation in

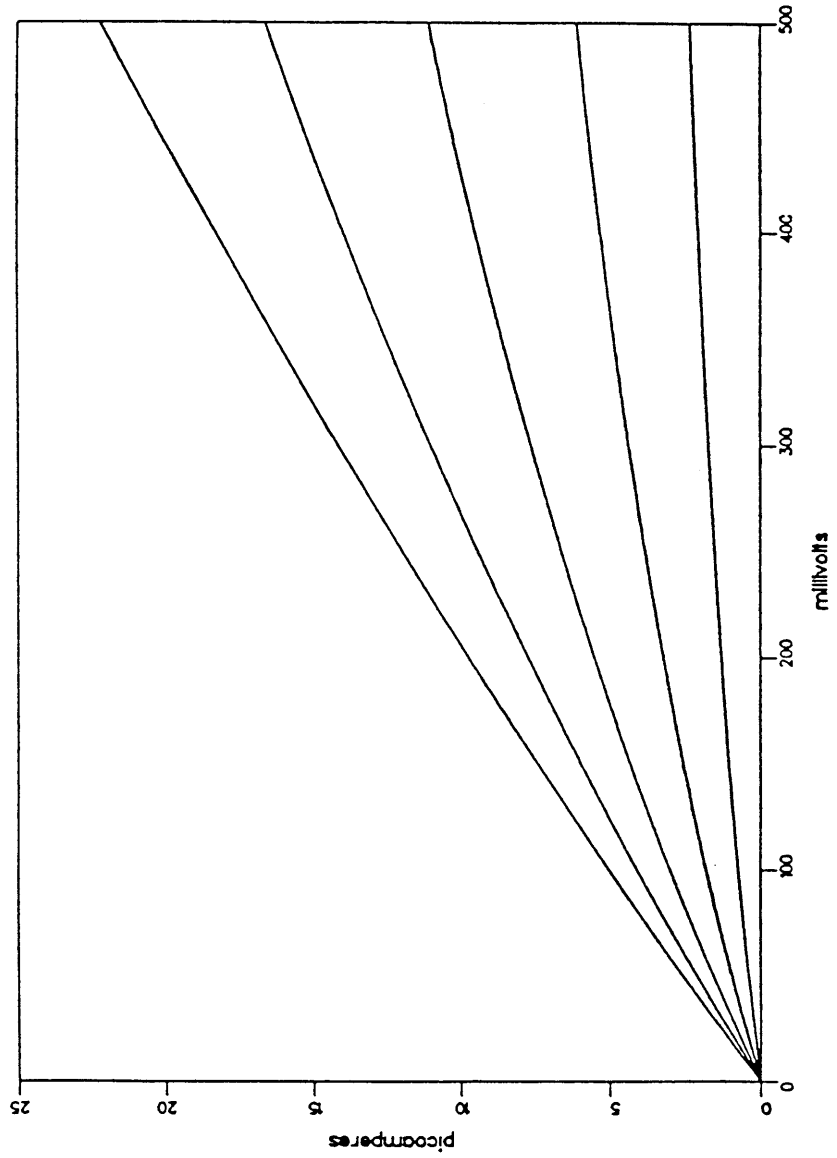


FIGURE 3. The current through the constant-field channel when ion-ion interactions are modeled with the assumption that at most one ion can enter the channel. The parameters are the same as for Fig. 2.

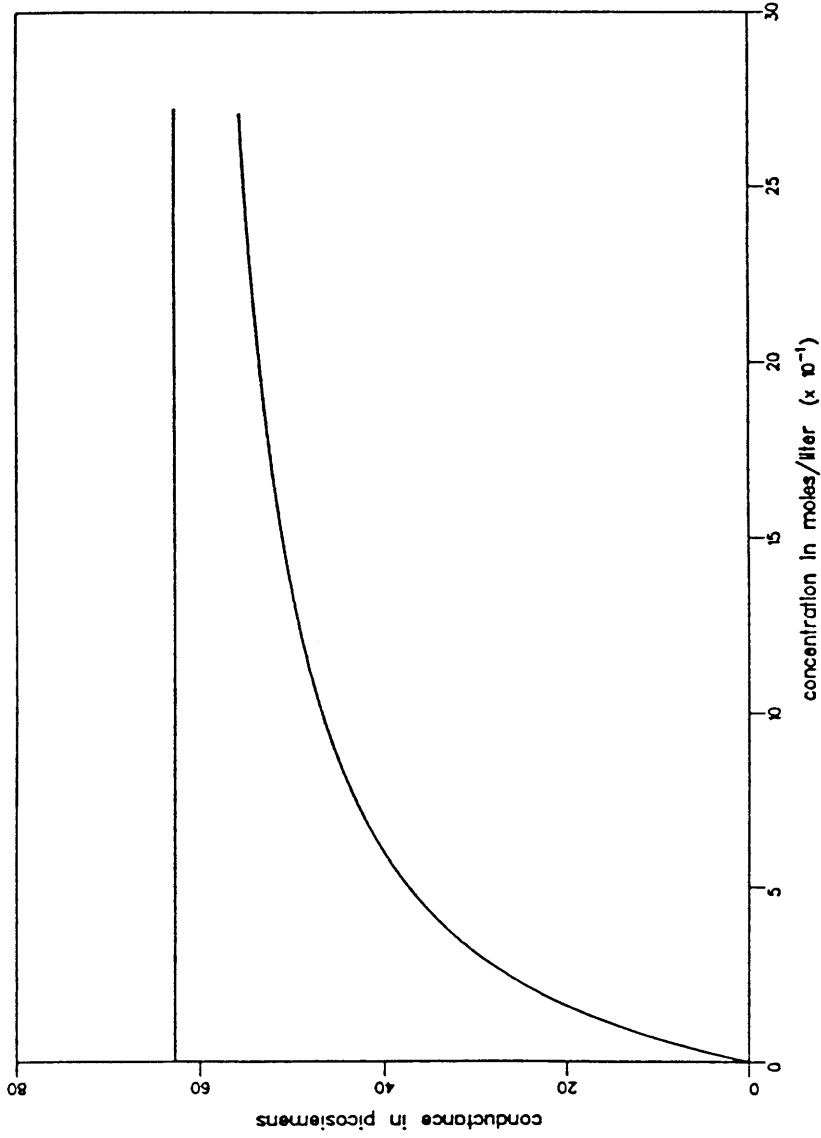


FIGURE 4. The small signal conductance of the constant-field channel under the one-ion occupancy assumption [defined in Eq. (29)] with a half saturation value of 340 mM.

a way that would provide a more transparent connection with traditional theory.

We pursue this program by considering various classes of trajectories taken by ions that encounter the channel. This classification of trajectories will form the basis of a kinetic scheme that can be demonstrated to be equivalent to the above result. The classification of trajectories will also lead us in a natural way to interpret the integral expressions in the empty probability in terms of the theory of passage times (Weiss, 1966; Schulten *et al.*, 1981; Goel and Richter-Dyn, 1974; Gardiner, 1983; Cooper *et al.*, 1988).

3. CHANNEL STATES AND TRANSITION RATES

The previous calculation suggests that the details of the mechanism by which an ion moves inside the channel may be considered separately from transitions between states characterized by the number of ions that occupy the channel. In order to pursue this idea, we will consider trajectories of individual ions as they move in the channel or the bath outside the channel. Such trajectories and their properties can be related in a natural way to machine calculations of the equations of motion of an ion in a channel (Jordan, 1987). In general, we can represent the position of the ion in the channel as a function of time $x(t)$. We expect that $x(t)$ will be continuous (no instantaneous jumps) as illustrated in Fig. 5.

3.1. Trajectories in a One-Ion Pore

If we consider the geometry of a channel, trajectories of ions can be classified into several possible categories. For the moment, consider an ion whose initial position is at the left end of the channel. If we wait for a length of time much longer than the mean time an ion spends in the channel, then

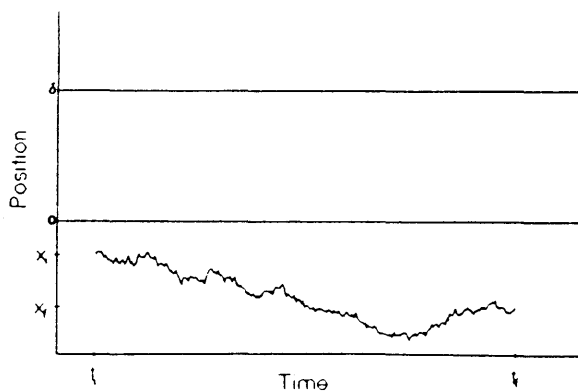


FIGURE 5. Trajectory in one spatial dimension as a function of time.

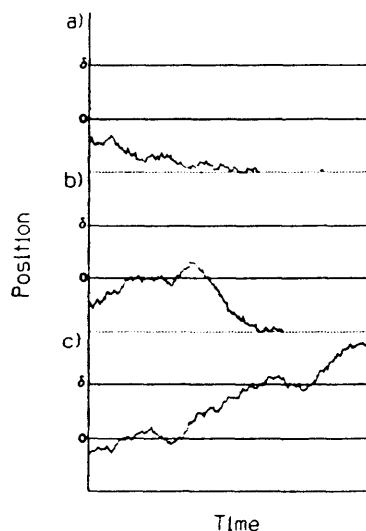


FIGURE 6. The three possible classes of trajectories associated with a one-ion channel where the initial position is on the left ($x < 0$) side of the channel. Panel (a) is a trajectory that never encounters the channel, panel (b) is a cis trajectory whose initial and end points are on the left side of the channel, and panel (c) is a trans trajectory whose initial point is on the left side of the channel and whose end point is on the right side of the channel.

we are likely to find the ion either in the bath on the left side or on the right side of the channel. Hence, an ion that encounters the channel will eventually end up either on the same side from which it entered or on the opposite side. These two outcomes will be designated cis and trans, respectively. If we consider an ion whose initial position is in the bath on the left side of the channel, we have a total of three possible outcomes: the ion may not encounter the channel, the ion may encounter the channel and be a cis trajectory, or the ion may encounter the channel and be a trans trajectory. These three classes of trajectories are illustrated in Fig. 6. If we then include those trajectories that begin in the bath on the right end of the channel, we have a total of six categories of trajectories. These categories are a natural consequence of the channel geometry and the assumption that at most one ion may enter the channel at a time.

3.2. Three-State Model

These categories of trajectories can now be used to generate a formalism by which the steady-state diffusion models can be combined with the concept of occupancy states. If we consider the initial positions of ions in the system, they are either on the right or left side of the channel. Thus, the state characterized by single ion occupancy can be divided into two states depending on the point at which the trajectory originated. If we add to this the empty state, we have a total of three occupancy states. Associated with these states are the probabilities (dimensionless) of finding the channel in each of the states:

State Probabilities

P_0	The probability that the channel is empty
$P(l)$	The probability that the channel is occupied by an ion whose trajectory started on the left side of the channel
$P(r)$	The probability that the channel is occupied by an ion whose trajectory started on the right side of the channel

Transitions between these states are then modeled through the use of a set of transition rates. These transition rates fall into two categories: rates at which ions fill the channel and rates at which ions empty from the channel. As in the case of the states characterized by the channel being occupied, the filling rates fall into two categories, each depending on the point at which the trajectory originated. These filling rates select for those trajectories that encounter the channel:

Filling Transition Rates

$F(l)$	The rate at which ions (that have trajectories starting in the left bath) encounter the capture radius on the left end of the channel
$F(r)$	The rate at which ions (that have trajectories starting in the right bath) encounter the capture radius on the right end of the channel

Having selected for those ions that encounter the channel, we can further select between the cis and trans trajectories through the use of conditional exit rates. Given that we have eliminated all trajectories that do not encounter the channel through the use of the filling rates, we are left with four remaining categories of trajectories. We associate each of these categories with an exit rate:

Exit Transition Rates

Cis

$E(l l)$	The rate at which ions (having trajectories that start and end in the left bath) exit the left end of the channel
$E(r r)$	The rate at which ions (having trajectories that start and end in the right bath) exit the right end of the channel

Trans

$E(l r)$	The rate at which ions (having trajectories that start in the left bath and end in the right bath) exit the right end of the channel
$E(r l)$	The rate at which ions (having trajectories that start in the right bath and end in the left bath) exit the left end of the channel

These rates describe transitions between the three different states of the channel. It is clear, by the arguments given above, that these transition rates describe all possible trajectories. But we have not yet determined how the transition rates depend on the channel structure, i.e., the component of $\varphi(x)$ due to ion-channel interactions.

The three-state kinetic scheme can be summarized in the single kinetic diagram in Fig. 7. The following set of equations describe the system:

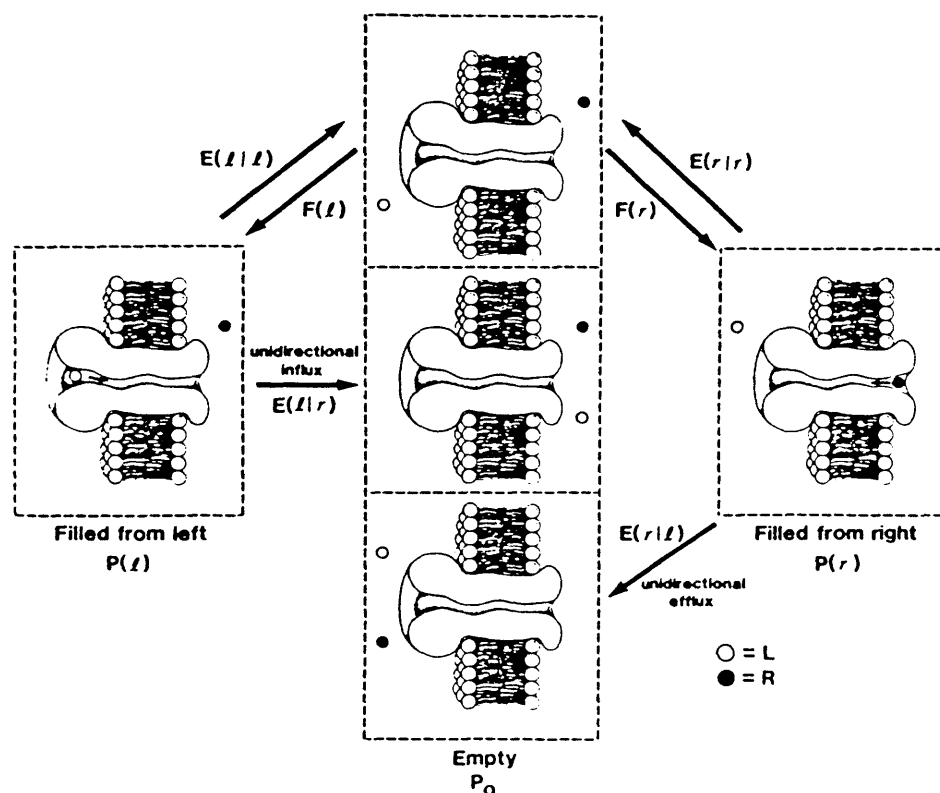


FIGURE 7. Characterization of the three possible occupancy states of a one-ion channel and the transitions between the states. P_0 is the probability that the channel is empty. $P(l)$ and $P(r)$ are the probabilities the channel is occupied by an ion that started on the left and right side, respectively. $F(l)$ and $F(r)$ are the rates (units seconds⁻¹) at which ions fill the channel from the left and right side, respectively. $E(l|l)$ and $E(r|r)$ are the rates (units seconds⁻¹) at which the channel empties on the same side it fills. $E(l|r)$ and $E(r|l)$ are the rates at which the channel empties on the opposite side from which it filled. Only the *trans* trajectories contribute to the unidirectional fluxes.

$$F(l)P_0 - [E(l|l) + E(l|r)]P(l) = dP(l)/dt \quad (30)$$

$$F(r)P_0 - [E(r|r) + E(r|l)]P(r) = dP(r)/dt \quad (31)$$

$$P_0 + P(l) + P(r) = 1 \quad (32)$$

In the steady state, the derivatives with respect to time are zero and Eqs. (30) through (32) yield the following matrix equation:

$$\begin{bmatrix} -E_l & 0 & F_l \\ 0 & -E_r & F_r \\ 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} P(l) \\ P(r) \\ P_0 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} \quad (33)$$

where $E_l = [E(l|l) + E(l|r)]$ is the sum of the rates at which ions originating on the left end of the channel exit from the channel and $E_r = [E(r|r) + E(r|l)]$ is the equivalent expression for the right end of the channel. Making use of these definitions, the solution of the above matrix equation is

$$P_0 = \frac{E_l E_r}{E_l E_r + F_l E_r + F_r E_l} \quad (34)$$

$$P(l) = \frac{F_l E_r}{E_l E_r + F_l E_r + F_r E_l} \quad (35)$$

$$P(r) = \frac{E_l F_r}{E_l E_r + F_l E_r + F_r E_l} \quad (36)$$

We now have relationships between the state probabilities and the transition rates between the states. In order to completely specify the model, we need expressions relating the transition rates to the ion-channel interaction potential and the concentrations of permeant ions on either side of the channel. In principle, we could, at this point, make use of any number of models for the dynamics of ion movement up to and through the channel. Thus, Eqs. (34) through (36) reflect the basic topology of a one-ion channel and therefore transcend any specific model for the dynamics of ion movement in the channel. For a discussion of the relationship between the three-state model and more traditional chemical kinetic schemes, see Section 8.

4. ELECTRODIFFUSION INTERPRETATION OF TRANSITION RATES

4.1. Preliminaries

Before continuing we make the following definitions. Define an electrodiffusive length as follows:

$$L_a^b = \int_a^b e^{\psi(x)} dx \quad (37)$$

Notice that negative potentials tend to reduce the electrodiffusive length below the physical length while positive potentials increase the electrodiffusive length above the physical length. From this, we can define an electrodiffusive resistance:

$$R_a^b = L_a^b / AD \quad (38)$$

The electrodiffusive resistance is proportional to a voltage-dependent generalization of the electrical resistance, the proportionality constant being

$(zq)^2 n/k_b T$. The reciprocal of Eq. (38) has the units of velocity times area and so could be thought of as a voltage-dependent permeability. The electrodiffusive resistance provides a convenient and intuitive way in which to write the integrated form of the Nernst–Planck equation and will be used throughout the remaining presentation.

4.2. Derivations

We now develop connections between the transition rates and electrodiffusion equations, obtaining a set of identities between the six transition rates of our kinetic scheme and their corresponding electrodiffusion theory interpretations. Let us begin with the entrance rates, $F(l)$ and $F(r)$. We will concentrate on $F(l)$, the arguments for $F(r)$ being completely analogous. $F(l)$ is the rate at which ions that start in the left bath find the mouth of the channel (i.e., arrive at ρ). The rate of such encounters was first calculated by Smoluchowski (1917) and later extended by numerous authors (reviewed in Rice, 1985). The technique used put a source of particles far from the encounter surface and set the concentration on the encounter surface to zero [making it a so-called absorbing boundary; see Eqs. (7)–(9)]. Using these boundary conditions in the diffusion equation, we equate the encounter rate with the flux (unidirectional) through the system. Denote such a flux by $J(a|b)$, where a is the source location and b is the absorbing boundary location. Using Eq. (1) such a flux can be shown to be

$$J(a|b) = n(a)e^{-\psi(a)/R_p^b} \quad (39)$$

In our case, on the left end of the channel, the source is at $-\infty$ and the absorbing boundary is at the capture radius ($-\rho$). This yields

$$F(l) = J(-\infty|-\rho) = n(-\infty)/R_p^- \quad (40)$$

Analogously for the right side we obtain

$$F(r) = -J(\infty|\rho) = n(\infty)e^{\psi(\infty)/R_p^+} \quad (41)$$

Let us now find an expression for the trans exit rates $E(l|r)$ and $E(r|l)$. As above, we will concentrate on $E(l|r)$ and note that the arguments for $E(r|l)$ are analogous. The starting point for this derivation is the net flux equation as interpreted from the kinetic scheme:

$$J = E(l|r)P(l) - E(r|l)P(r) \quad (42)$$

Note this is the difference between two unidirectional fluxes. The diffusion equation can be written likewise:

$$J = J(-\infty|\infty) - J(\infty|-\infty) \quad (43)$$

where

$$J(-\infty|\infty) = (P_0 n(-\infty)/R_{-\infty}^{\infty}) \quad (44)$$

$$-J(\infty|-\infty) = P_0 n(\infty) e^{\Delta\varphi}/R_{-\infty}^{\infty} \quad (45)$$

Here, we define $R_{-\infty}^{\infty} = R_{-\infty}^{\infty} + R_0^{\delta} + R_p^{\infty}$. Equating the unidirectional fluxes we obtain

$$E(l|r)P(l) = J(-\infty|\infty) \quad (46)$$

Using the above we can arrive at the following:

$$E(l|r) = P_0 n(-\infty)/[P(l)R_{-\infty}^{\infty}] \quad (47)$$

This equation establishes the relationship between the *trans* exit transition rate and the bulk electrochemical potential. To proceed, we require an expression for the occupancy probability $P(l)$ in terms of the electrodiffusive resistances. Analogous to our earlier treatment [see Eqs. (23) and (24)]. Since we have assumed a steady state, we can integrate from an arbitrary position x in the channel up to the right end of the channel or similarly from the left end of the channel up to an arbitrary point x , yielding

$$n(x) = \left[n(\delta) e^{\varphi(\delta)} + JR_x^{\delta} \right] e^{-\varphi(x)} \quad (48)$$

$$n(x) = \left[n(0) e^{\varphi(0)} - JR_0^x \right] e^{-\varphi(x)} \quad (49)$$

At this point we need expressions for J and for $n(0)e^{\varphi(0)}$ to obtain $P(l)$. We can use Eq. (45) for the flux. To obtain an expression for the electrochemical potential at the mouth requires a little more work. Such an expression can be obtained as follows. Note that the flux, $J(-\infty|\infty)$, can be written in terms of the net flux in the bath on the left end of the channel where the concentration in the bath on the right end of the channel is set to zero [analogous to Eq. (19)]:

$$\begin{aligned} J(-\infty|-\rho)P_0 - J(-\rho|-\infty) &= J(-\infty|\infty) \\ (1/R_{-\infty}^{\infty})[P_0 n(-\infty) - n_1(-\rho) e^{\varphi(-\rho)}]/R_{-\infty}^{\infty} &= P_0 n(-\infty)/R_{-\infty}^{\infty} \end{aligned} \quad (50)$$

We have introduced the subscript l in the concentration to emphasize that ions contributing to this concentration have originated in the bath on the left side of the channel. Solving for $n_1(-\rho)e^{\varphi(-\rho)}$ yields

$$n_1(-\rho) e^{\varphi(-\rho)} = P_0 n(-\infty) R_0^x / R_{-\infty}^{\infty} \quad (51)$$

If we associate the concentration at the capture radius on the left end of the channel with the concentration at $x = 0$ inside the channel, Eq. (51) can be combined with Eq. (49) to yield an expression for the concentration at x in terms of the bath concentration:

$$n_l(x) = [P_0 n(-\infty) / R_{-\infty}^x] R_x^x e^{-\varphi(x)} \quad (52)$$

Strictly speaking, we should consider a three-dimensional treatment of the entrance process. Here, we are assuming that such considerations can be absorbed into the choice of the capture radius, which depends only weakly on the other parameters of the model. Integrating from 0 to δ and multiplying by the cross-sectional area yields the occupancy probability due to ions that started in the left bath:

$$P(l) = A \int_0^\delta n_l(x) dx = \frac{P_0 A n(-\infty)}{R_{-\infty}^x} \int_0^\delta R_x^x e^{-\varphi(x)} dx \quad (53)$$

and the corresponding result for ions that started in the right bath:

$$P(r) = A \int_0^\delta n_r(x) dx = \frac{P_0 A n(\infty) e^{\Delta\varphi}}{R_{-\infty}^x} \int_0^\delta R_{-\infty}^x e^{-\varphi(x)} dx \quad (54)$$

This expression for $P(l)$ can now be substituted into Eq. (47) to yield an expression for the trans exit transition rate in terms of the bulk electrochemical potential and the channel structure:

$$E(l|r) = \left(A \int_0^\delta R_x^x e^{-\varphi(x)} dx \right)^{-1} \quad (55)$$

An analogous equation for $E(r|l)$ can be derived, yielding

$$E(r|l) = \left(A \int_0^\delta R_{-\infty}^x e^{-\varphi(x)} dx \right)^{-1} \quad (56)$$

We now have the desired expressions for four of the six rate expressions. Our final job is to find expressions for the cis exit rates, $E(l|l)$ and $E(r|r)$. As above, we will concentrate on $E(l|l)$ and note that $E(r|r)$ can be obtained by analogous arguments. The derivation begins with Eq. (30) derived earlier from the kinetic scheme:

$$F(l)P_0 = P(l)[E(l|l) + E(l|r)] \quad (57)$$

This can be rearranged to yield

$$E(l|l) = [F(l)P_0/P(l)] - E(l|r) \quad (58)$$

If we now substitute in the expressions above for $F(l)$, $P(l)$, and $E(l|r)$, we obtain our desired result:

$$E(l|l) = \frac{R_0^x}{AR_0^x \int_0^{\delta} R_x^z e^{-\varphi(x)} dx} \quad (59)$$

and by analogous arguments we obtain

$$E(r|r) = \frac{R_{-x}^{\delta}}{AR_{\delta}^z \int_0^{\delta} R_x^z e^{-\varphi(x)} dx} \quad (60)$$

If Eqs. (53)–(56) are substituted into Eq. (42), we will recover (after some rearrangement) our earlier result [Eq. (27)] showing the consistency of the two approaches. There still remain questions concerning the connection between the above rates and the occupancy times in the channel. These issues will involve a considerable digression where we will build, in several steps, from a reduced problem that is standard in the theory of passage times up to the exit transition rates derived above. This process will involve generalizing the mean first passage times (MFPTs), which are standard results in the literature (Weiss, 1966; Goel and Richter-Dyn, 1974; Gardiner, 1983; Cooper *et al.*, 1988), to what we will refer to as mean occupancy times.

5. TRANSITION RATES AS MEAN FIRST PASSAGE TIMES

The first passage time is the time that a diffusing particle spends in a designated region before encountering a target defined by an absorbing boundary. Because the target is absorbing, the first encounter with the target results in the particle being removed from the region, hence the designation first passage time. The use of the first moment of the distribution of passage times as a model for a rate constant in chemical kinetics has been shown to be a useful approximation (Schulten *et al.*, 1981). This type of model has also been suggested for the rate constants traditionally used in modeling transport in ion channels (Jakobsson and Chiu, 1987; Gates *et al.*, 1987; Cooper *et al.*, 1988). In our treatment above, we started with a steady-state diffusion equation and in the process of accounting for discrete occupancy states we generated expressions similar to standard results in the theory of passage times. On discovering these similarities we began to pursue a relationship between the two results. Here, rather than starting with the transition rates and attempting to interpret them in terms of passage times, we will start with calculations of passage times in reduced problems and slowly build to expressions that can be directly related to the transition rates in our model of a channel. In the calculation of passage times, we make use of

artificial “channels” confined between absorbing and reflecting boundaries. These seem in conflict with our intuition concerning the nature of trajectories of ions entering and leaving a channel, but we shall see in the following series of derivations that the simpler trajectories associated with passage times provide the information needed to calculate the time an ion occupies a channel.

5.1. The Steady State and Mean First Passage Times

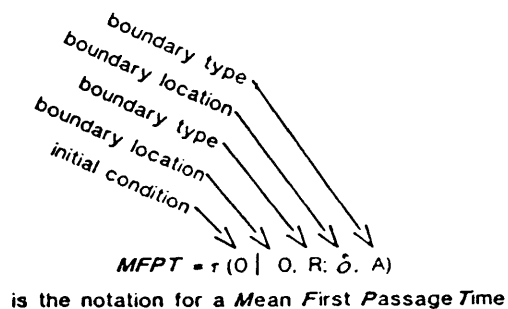
It has been shown (Hardt, 1981; Cooper et al., 1988) that MFPTs can be related to steady-state fluxes. In this approach we define the “holdup” of a system to be the average number of particles in the system under a given set of steady-state conditions. In one dimension, these conditions consist of either an absorbing and reflecting boundary or two absorbing boundaries. If we then assume that the dynamics of the particles in the system are independent of one another, the mean time of passage of a particle through the system is the holdup divided by the steady-state rate at which ions are injected into the system:

$$\tau = N/I \quad (61)$$

where τ is the MFPT, N is the holdup, and I is the steady-state rate of injection. We can now make use of Eq. (39) where the source of the unidirectional flux is the point of injection and the sink is at an absorbing boundary. In the case where we have an absorbing and reflecting boundary, there is a unidirectional flux from the point of injection to the absorbing boundary and no net flux between the source and the reflecting boundary. In the case where we have two absorbing boundaries, we make use of two unidirectional flux equations of the form of Eq. (39), each with the source at the point of injection and the sinks at the two absorbing boundaries. The holdups are obtained through the use of equations analogous to Eqs. (23), (48), and (49). In our system, the one-ion assumption requires that the holdup be no greater than one (the channel always occupied). Equivalently, the holdup can be considered to be the probability the channel is occupied. In our notation, Eq. (61) becomes

$$\tau = \frac{P(l) + P(r)}{I} = \frac{1 - P_0}{I} \quad (62)$$

Equation (62) provides a method by which the first moment of the passage time can be calculated from steady-state diffusion. We will begin with a very simple set of boundary conditions that will yield a standard result in the theory of MFPTs. After having done so, we will then proceed to generalize the boundary conditions until we obtain a result that is the reciprocal of the exit transition rates of the previous section. Along the way we will discover several interesting properties of the mean time an ion occupies the channel, and we will relate these properties to the trajectories of individual ions.



with initial_i and boundary_b conditions

$(x_i = 0 | \text{reflecting boundary at } x_b = 0; \text{absorbing boundary at } x_b = \delta)$

FIGURE 8. The notation we use for the mean first passage time τ and the mean occupancy time t indicates the initial position of the diffusing ion followed by the locations and attributes of the system.

5.2. Mean First Passage Time

In the calculation of MFPTs there are three parameters: the initial position of the diffusing particle, and the two boundaries of the spatial domain. One of the boundaries must be absorbing while the other can be either absorbing or reflecting. The notation we use indicates the initial position of the diffusing particle followed by the locations and attributes of the boundaries of the system (see Fig. 8):

$$MFPT = \tau(x_i | O, R; \delta, A)$$

with initial and boundary conditions:

$(x_i = 0 | \text{Reflecting boundary at } x = 0; \text{Absorbing boundary at } x = \delta)$

First consider a one-dimensional box with the above boundary conditions. Recalling the recipe for the MFPT, we must obtain expressions for the steady-state rate of injection and the steady-state holdup. We begin with the injection rate by applying the above boundary conditions to Eq. (39):

$$I = J(0|\delta) = n(0)e^{\phi(0)/R_0\delta} \tag{63}$$

We now obtain the holdup by integrating the steady-state concentration over the length of the box:

$$P_{occ} = 1 - P_0 = A \int_0^\delta n(x) dx \tag{64}$$

The holdup is, of course, the occupancy. We combine Eq. (49) with Eq. (63) to obtain an expression for $n(x)$:

$$n(x) = [n(0)e^{\varphi(0)/R_0^\delta}]R_x^\delta e^{-\varphi(x)} \quad (65)$$

This result can be compared with Eq. (52). Integrating Eq. (65):

$$P_{\text{occ}} = \frac{An(0)e^{\varphi(0)}}{R_0^\delta} \int_0^\delta R_x^\delta e^{-\varphi(x)} dx \quad (66)$$

Taking the ratio of the holdup and the flux eliminates the boundary condition at $x = 0$ and yields the passage time. After a change in the order of integration, we have a result previously obtained by others with more sophisticated approaches (Weiss, 1966; Goel and Richter-Dyn, 1974; Schulten *et al.*, 1981; Gardiner, 1983; Cooper *et al.*, 1988):

$$\tau = A \int_0^\delta R_x^\delta e^{-\varphi(x)} dx = \frac{1}{D} \int_0^\delta e^{\varphi(s)} \int_0^s e^{-\varphi(x)} dx ds \quad (67)$$

Notice that this expression is equal to the second term on the right-hand side of Eq. (25). As mentioned earlier, this observation motivated what follows. In summary, we obtain a passage time by representing the steady-state flux and holdup in terms of the electrochemical potential at the initial position of the ion in the system of interest. We then take the ratio of the holdup to the flux yielding the passage time. We must now extend this calculation in a way that more accurately reflects the trajectories of ions associated with the "real" channel geometry described previously.

5.3. Mean Occupancy Times in Terms of Mean First Passage Times

It is clear that the trajectories associated with the calculation of the MFPT are quite different from those associated with the one-ion channel described in Section 3, and so we must modify the boundary conditions to more accurately reflect an ion channel. In the calculation of the MFPT of the previous section, we injected ions into the box by placing each ion at the reflecting boundary. As already discussed, in the case of a pore, ions may leave from the same side that they entered, something not permitted by the reflecting boundary of the previous calculation. In fact, it would be expected that any ion that encountered the channel would undergo many crossings at the boundary of the channel. As a consequence, the durations we calculate under the steady-state assumption in this section will be referred to as mean occupancy times (MOTs). Both the MOT (denoted by t) and the MFPT (denoted by τ) are the average times that a diffusing particle spends in a region before encountering an absorbing boundary. In the case of an MFPT, the

target is on the boundary of the system and (because the target is absorbing) the first encounter with the target results in the particle being removed from the system, hence the designation mean first passage time. In the case of an MOT, we are interested in the time a particle spends in a region which is a proper subset of the system and consequently a particle can encounter a boundary of the region, leave the region, and later reenter before finally being absorbed at the target. Because the time the particle spends outside the region is counted in an MFPT analysis, but not in an MOT analysis, an MFPT $>$ MOT for the equivalent geometry of the system. The MOT is related to the MFPT through the ratio of the holdup of the region to the holdup of the system.

The strategy of the following sections will be to extend the calculation of the MFPT in three steps. We will first modify the reflecting boundary of the MFPT calculation to more accurately represent a channel. We will then modify the absorbing boundary of the MFPT. Finally we will combine these results to yield an expression that can be directly compared with the exit transition rates. After each MOT is obtained, we will interpret the MOT in terms of the MFPT of the previous section through the use of arguments based on averages of the durations of trajectories. These arguments follow the spirit of the presentation of Chandrasekhar (1943). For the MOTs we will, by convention, always refer to the occupancy time in the channel, i.e., the time the ion spends in the interval $(0, \delta)$. As in the case of the MFPTs, we must then specify the locations and attributes of the boundaries of the system, and the initial condition.

5.3.1. MOT #1 ($x_i = 0$ | $x = -\lambda$, Absorbing; $x = \delta$, Absorbing)

In the MFPT calculation, we forced the ion to exit the right $x = \delta$ end of the interval by placing a reflecting boundary at the left end at $x = 0$. In an ion channel we know that an ion that encounters the left end of the channel may exit from either end. To approximate this possibility more realistically, there must be two absorbing boundaries in the system. Thus, we place an absorbing boundary at an arbitrary position $(-\lambda)$ in the bath to the left of the interval. We now have two possible outcomes: the ion may be absorbed in the bath at $x = -\lambda$ [and contribute to $J(0|-\lambda)$] or pass through the channel to be absorbed at $x = \delta$ [and contribute to $J(0|\delta)$].

Thus, in comparison with the calculation of the MFPT, we have extended the system out into the bath, and what used to be the system has become a subregion of the new system. Consequently, the holdups of the two calculations are obtained by integrating over the same interval, while the rate of injection in the MOT calculation includes the additional flux to the absorbing boundary at $x = -\lambda$. This additional flux is obtained by writing Eq. (39) with $a = -\lambda$ and $b = 0$. The rate of injection is then the sum of the absolute value of the unidirectional fluxes to the two absorbing boundaries in the system:

$$I = J(0|\delta) - J(0|-\lambda) = n(0)e^{\varphi(0)} R_{-\lambda}^{\delta}/(R_{-\lambda}^0 R_0^{\delta}) \quad (68)$$

Since the holdup for the MOT is obtained by integrating over the same interval as was the holdup for the MFPT, we can use Eq. (66). We now divide the holdup by the new expression for the flux to obtain the new expression for the MOT:

$$t(0|-\lambda, A; \delta, A) = \frac{R_{-\lambda}^0}{R_{-\lambda}^{\delta}} A \int_0^{\delta} R_x^{\delta} e^{-\varphi(x)} dx \quad (69)$$

Comparing this expression with the expression for the MFPT [Eq. (67)] yields

$$t(0|-\lambda, A; \delta, A) = (R_{-\lambda}^0/R_{-\lambda}^{\delta})\tau(0|0, R; \delta, A) \quad (70)$$

Let us pursue a better understanding of this result. First note that the ratio of electrodiffusive resistances in Eq. (70) is precisely the ratio of the rate at which ions pass through the channel to the rate at which ions are supplied to the source (the sum of the rates that the ions are removed at the two absorbing boundaries). This is the probability that an ion that starts out at the source will eventually be absorbed at the opposite end of the channel. Hence, we define a probability that we will call the *trans* probability:

$$P_{trans} = J(0|\delta)/[J(0|\delta) - J(0|-\lambda)] = R_{-\lambda}^0/R_{-\lambda}^{\delta} \quad (71)$$

We can also account for the remaining ions by defining a *cis* probability:

$$P_{cis} = R_0^{\delta}/R_{-\lambda}^{\delta} \quad (72)$$

Applying the definition of the *trans* probability to Eq. (70) yields the result that the channel MOT under these boundary conditions is given by the product of the MFPT and the probability that an ion exits the opposite end of the channel:

$$t(0|-\lambda, A; \delta, A) = P_{trans}\tau(0|0, R; \delta, A) \quad (73)$$

In order to understand the above result, we now consider an ensemble of trajectories. These trajectories are of two categories: the first category (*cis*) is the set of trajectories that terminate at the absorbing hemisphere at $x = -\lambda$, and the second category (*trans*) is the set of trajectories that reach the *trans* absorbing boundary at $x = \delta$ and terminate there. Consider a trajectory from the first category. If we discard those times during which the ion is outside of the channel, we are left with a trajectory that begins and ends at the *cis* end of the channel and in between appears to reflect off of the *cis* boundary at every encounter. To see this, notice that every time the trajectory encounters the boundary of the channel at $x = 0$ it will be joined with a later part of the

trajectory where the ion reenters the channel, thus giving the appearance of reflecting off the boundary at $x = 0$. In this way we can define an accumulated duration that a given ion spends in the channel. We can do likewise for trajectories from the second category. If we enumerate the members of the ensemble with the index k we have for the MOT:

$$t(0|-\lambda, A; \delta, A) = \lim_{N \rightarrow \infty} \frac{1}{N} \sum_{k=1}^N \Delta t_k \quad (74)$$

where Δt_k is the accumulated duration the k th ion spends in the channel.

Let us consider a different system of indexing the trajectories of the ensemble. In the above discussion we identified each trajectory with an index k . We now define two indices i and j . The index i enumerates the trajectories from the second category, those that are absorbed at the *trans* end of the channel. Each trajectory from the second category defines the end of a partial sequence of trajectories. The index j enumerates the trajectories within a partial sequence. We allow i to range from 1 to M , the number of trajectories from the second category, which is also the total number of partial sequences. The index j ranges from 1 to n_i , the number of trajectories in the i th partial sequence (see Fig. 9).

Consider the i th partial sequence of trajectories. The first $n_i - 1$ edited trajectories (first category) begin and end at the *cis* end of the channel and in between appear to reflect back into the channel at every encounter of the *cis*

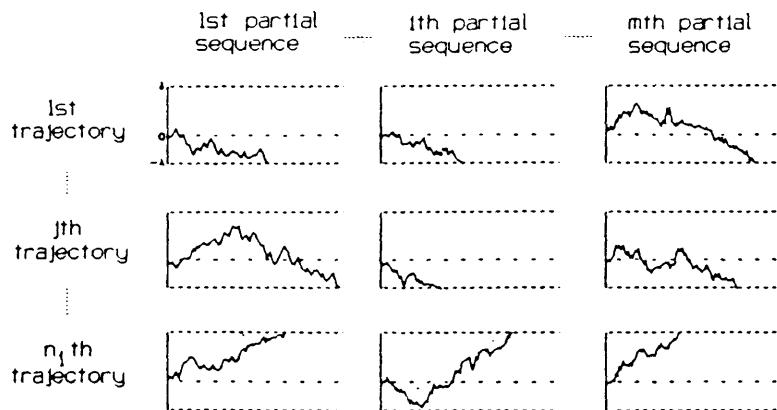


FIGURE 9. An ensemble of trajectories can be enumerated in such a way that after editing out those parts of the trajectories that lie outside of the channel, the trajectories that remain can be appended to compose a sample trajectory from an MFPT. This is done by dividing the ensemble into partial sequences that are enumerated by the index i . Within each partial sequence the trajectories are enumerated by the index j . The i th partial sequences begins with $n_i - 1$ trajectories of the first kind (absorbed at $x = -\lambda$) and ends with a trajectory of the second kind (absorbed at $x = \delta$).

channel boundary, never reaching the *trans* absorbing boundary. The n_i th edited trajectory (second category) begins at 0, also appears to reflect back into the channel at every encounter with the boundary at 0, and ends at the *trans* absorbing boundary. If we now append these n_i edited trajectories, we will obtain a single accumulated trajectory that is indistinguishable from a sample trajectory where there is a reflecting boundary at the *cis* end of the channel and an absorbing boundary at the *trans* end of the channel. The total time, Δt_i , for the accumulated trajectory associated with the i th partial sequence of trajectories will be the sum of the durations Δt_j of the edited trajectories:

$$\Delta t_i = \sum_{j=1}^{n_i} \Delta t_j \quad (75)$$

We can also consider the average of the Δt_i given by

$$\tau = \lim_{M \rightarrow \infty} \frac{1}{M} \sum_{i=1}^M \Delta t_i \quad (76)$$

Consider now that for each k there exists an i, j that identifies the same trajectory. Therefore, Eq. (74) for the MOT can be written in terms of the indices i, j . Furthermore, we can write N as the product of M and the average of the n_i to yield

$$t(0|-\lambda, A; \delta, A) = \lim_{M \rightarrow \infty} \frac{1}{\bar{n}M} \sum_{i=1}^M \Delta t_i = \frac{\tau}{\bar{n}} \quad (77)$$

$\bar{n} = \frac{1}{m} \sum_{i=1}^M$ is the average number of trajectories we must accumulate to com-

pose a sample trajectory enumerated by the index i . Recall that in the i th sequence of trajectories the first $n_i - 1$ ions in the sequence were absorbed at the hemispherical surface at $-\lambda$ and only the last ion successfully made the trip through the channel to be absorbed at δ . Hence, one out of the total of the n_i ions in the i th sequence passes through to the *trans* end of the channel. On the average we would expect that one out of \bar{n} ions would successfully pass through to the *trans* side, which is precisely the *trans* probability. With this in mind a comparison of Eq. (73) and (77) reveals that the average of the Δt_i is equal to the MFPT of Section 5.2. Hence, by editing and appending an ensemble of trajectories associated with a channel, we can compose trajectories whose mean duration is identical to the mean duration of an ensemble of trajectories associated with a reflecting boundary. In this way we can see that the form of Eq. (73) is merely a reflection of the fact that the average accumu-

lated time a partial sequence of trajectories spends in transporting an ion from the left to the right (absorbing) end of a channel is exactly the MFPT.

5.3.2. MOT #2 ($x_i = 0 \mid x = 0$, Reflecting; $x = \lambda$, Absorbing)

Having considered a more realistic boundary for the left end of the channel, let us turn to a consideration of the right boundary. For simplicity we will return to the MFPT calculation as a starting point. In calculating the MFPT, δ was taken to be an absorbing boundary. We would now like to allow for the possibility that an ion can return to the channel after reaching the boundary at δ for the first time. We know that the average time required, starting from 0, to reach δ for the first time is the MFPT. An estimate of the additional time spent in the channel can be obtained by starting the ion at δ and calculating the MOT with 0 a reflecting boundary and an absorbing boundary in the bath to the right of the interval $(0, \delta)$ at an arbitrary point $x = \delta + \lambda$. We will therefore calculate the contribution due to reentries and add the result to the MFPT, i.e., $t(0|0,R;\lambda,A) + \tau(0|0,R;\delta,A) = t(\delta|0,R;\lambda,A)$.

Now we will calculate $t(\delta|0,R;\lambda,A)$. As before, we start by obtaining the steady-state rate of injection. In this case, the only way that an ion can leave the system is through the absorbing hemispherical surface at $x = \delta + \lambda$. Thus, we have

$$I = J(\delta|\delta+\lambda) = n(\delta)e^{\varphi(\delta)}/R_{\delta}^{\delta+\lambda} \quad (78)$$

Consider that there can be no net flux between a reflecting boundary and the source at $x = \delta$. Applying this to Eq. (48) yields Boltzmann's equilibrium distribution:

$$n(x) = n(\delta)e^{\varphi(\delta) - \varphi(x)} \quad (79)$$

For the holdup, as before, we integrate Eq. (79) times the cross-sectional area:

$$P_{\text{occ}} = An(\delta)e^{\varphi(\delta)} \int_0^{\delta} e^{-\varphi(x)} dx \quad (80)$$

Taking the ratio of the holdup to the flux yields an occupancy time:

$$t(\delta|0,R;\delta + \lambda,A) = AR_{\delta}^{\delta+\lambda} \int_0^{\delta} e^{-\varphi(x)} dx \quad (81)$$

$$R_{\delta}^{\delta+\lambda} = \frac{1}{2\pi D_b} \int_{\rho_r}^{\delta+\lambda} r^{-2} e^{\varphi(r)} dr$$

Comparing Eq. (81) to earlier results, we notice that this expression for the MOT seems simpler because it does not involve an iterated integral. This is a consequence of the use of the Boltzmann distribution for the concentration.

However, the interpretation of this expression in terms of MFPTs and subsequently in terms of ensembles of trajectories will prove to be more involved than in the previous calculation. To obtain this connection we make a brief digression.

To understand the above result we will need an expression for the MFPT from a reflecting boundary at $x = \delta$ to an absorbing boundary at $x = 0$. This is accomplished by replacing Eq. (63) with the unidirectional flux from a source at $x = \delta$ to a sink at $x = 0$ and using Eq. (48) for the concentration instead of Eq. (49). We then proceed as before to obtain

$$\tau(0|\delta) = A \int_0^\delta R_x^\delta e^{-\varphi(x)} dx \quad (82)$$

$$\tau(\delta|0) = A \int_0^\delta R_0^\delta e^{-\varphi(x)} dx \quad (83)$$

Here, our notation for the MFPTs is the same as for the unidirectional fluxes [see Eq. (39)] reflecting the fact that the initial position of the ion is equal to the position of the reflecting boundary. If we now take the sum of Eq. (82) and (83), we can add the arguments of the two integrals since the range of integration is the same and obtain

$$\tau(0|\delta) + \tau(\delta|0) = AR_0^\delta \int_0^\delta e^{-\varphi(x)} dx \quad (84)$$

Comparison of Eq. (81) for the MOT with Eq. (84) yields

$$\begin{aligned} t(0|0,R;\delta+\lambda,A) &= (R_0^{\delta+\lambda}/R_0^\delta)[\tau(0|\delta) + \tau(\delta|0)] \\ &= (P_{rl}/P_{rr})[\tau(0|\delta) + \tau(\delta|0)] \end{aligned} \quad (85)$$

where P_{rl} and P_{rr} are the analogues to the trans and cis probabilities respectively defined in Eq. (71) for the initial position at the right end of the channel. We have obtained an expression for the additional time an ion spends in the channel due to reentries in terms of MFPTs and the trans probability. We now add the occupancy time due to reentries to $\tau(0|\delta)$ to obtain the total occupancy time starting with the ion at $x = 0$:

$$t(\delta|0,R;\delta+\lambda,A) = \tau(0|\delta) + (P_{rl}/P_{rr})[\tau(0|\delta) + \tau(\delta|0)] \quad (86)$$

Returning to an analysis of the trajectories of ions in the channel, we present an extension of the argument we used to interpret the expression obtained in the last section for MOT #1. We first consider only the additional time an ion spends in the channel due to reentries. Since we are starting the ion at the right end of the channel, the *cis* boundary is at δ and the *trans* boundary is at 0. As before we divide the trajectories into two

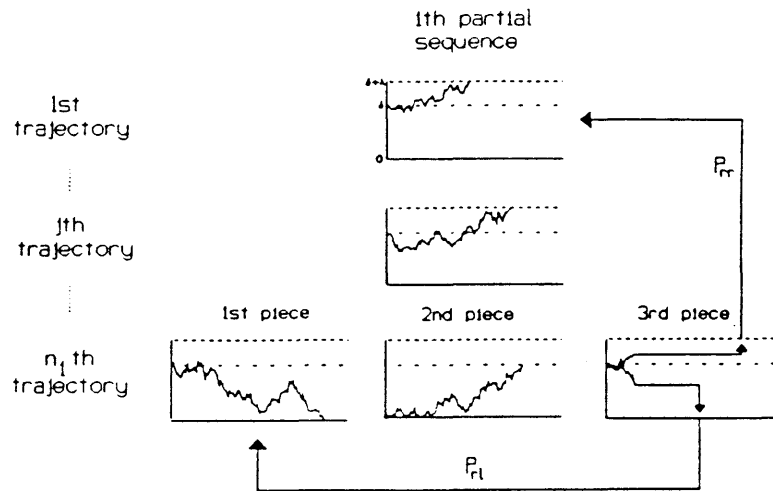


FIGURE 10. The additional time an ion spends in the channel due to reentries can also be related to MFPTs. An ensemble of trajectories whose initial position is at the right end of the channel ($x = \delta$) is again enumerated with the indices i and j (see Fig. 9). As in the argument for MOT #1, the first $n_i - 1$ trajectories of the i th partial sequence never reach the *trans* boundary of the channel ($x = 0$ in this case). The first piece of the n_i th trajectory is also analogous to the n_i th trajectory of MOT #1. The second piece of the n_i th trajectory is equivalent to a sample trajectory of an MFPT. The third piece is exactly a sample trajectory for the MOT we are calculating, thus leading to a consistency relationship that can be solved for the desired MOT.

categories. The first category (*cis*) is the set of trajectories that encounter the absorbing boundary at $\delta + \lambda$ without ever encountering the reflecting boundary at 0. The second category is the set of trajectories that encounter the reflecting boundary at 0 at least once before being absorbed at $\delta + \lambda$. Let us consider the second category in greater detail. The first piece (a *trans* trajectory), starting at δ and ending at the first contact with the reflecting boundary at 0, is analogous to a sample from the second category of trajectories of MOT #1 (with the right and left ends of the channel reversed). The second piece, starting at the first contact with the reflecting boundary and ending at δ , is a sample trajectory from $\tau(0|\delta)$ of Eq. (82). The third piece, starting at δ and being absorbed at $\delta + \lambda$, is a sample trajectory from the MOT we are considering here due to reentries (see Fig. 10).

We begin by applying the same argument used for MOT #1 to obtain the average time a sequence of n_i trajectories spends in the channel ending in contact with the *trans* absorbing boundary (in this case the first contact with the reflecting boundary at δ). We then add to this $\tau(0|\delta)$. This yields the average time a sequence of trajectories spends in the channel where the first $n_i - 1$ ions are absorbed at $\delta + \lambda$ without ever encountering the reflecting boundary at 0 and where the n_i th ion reaches δ for the first time after encountering the reflecting boundary at 0 at least once. On the average, the additional time the ion spends in the channel due to the third piece of the n_i th

trajectory is precisely the MOT we are trying to calculate, thus leading to a consistency relationship that we can solve for the MOT. For clarity, we will begin by neglecting the third piece of the n_i th trajectory and calculate the accumulated time spent in the channel by a sequence of n_i trajectories to the first encounter with the point at $x = \delta$ after an intervening encounter with the reflecting boundary at $x = 0$. After having done so, we introduce the third piece and generate a consistency relationship that we will solve for the MOT.

Formally, neglecting the third part of the n_i th trajectory, we have:

$$\text{Total accumulated time} = \sum_{j=1}^{n_i-1} \Delta t_j + \Delta t_{j=n_i} = \sum_{j=1}^{n_i} \Delta t_j \quad (87)$$

where the n_i th Δt is now the n_i th Δt associated with the second category of MOT #1 (an absorbing boundary at $x = 0$) plus a $\Delta t'_i$ required for the ion to return and visit the point at $x = \delta$ for the first time (be absorbed at $x = \delta$):

$$\Delta t_{n_i}(0, \text{Ref}) = \Delta t_{n_i}(0, \text{Abs}) + \Delta t'_i \quad (88)$$

Consider the average time spent in the channel by each ion neglecting the third piece of the n_i th trajectory. This is the average of the Δt_k :

$$t = \lim_{N \rightarrow \infty} \frac{1}{N} \sum_{k=1}^N \Delta t_k \quad (89)$$

We again write N as the product of M and the average of the n_i , factor the reciprocal of \bar{n} (the trans probability) from the limit, and write the sum in terms of a sum over the index i :

$$t = P_{rl} \lim_{M \rightarrow \infty} \frac{1}{M} \sum_{i=1}^M (\Delta t_i + \Delta t'_i) \quad (90)$$

Here we make use of a similar definition for Δt_i as we did in Eq. (75), i.e., the duration of an appended sequence of n_i edited trajectories ending with an absorption at $x = 0$. Recall that we concluded that Δt_i was the average of the MFPT ($x = 0$, reflecting; $x = \delta$, absorbing for MOT #1 and $x = 0$, absorbing; $x = \delta$, reflecting here) and that $\Delta t'_i$ was a duration associated with a sample trajectory of a left-to-right MFPT. Hence, Eq. (90) can be rewritten in terms of the MFPTs:

$$t = P_{rl} [\tau(0|\delta) + \tau(\delta|0)] \quad (91)$$

We now introduce the third piece of the trajectory and use Eq. (91) to obtain a consistency relationship for the MOT. Recall that the n_i th ion in the

sequence terminated at the *cis* end of the channel ($x = \delta$), effectively neglecting the third piece of the n_i th trajectory. Thus, the n_i th ion in the sequence still has the opportunity to spend more time in the channel. We can account for this by noting again that at the end of the above accumulated time t , the ion is at the initial position with the same boundary conditions for a sample trajectory we are calculating here. We would therefore expect that, on the average, the n_i th ion would spend an additional MOT in the channel before being absorbed at $x = \delta + \lambda$. Adding the additional MOT to the duration of the n_i th ions' occupancy brings the average time on the left-hand side (91) up to the MOT. We again average and make use of the definition of the MFPTs in terms of the Δt_i 's to yield

$$t(\delta|0,R;\delta+\lambda,A) = P_{rl}[\tau(0|\delta) + \tau(\delta|0) + t(\delta|0,R;\delta+\lambda,A)] \quad (92)$$

We can now solve Eq. (92) for MOT #2 to obtain Eq. (85). To obtain the average time an ion occupies the channel starting at $x = 0$ with a reflecting boundary at $x = 0$ and an absorbing boundary at $x = \delta + \lambda$, we add the correction due to reentries given by Eq. (85) to the MFPT:

$$t(0|0,R;\delta+\lambda,A) = \tau(0|\delta) + (P_{rl}/P_{rr})[\tau(0|\delta) + \tau(\delta|0)] \quad (93)$$

5.3.3. MOT #3 ($x_i = 0 \mid x = -\lambda$, Absorbing; $x = \delta + \lambda$, Absorbing)

Finally we would like to allow for the possibility that the ion will exit either end of the channel and subsequently reenter. This is accomplished by placing absorbing hemispherical surfaces at a radial distance λ from both ends of the channel. We will start with the ion at $x = 0$ for ease of comparison with the previous calculations.

For the flux we need only modify the flux used in the second MOT by moving the absorbing boundary from $x = \delta$ to $x = \delta + \lambda$:

$$J(0|\delta+\lambda) - J(0|-\lambda) = n(0)e^{\psi(0)}(R_+^{\delta+\lambda}/R_-^{\delta+\lambda}) \quad (94)$$

An expression for the concentration can be obtained by using the unidirectional flux from the source at $x = 0$ to the absorbing boundary at $x = \delta + \lambda$ for the flux in Eq. (49):

$$n(x) = n(0)e^{\psi(0) - \psi(x)}R_x^{\delta+\lambda}/R_0^{\delta+\lambda} \quad (95)$$

Integrating Eq. (95) and multiplying by the cross-sectional area yields the holdup:

$$P_{occ} = \frac{An(0)e^{\psi(0)}}{R_0^{\delta+\lambda}} \int_0^{\delta} R_x^{\delta+\lambda} e^{-\psi(x)} dx \quad (96)$$

Dividing by the total rate at which ions leave the channel, Eq. (94) yields the MOT:

$$t(0|-\lambda, A; \delta + \lambda, A) = \frac{R_{-\lambda}^0}{R_{-\lambda}^{\delta+\lambda}} \int_0^{\delta} R_x^{\delta+\lambda} e^{-\phi(x)} dx \quad (97)$$

If we again make use of the definition of the *trans* probability as the ratio of the rate at which ions are absorbed at $\delta + \lambda$ to the total rate at which ions are supplied to the source, we will discover that the coefficient of the integral on the right-hand side of Eq. (97) is the *trans* probability. Some algebra also reveals that the integral in Eq. (97) is the second MOT [Eq. (93)]. Applying these results yields

$$t(0|-\lambda, A; \delta + \lambda, A) = P_{lr} \left[\tau(0|\delta) + (P_{rl}/P_{rr})[\tau(0|\delta) + \tau(\delta|0)] \right] \quad (98)$$

The trajectory argument for this result is trivial given the results already obtained. We need only apply the argument used in the first MOT (absorbing boundaries at $-\lambda$ and δ) to the second MOT [$\tau(0|\delta)$ plus a correction term for reentries in the *trans* end of the channel], rather than to $\tau(0|\delta)$. Notice that if we discard the correction term to $\tau(0|\delta)$ of Eq. (98) due to reentries, we will recover the result obtained for the first MOT. Intuitively, Eq. (98) states that the average time an ion occupies the channel is the total accumulated time a partial sequence of ions occupies the channel ending in an ion being absorbed at the hemispherical surface $x = \delta + \lambda$, times the probability that a given ion in the sequence will be absorbed at $\delta + \lambda$. Similarly, the occupancy time for ions whose point of injection is at the right end of the channel is given by

$$t(\delta|-\lambda, A; \delta + \lambda, A) = P_{rl} \left[\tau(\delta|0) + (P_{lr}/P_{ll})[\tau(0|\delta) + \tau(\delta|0)] \right] \quad (99)$$

5.4. Mean Occupancy Times and Exit Transition Rates

Now let us consider the relationship between the MOTs we have derived and the problem of ion permeation through a channel. Recall that in the calculation of the transition rates we defined the electrodiffusive resistances in terms of improper integrals. In Section 3.1 we argued loosely that once a trajectory ends up a large distance away from the channel, it can be classified into one of the four categories associated with the exit transition rates. This suggests that a semi-infinite volume could be represented by taking the limit as the radius of an absorbing hemispherical surface goes to infinity. We now return to this issue. Consider the electrodiffusive resistance offered by the semi-infinite volume on the right end of the channel. Let us write this resistance as the sum of the resistance from the capture radius ρ to λ and the resistance from λ to infinity:

$$R_{\rho}^{\infty} = R_{\rho}^{\lambda} + R_{\lambda}^{\infty} = \frac{1}{2\pi D} \left[\int_{\rho}^{\lambda} r^{-2} e^{\varphi(r)} dr + \int_{\lambda}^{\infty} r^{-2} e^{\varphi(r)} dr \right] \quad (100)$$

We take the reference for the potential to be at infinity, i.e., $\varphi(\infty) \equiv 0$, and further assume that the influence of the channel on the dimensionless potential is local in nature. This is equivalent to the mathematical statement that for all $\epsilon > 0$ there exists a $\lambda(\epsilon)$ such that $|\varphi(r)| < \epsilon$ for all $r < \lambda(\epsilon)$. The dependence of λ on ϵ is determined by $\varphi(r)$. For instance, if $|\varphi(r)|$ decays at least as fast as $1/r$, we have:

$$|\varphi(r)| < \rho|\varphi(\rho)|/r \quad (101)$$

where $\varphi(\rho)$ is the potential at the capture radius and then we must have:

$$\lambda(\epsilon) > \rho|\varphi(\rho)|/\epsilon \quad (102)$$

Under this assumption, $\varphi(r)$ in the second integral of Eq. (100) is bounded above by ϵ . If we take ϵ less than one and assume that Eq. (101) holds, we have the following bound on the second integral of Eq. (100):

$$\int_{\lambda}^{\infty} r^{-2} e^{\varphi(r)} dr < e^{\epsilon} \int_{\lambda}^{\infty} r^{-2} dr < \frac{e}{\rho|\varphi(\rho)|} \epsilon \quad (103)$$

If we now take the limit as $\epsilon \rightarrow 0$ (λ becomes large), then we see that the influence of the absorbing boundary on the electrodiffusive resistance can be made as small as we like. Furthermore, the only dependence of the MOTs on the position of the absorbing boundary is through the electrodiffusive resistances. We can then conclude that for λ sufficiently large, the MOTs calculated above can be made to arbitrarily well approximate the case where there is a semi-infinite bath and no absorbing boundary. This yields the following identifications between the MOTs and the emptying transition rates [see the definition of E_l following Eq. (33)]:

$$E_l = \frac{1}{t_l} = \lim_{\lambda \rightarrow \infty} [t(0|-\lambda, A; \delta + \lambda, A)]^{-1} \quad (104)$$

Under the constant-field assumption in the channel we have

$$E_l = \frac{1 + e^{\Delta\varphi} + \frac{k_i \delta e^{\Delta\varphi b}}{AD\Delta\varphi} (e^{\Delta\varphi} - 1)}{\frac{1}{D} \left(\frac{\delta}{\Delta\varphi} \right)^2 (e^{\Delta\varphi} - 1 - \Delta\varphi) + \frac{\delta AD e^{\Delta\varphi - \Delta\varphi b}}{k_i \Delta\varphi (e^{\Delta\varphi} - 1)} (e^{\Delta\varphi} + e^{-\Delta\varphi} - 2)} \quad (105)$$

$$E_r = \frac{1}{t_r} \lim_{\lambda \rightarrow \infty} [t(\delta|-\lambda, A; \delta + \lambda, A)]^{-1} \quad (106)$$

Again the constant-field result is

$$E_r = \frac{1 + e^{\Delta\varphi} + \frac{k_f \delta e^{\Delta\varphi b}}{AD\Delta\varphi} (e^{\Delta\varphi} - 1)}{\frac{1}{D} \left(\frac{\delta}{\Delta\varphi} \right)^2 e^{\Delta\varphi} (e^{-\Delta\varphi} - 1 + \Delta\varphi) + \frac{\delta AD e^{\Delta\varphi - \Delta\varphi b}}{k_f \Delta\varphi (e^{\Delta\varphi} - 1)} (e^{\Delta\varphi} + e^{-\Delta\varphi} - 2)} \quad (107)$$

The case where reentrances are negligible (Levitt, 1986) can be obtained from the constant-field result by taking the limit where k_f becomes large holding $\Delta\varphi_b$ fixed. Since $\Delta\varphi_b$ is proportional to the logarithm of the dissociation constant, allowing k_f to get large implies that the off rate increases proportionally and ions seldom reenter. In this limit the last term in the numerators and first term in the denominators of Eqs. (105) and (107) dominate. Thus, one of the implications of Levitt's (1986) result is that *trans* trajectories are absorbed at the *trans* end of the channel.

6. STANDARD RESULTS

6.1. Binding Affinity

By rearranging Eq. (34) the probability the channel is empty can be written

$$P_0 = \left[1 + \frac{F_l}{E_l} + \frac{F_r}{E_r} \right]^{-1} \quad (108)$$

If we now make use of the definitions of the filling transition rates as the concentration times the second-order filling rate coefficient and exit transition rates as reciprocal MOTs, we have

$$P_0 = [1 + n(-\infty)t_l k_{fl} + n(\infty)t_r k_{fr}]^{-1} \quad (109)$$

This form suggests that P_0 can be written as

$$P_0 = (1 + \bar{n} \cdot \bar{K}_a)^{-1} \quad (110)$$

where $\bar{n} \cdot \bar{K}_a$ is the scalar product of the vectors

$$\bar{n} = [n(-\infty), n(\infty)] \quad (111)$$

$$\bar{K}_a = [K_{al}, K_{ar}] \quad (112)$$

$$K_{al} = t_l k_{fl}, K_{ar} = t_r k_{fr} \quad (113)$$

Notice that the components of the affinity constant are given by the second-order rate coefficient (k_f) divided by the first-order rate coefficient ($1/t$). In the case of a symmetric channel with no applied voltage, the affinity vector will be 45° from the "left" and "right" coordinate axes. Thus, the deviation of the affinity vector from 45° can be thought of as an indication of the asymmetry of the potential energy profile in the channel. In symmetric solutions, Eq. (110) reduces to the Michaelis–Menten result for the probability of finding free enzyme where $n = n(-\infty) = n(\infty)$ is the substrate concentration and $K_{al} + K_{ar} = 1/K_m$, the Michaelis constant. The Michaelis–Menten result can also be obtained by setting the concentration on either end of the channel to zero. With $n(-\infty) = 0$, $K_{al} = 1/K_m$ and with $n(\infty) = 0$, $K_{ar} = 1/K_m$.

6.2. Multiple Current-Carrying Species

At this point, several additional quantities can be derived that are standard in experimental work on membrane channels. In most experimental protocols, several ionic species are present. In this case, the channel can be occupied by several different species and Eq. (32) becomes

$$P_0 + \sum_{k=1}^N [P_k(l) + P_k(r)] = 1 \quad (114)$$

where $P_k(l) + P_k(r)$ is the probability that the channel is occupied by the k th ionic species. Equations (40), (41), (53)–(56), (59), and (60) together with Eq. (114) allow us to obtain the analogue of Eq. (110) for the case with N permeant species. The concentration and affinity vectors are now given by

$$\bar{n} = [n_1(-\infty), \dots, n_N(-\infty), n_1(\infty), \dots, n_N(\infty)] \quad (115)$$

$$\bar{K}_a = [K_{al1}, \dots, K_{alN}, K_{ar1}, \dots, K_{arN}] \quad (116)$$

The current through the channel in this case would be given by

$$I = P_0 \sum_{k=1}^N z_k e J_{ik} \quad (117)$$

where J_{ik} is the flux through the channel of the k th ionic species under the assumption of independence and z_k is the valence of the k th ionic species:

$$-J_{ik} = (1/R_k)[n_k(\infty)e^{\varphi_k(\infty)} - n_k(-\infty)e^{\varphi_k(-\infty)}] \quad (118)$$

where $R_k = R_{-z_k}$ is the electrodiffusive resistance over the entire range of integration for the k th species of ion and can be considered the reciprocal of an effective permeability.

6.3. Reversal Potential

The reversal potential in this model can be obtained by setting the current equal to zero and solving for the potential:

$$\sum_{k=1}^N \frac{z_k}{R_k} [n_k(-\infty) - n_k(\infty)e^{z_k\Delta\psi}] = 0 \quad (119)$$

where $\Delta\psi$ is the dimensionless reversal potential. Equation (119) can be rearranged to yield a polynomial in $e^{\Delta\psi}$. An interesting special case of Eq. (119) occurs when we have a monovalent and a divalent cation on either side of the channel:

$$\frac{2n_2(\infty)}{R_2} u^2 + \frac{n_1(\infty)}{R_1} u - \left[\frac{2n_2(-\infty)}{R_2} + \frac{n_1(-\infty)}{R_1} \right] = 0 \quad (120)$$

where $u = e^{\Delta\psi}$, the subscript 2 indicates the divalent, and the subscript 1 indicates the monovalent. Thus, we have

$$\Delta\psi = \ln \frac{-\frac{n_1(\infty)}{R_1} + \sqrt{\left[\frac{n_1(\infty)}{R_2}\right]^2 + \frac{8n_2(\infty)}{R_2} \left[\frac{2n_2(-\infty)}{R_2} + \frac{n_1(-\infty)}{R_1}\right]}}{\frac{4n_2(\infty)}{R_2}} \quad (121)$$

A more familiar expression (the Goldman–Hodgkin–Katz equation) is obtained when one considers two different monovalent species:

$$\Delta\psi = \ln \left[\frac{P_1 n_1(-\infty) + P_2 n_2(-\infty)}{P_1 n_1(\infty) + P_2 n_2(\infty)} \right] \quad (122)$$

Here, we define the permeability as the reciprocal of the total electrodiffusive resistance. Finally, as a special case of Eq. (122), we consider a bi-ionic experiment with two monovalent species. The voltage is measured at zero current with the concentration of ion #1 on the left side equal to the concentration of ion #2 on the right side and the concentration of ion #1 right and #2 left equal zero to yield:

$$\Delta\psi = \ln(R_2/R_1) = \ln(P_1/P_2) \quad (123)$$

6.4. Block

Ionic channel blockers, in the case of a one-ion channel, are a special case of Eq. (117) where the blocking ion has a much lower permeability than

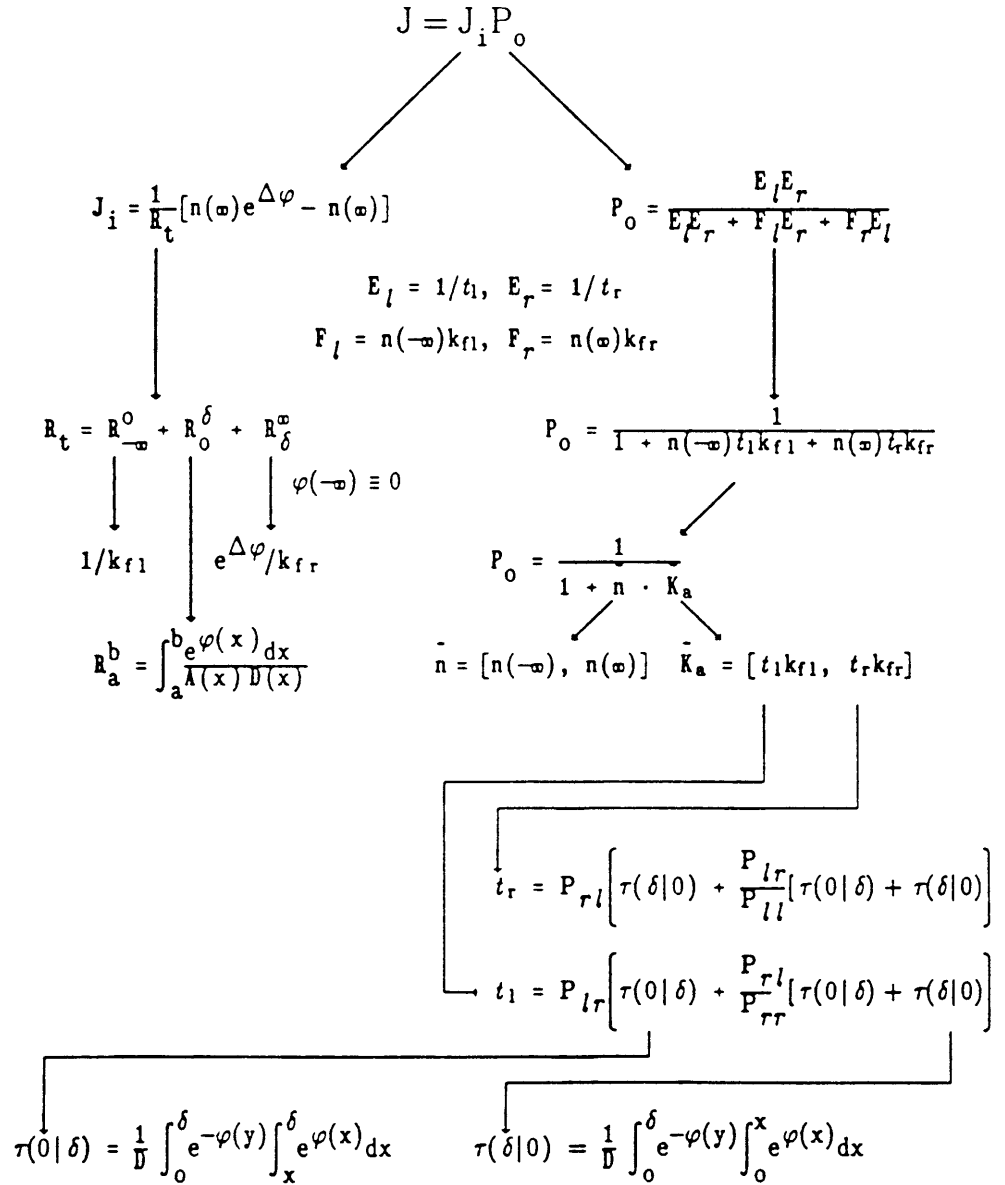


FIGURE 11. Summary of important results for a diffusion model of a one-ion channel. Starting at the top of the figure we have from Eq. (22) that the flux through a one-ion channel is the product of the empty probability and the independence flux. Moving down the left-hand column we have from Eqs. (11), (38), and (118) the independence flux as the ratio of the difference of exponentials of the electrochemical potential and the total electrodiffusive resistance. The total electrodiffusive resistance can in turn be decomposed into components associated with the bath access region on the left end of the channel, the channel itself, and the bath access region on the right end of the channel. For the right-hand column beginning with the second row we have from Eq. (34) the empty probability in terms of the filling and emptying transition rates between the channel occupancy states. In the middle below the second row we have Eqs. (40)

other ions in the solution. In this limiting case we can assume that none of the current is carried by the blocker, but the blocker does change the probability that the channel is empty. Hence, the sum in Eq. (117) would only include the permeant ions while the concentration and affinity vectors used in calculating P_0 would include any ions that could enter the channel. In Fig. 11 we have summarized the one-ion model.

7. DISCUSSION

7.1. Assumptions

7.1.1. Constant-Field Assumption

In the numerical calculations we have assumed that the contribution to the potential energy profile from the transmembrane voltage is linear. In this section we will examine this assumption and show by dimensional arguments that the approximation is a natural consequence of the one-ion assumption (given a homogeneous dielectric medium).

At equilibrium the relationship between the electrostatic field and the concentration in an ionic solution is given by the Poisson–Boltzmann theory (McQuarrie, 1976, Chapter 15):

$$\nabla^2\psi(r_1, r) = \frac{q}{\kappa\epsilon_0} \sum_k n_k z_k e^{-\beta q\psi(r_1, r)} \quad (124)$$

where $\psi(r_1, r)$ is the electrostatic Helmholtz free energy at a position r , given that there is a charged particle at position r_1 , κ is the dielectric constant, ϵ_0 is the permittivity of free space, n_k and z_k are the number density and valence of the k th ionic species in solution, and β is the reciprocal of the mean thermal kinetic energy $k_b T$. In this context we are interested in ion channels that are highly selective. We will assume that the channel will only admit one type of monovalent cation. Equation (124) then becomes

$$\nabla^2\psi = (qne^{-\beta q\psi})/\kappa\epsilon_0 \quad (125)$$

and (41) the filling transition rates in terms of the bath concentrations and second-order filling rate constants. Also from Eqs. (104) and (106) we have the emptying transition rates in terms of the mean channel occupancy times (MOTs). From Eqs. (109)–(113) we have the empty probability in terms of the channel occupancy times and second-order filling rate constants followed by an expression in terms of the scalar dot product of the concentration and affinity vectors. From Eqs. (98) and (99) we also have the channel occupancy times in terms of mean first passage times and *cis* and *trans* probabilities. Finally, from Eqs. (67), (82), and (83) we have expressions for the mean first passage times in terms of the diffusion coefficient, the channel length, and the dimensionless potential in the channel.

Now assume that the potential energy profile in the channel can be obtained from the superposition of image charge effects, specific interactions between the permeating ions and the channel protein, and the applied transmembrane voltage. We will use Eq. (125) for the contribution to the potential energy due to the capacitive charge arising from the transmembrane voltage. The following nondimensional variables can be introduced:

$$\varphi = \beta q \psi, \quad \gamma = \beta q^2 \delta^2 n / \kappa \epsilon_0, \quad \xi = x / \delta \quad (126)$$

Making these substitutions, Eq. (125) becomes

$$d^2 \varphi / d \xi^2 = \gamma e^{-\varphi} \quad (127)$$

Notice that in the limit where the interaction potential φ is large (the one-ion occupancy assumption), the right-hand side of Eq. (127) is small and the potential is approximately linear. Also it is clear that in the low-concentration limit, γ becomes small and again we conclude that the potential is linear (the thin-membrane assumption; Moore, 1977). These two limiting cases also lead to the result that the channel never contains more than one ion. More sophisticated electrostatic calculations (Levitt, 1978; Jordan, 1984) are in qualitative agreement with this result when one ion is in the channel. In summary, we conclude that we can adequately approximate the contribution due to the applied transmembrane potential by the following linear function:

$$\varphi(x) = zq\beta\Delta V_m x/\delta \quad 0 < x < \delta \quad (128)$$

where ΔV_m is the applied transmembrane voltage. We also conclude that this is consistent with the assumption that at most one ion can enter the channel at a time.

7.1.2. One-Ion Occupancy

In this section we will develop a simple model to estimate the validity of assuming that at most one ion can occupy a membrane channel. The strategy will be to assume that the probability of finding the channel in a given configuration is approximated by the equilibrium distribution. We will then proceed by assuming that the channel is in one of three states: empty, occupied by one ion, or occupied by two ions. The equilibrium density of a system that contains a variable number of particles is known as the grand canonical ensemble density function (McQuarrie, 1976):

$$p(N; \mathbf{x}) = \frac{e^{-\beta U(\mathbf{x})} e^{\beta \mu N}}{\sum_{N=0}^{\infty} \int \dots \int e^{-\beta U(\mathbf{x}')} e^{\beta \mu N} dx'_1 \dots dx'_{jN}} \quad (129)$$

where N is the number of particles in the system, $U(\mathbf{x})$ is the potential energy of the system, $\mathbf{x} = (x_1, x_2, \dots, x_{3N})$ is the configuration space vector of the system (x_{3i-2} , x_{3i-1} , and x_{3i} are the three spatial coordinates of the i th particle in the system), and $\mu = k_b T \ln(n)$ is the chemical potential of the bath. The assumption that at most two ions can enter the channel is equivalent to neglecting all of the terms in the grand partition function [denominator of Eq. (129)] past $N = 2$. If we further assume that the channel cross-sectional area is uniform and the potential depends only on the coordinate normal to the membrane, the probability $P(2)$ that the channel is occupied by two ions can be obtained by integrating the density $p(N; \mathbf{x})$ over all states with two ions:

$$P(\text{two ions}) = \frac{(nA)^2 \int_{2r}^{\delta} \int_0^{x_1-2r} e^{-\varphi_2(x_1, x_2)} dx_2 dx_1}{1 + nA \int_0^{\delta} e^{-\varphi_1(x)} dx + (nA)^2 \int_{2r}^{\delta} \int_0^{x_1-2r} e^{-\varphi_2(x_1, x_2)} dx_2 dx_1} \quad (130)$$

where φ is the dimensionless potential βU , x_1 is the distance from the left end of the channel of the rightmost ion and x_2 is the distance from the left end of the channel of the other ion (only half of the available configurations are included since there are two ways to label the two particles), $2r$ is the closest distance of approach between the two ions, A is the cross-sectional area of the channel that is accessible to the permeant ion, and n is the concentration of permeant ion in the bath at the reference potential ($\varphi = 0$).

We must now specify the potential functions in Eq. (130). The first assumption is that the interaction potential between the channel and each ion superposes with the potential of interaction between two ions (i.e., the binding of the two ions is not cooperative in any way). Thus, the one-ion potential φ_1 is the ion-channel interaction potential. The two-ion potential φ_2 is the sum of the ion-channel interaction potential at x_1 plus the ion-channel interaction potential at x_2 plus the ion-ion interaction potential $\varphi_i(x_1, x_2)$. Thus, we have

$$\varphi_2(x_1, x_2) = \varphi_1(x_1) + \varphi_1(x_2) + \varphi_i(x_1, x_2) \quad (131)$$

We proceed by allowing φ_1 to remain unspecified, but identical to the ion-channel interaction potential during single occupancy. This last assumption is motivated by convenience rather than sound physical reasoning. We are left with φ_i , the potential of interaction between ions. We construct the interaction potential by first placing an ion at the position x_1 and calculating the resulting field. The interaction potential is then obtained through the use of a line integral up to position x_2 . Following Levitt (1985) we will take the limit where the dielectric constant of the water-filled pore is considered to be much greater than that of the surrounding protein and lipid. In this limit the component of the electric field normal to the channel lumen is negligible

relative to the axial component. We will use a simplification of Levitt's results by allowing the cross-sectional area to undergo a jump discontinuity from the circular disk ($A = \pi r^2$) at the mouth of the channel to the hemispherical surface ($A = 2\pi r^2$) defined by the capture radius. This simplification yields more manageable expressions that have qualitatively similar properties to Levitt's more realistic expressions. Under these assumptions, the component of the electric field in the channel due to an ion in the channel is uniform and given by

$$E(x < x_1) = \frac{zq}{\pi a^2 \kappa \epsilon_0} \left[\frac{\eta + \delta - x_1}{2\eta + \delta} \right] \quad (132)$$

$$E(x > x_1) = \frac{zq}{\pi a^2 \kappa \epsilon_0} \left[\frac{\eta + x_1}{2\eta + \delta} \right] \quad (133)$$

where η is a length defined by

$$\eta = (1/\lambda + 1/\rho)^{-1}$$

and λ is the Debye length. The interaction potential at x_2 given an ion at x_1 can now be obtained by adding the potential at the left mouth of the channel to the integral of the field from the mouth up to x_2 [remember that the limits in Eq. (130) require $x_2 < x_1$]:

$$\varphi_i(x_1, x_2) = \frac{\beta z_1 z_2 e^2}{\pi a^2 \kappa \epsilon_0} \left[\frac{(\eta + x_2)(\eta + \delta - x_1)}{2\eta + \delta} \right] \quad (134)$$

We compare the results for a centrally located well versus a centrally located barrier versus the constant field where we have constrained the equilibrium one-ion affinity to be the same for each potential (see Fig. 12). If we take the dielectric constant to be 40 and require the probability of double occupancy to be less than or equal to 0.1 with a one-ion affinity constant of $(340 \text{ mM})^{-1}$, the highest concentration for which the one-ion assumption is valid is 0.71 M for the central barrier, 1.56 M for the constant field, and 12.2 M for the central well. The reader should be cautioned that this is only an order-of-magnitude estimate of the effects of double occupancy. For example, multiple occupancy in the well potential would be expected to have a greater influence on the flux than in the barrier potential.

7.1.3. Transients

Next we examine the validity of the use of the steady-state approximation for the rate at which ions enter the channel. The issue is whether the potential of interaction, which we assume excludes a second ion from entering the channel, influences the rate at which an ion will enter after the ion in the channel has left. To address this issue we will consider the charge relaxation time in a uniform conductor.

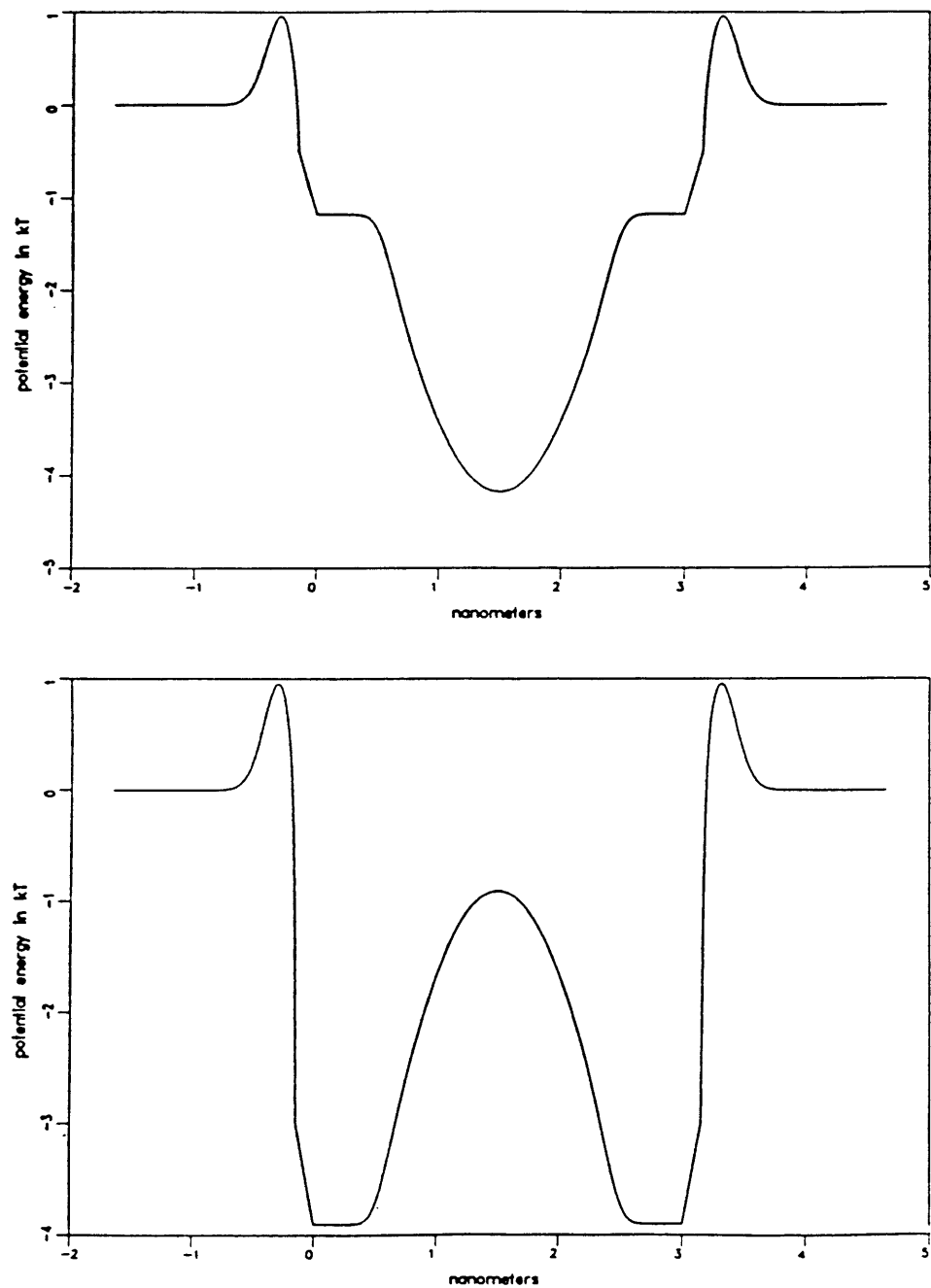


FIGURE 12. Plots of the ion-channel interaction potential where the form of the potential is varied from a centrally located $3k_bT$ well to a centrally located $3k_bT$ barrier. The offset in each case has been chosen to give a 340 mM equilibrium dissociation constant for comparison with the constant-field potential in Fig. 1.

We begin by calculating the product of the steady-state diffusion to capture up to a hemispherical surface of radius $r = \rho$ and the charge relaxation time constant in a conductor. In making use of the charge relaxation time of a conductor, we are assuming that charge in the bath access region can be represented in terms of a continuous density function and that the conductivity is a constant independent of the time and spatial coordinates. We will return to these issues again after concluding the analysis.

It is a standard result in electromagnetic theory that the time course of relaxation of a charge distribution Λ in a dielectric medium is given by (Lorrain and Corson, 1970, p. 424)

$$\Lambda = \Lambda_0 e^{-(\sigma/\kappa\epsilon_0)t} \quad (135)$$

where Λ_0 is the initial charge distribution and σ is the conductivity of the medium. Thus, for the relaxation time constant we have

$$t_r = \frac{\kappa\epsilon_0}{\sigma} = \frac{\kappa\epsilon_0 k_b T}{q^2 [\sum_i z_i^2 D_i n_i(\infty) + \sum_j z_j^2 D_j n_j(\infty)]} \quad (136)$$

where the index i refers to all those ionic species that are able to permeate the channel and the index j refers to those ionic species that are impermeant.

From the steady-state Nernst–Planck equation, we have for the flux up to an absorbing hemispherical surface of radius ρ :

$$J_f = \sum_i 2\pi D_i n_i(\infty) \left[\int_{\rho}^{\infty} r^{-2} e^{\phi(r)} dr \right]^{-1} \quad (137)$$

Equation (137) is the average rate at which ions diffusing in a radially symmetric field ϕ encounter the surface at $r = \rho$. Since only those ions that can enter the channel can contribute to a net flux, we sum only over the index i . Thus, the average time t_f between encounters is given by the reciprocal of Eq. (137):

$$t_f = 1/J_f \quad (138)$$

Consider several special cases of the product of Eqs. (136) and (138). First consider that ϕ is everywhere zero and there is a single ionic species of valence one. We have for the dimensionless ratio of times:

$$R_0 \equiv t_f/t_r = q^2/2\pi\rho\kappa_w\epsilon_0 k_b T \quad (139)$$

where κ_w is the dielectric constant of water, taken to be 80. At 300° the numerical value of Eq. (139) is approximately $14/\rho$ where ρ is measured in angstroms. Thus, we have a separation of time scales only if the channel capture radius is say 2\AA or less.

Next consider the case where there is a charge Q distributed uniformly

across the hemispherical surface (assume that the entire flux of the electric field is directed radially outward into the semi-infinite volume). At infinite dilution the potential will be given by

$$\varphi(r) = Qq/2\pi\kappa_b T\kappa_w\epsilon_0 r \quad (140)$$

Equation (139) now becomes

$$t_f/t_r = q^2[e^{\varphi(\rho)} - 1]/2\pi a\varphi(\rho)\kappa_w\epsilon_0 k_b T \quad (141)$$

$$\varphi(\rho) = Qq/2\pi\rho\kappa_w\epsilon_0 k_b T \quad (142)$$

For $\varphi(\rho) \ll 1$ we have

$$t_f/t_r \cong R_0 + [Q\varphi^2(\rho)/2q] \quad (143)$$

Hence, if Q is negative (the channel is at a lower potential energy than the bath), the ratio is smaller still. This case is relevant in light of the fact that most ion-specific channels exhibit high affinities for the ions that permeate them. Equation (143) is an overestimate of the effect of a potential difference between the bath and the channel. At physiological concentrations, the charge Q would be screened by counterions, but qualitatively the effect would be the same.

This analysis suggests that the steady-state assumption for the filling rate constant would only be valid in two limiting cases: the case where there is a high concentration of impermeant ions and a low concentration of permeant ions, and second, in the limit where the capture radius goes to zero. In the absence of these assumptions, one would conclude that the steady-state assumption for the filling rate constant might underestimate the effect of bath access limitations especially in the case where the channel was at a potential below that of the bath.

The above method is based on a continuum macroscopic theory, the assumptions of which are suspect in the case of a molecular ion channel. A more accurate treatment requires the use of the time-dependent diffusion equation whose validity at the microscopic level is on firmer grounds (Gates, 1988).

7.2. Constant-Field Diffusion Model versus Eyring Rate Theory Models

We will begin by applying the assumption of a linear form for the potential in the channel (constant field) in the diffusion model [Eq. (4)]. As discussed in Section 2.2, we have added an offset in the potential to account for the affinity the channel has for the permeant ion. Combining Eqs. (28) and (12) yields

$$-J_i = nk_f \frac{e^{\Delta\varphi} - 1}{1 + e^{\Delta\varphi} + \frac{k_f \delta^2}{D\Delta\varphi K_a} (e^{\Delta\varphi} - 1)} \quad (144)$$

For the probability that the channel is empty we have

$$P_0 = \frac{1 + e^{\Delta\varphi} + \frac{k_f \delta^2}{D\Delta\varphi K_a} (e^{\Delta\varphi} - 1)}{1 + e^{\Delta\varphi} + \frac{k_f \delta^2}{D\Delta\varphi K_a} (e^{\Delta\varphi} - 1) + nk_f t} \quad (145)$$

$$t = \frac{\delta^2}{D\Delta\varphi} (e^{\Delta\varphi} - 1) + \frac{2K_a e^{\Delta\varphi}}{k_f \Delta\varphi (e^{\Delta\varphi} - 1)} (e^{\Delta\varphi} + e^{-\Delta\varphi} - 2) \quad (146)$$

Equations (144) through (146) suggest the definition of three dimensionless numbers:

$$R = \frac{\delta^2 / (DK_a)}{1/k_f} = \frac{R_c}{R_b} \Big|_{\Delta\varphi = 0} \quad (147)$$

$$T = \frac{\delta^2 / D}{1/(nk_f)} = \frac{\tau_c}{\tau_b} \Big|_{\Delta\varphi = 0} \quad (148)$$

$$V = nK_a = \frac{v_c}{v_{sp}} e^{-\Delta\varphi_b} \Big|_{\Delta\varphi = 0} \quad (149)$$

where v_{sp} is the specific volume of the permeant ion in bulk solution, v_c is the channel volume, τ_c is the sum of the MFPTs for ions injected at the right and left ends of the channel at $\Delta\varphi = 0$ under the constant-field assumption [see Eq. (84)], and τ_b is the characteristic time an ion takes to find the channel [see Eq. (137)]. Applying the constant-field assumption to Eq. (93) reveals that the dimensionless volume is associated with the additional occupancy time due to reentrances.

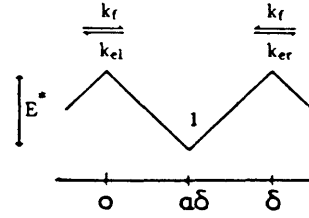
7.2.1. One-Site Rate Theory Model

We now turn to rate theory models. We begin with a one-site model to demonstrate that many of the properties of a one-ion channel can be captured by thinking of the channel interior as being a single site. The model is shown in Fig. 13. For this model the independence flux and the empty probability are

$$-J_i = nk_f [(e^{\Delta\varphi} - 1) / (e^{\Delta\varphi} + 1)] \quad (150)$$

$$P_0 = \frac{1 + e^{\Delta\varphi}}{1 + e^{\Delta\varphi} + (2nk_f/v) \exp[\beta E^* + (1 - \alpha)\Delta\varphi]} \quad (151)$$

FIGURE 13. A single-site rate theory model for a channel where the rate at which ions encounter the channel is voltage independent. k_f is the second-order filling rate constant for ion entry from the bath and k_{e1} and k_{er} are the first-order exit rate coefficients for the left and right ends of the channel, respectively. E^* is the potential energy difference (for $\Delta\varphi = 0$) between site 1 and the top of the barrier and α is the dimensionless position of site 1.



where α is the dimensionless electrical distance of the binding site from the left end of the channel. By analogy with the diffusion model, we define the dimensionless time:

$$T = \frac{1/(\nu \exp[-\beta E^*])}{1/nk_f} \quad (152)$$

Comparing Eqs. (148) and (152) identifies the characteristic time of escape from the well in the rate theory model with twice the time [one each for t_l and t_r , see Eqs. (105) and (107)] an ion takes to diffuse the length of the channel. We also see that the one-site rate theory model lacks the other two dimensionless numbers. If we compare Eqs. (144) and (150), we see that the independence flux has an additional term that is absent in the one-site rate theory model. This term is proportional to the dimensionless ratio of the electrodiffusive resistances. Hence, if we take the limit where the bath access to the channel dominates the resistance to permeation, the diffusion model goes over to the one-site rate theory model. The one-site rate theory model therefore exhibits the limiting steepness to saturation with voltage of the constant field diffusion model. Comparison of Eqs. (145) and (151) further reveals that the dimensionless ratio of volumes has been lost in the rate theory model, which therefore does not account for reentrances. An additional point worth noting is that we can cast the rate theory model in terms of the transition rates we defined for the diffusion model. This reveals that the exit rates are symmetrical: $E_l = E_r = k_{e1} + k_{er}$. Thus, the one-site model is memoryless in the sense that the side on which an ion exits the channel does not depend on the side it entered.

7.2.2. Symmetric Two-Site Rate Theory Model

We can gain a better understanding of the relationship between the two formalisms by including an additional site in the rate theory model. Since the diffusion model is symmetric with respect to the center of the channel, we will require the two-site rate model to be symmetric (see Fig. 14). For the independence flux and the empty probability, we have:

$$J_i = nk_f \frac{e^{\Delta\varphi} - 1}{e^{\Delta\varphi} + 1 + \gamma e^{\Delta\varphi/2}} \quad (153)$$

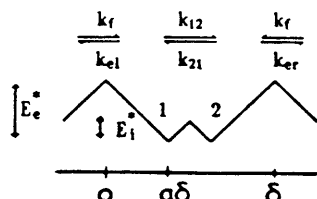


FIGURE 14. A two-site rate theory model for a channel where the rate at which ions encounter the channel is again assumed to be voltage independent. The two-site model now allows for the channel itself to offer a resistance to transport modeled by the rate coefficients k_{12} and k_{21} . E_e^* and E_i^* are the differences

(for $\Delta\phi = 0$) in potential energy between a site and the top of the barrier leading to exit from the channel and the height of the barrier separating the two sites, respectively. α is now the dimensionless position of site 1.

$$P_0 = \frac{e^{\Delta\phi} + 1 + \gamma e^{\Delta\phi/2}}{e^{\Delta\phi} + 1 + \gamma e^{\Delta\phi/2} + \nu n k_f [\gamma (e^{\alpha\Delta\phi} + e^{-\alpha\Delta\phi}) + 2(e^{\alpha\Delta\phi} + e^{(1-\alpha)\Delta\phi})]} \quad (154)$$

where α is now the dimensionless distance of the leftmost binding site from the left end of the channel and γ is defined by

$$\gamma = \exp[-\beta(E_e^* - E_i^*)] \quad (155)$$

with E_e^* the height of the barrier to ion exit from the channel and E_i^* the height of the barrier between the two sites in the channel. The constant-field diffusion model and the two-state rate theory model can now be made to agree at low concentrations near equilibrium through the following identification:

$$\gamma = k_f \delta^2 / DK_a \quad (156)$$

Better agreement between the two models can be obtained by increasing the number of sites in the rate theory model. In the limit where many barriers of equal height are introduced, the rate theory can be made to arbitrarily well approximate the low-concentration limit of the constant-field diffusion model (Lauger, 1979). In this limit the rate theory model would still fail to account for reentries. To account for reentries in the rate theory model would require a hybridization with a diffusion model for the bath (e.g., Lauger, 1976) or the addition of a system of barriers for the bath on either side of the channel.

7.3. Future Directions

In the following discussion we would like to suggest several ways that the model presented here can be generalized. These generalizations will be divided into two categories, the first being extensions of the one-ion theory and the second being a relaxation of the one-ion assumption itself.

7.3.1. Extensions of the Existing One-Ion Theory

7.3.1a. Relaxation of the Assumption of Instantaneous Transition Rates. As discussed in the previous section, the rate at which ions enter the

channel may require a more sophisticated treatment than the simple diffusion to capture presented here. The influence of past channel occupancy can result in a reduction of the rate at which ions enter the channel below what is predicted under the steady-state assumption. The qualitative effect of such transients can be investigated through the introduction of additional states. This can be done by defining a refractory state or states. When the channel is in such states, the rate at which ions enter is reduced to below the steady-state filling rate. These states are then allowed to decay to the normal empty state at a rate determined by a transient analysis. Such a kinetic model is consistent with the exponential time course of the transient described in the previous section and therefore suffers from the same approximations. These more complicated kinetic models can give rise to a voltage dependence in the effective rate at which ions enter the channel that is absent in the model presented here (Gates, 1988).

Another mechanism by which a voltage dependence in the filling rate can occur is through the ion injection effect (Walz *et al.*, 1969). This effect arises from the diffuse double layer of capacitive charge on the membrane. As the transmembrane voltage is increased, the capacitive charge increases and the local concentration of ions at the channel mouth increases. Although such effects are small in the physiological range of concentrations and voltages, they become important in high-voltage studies of model systems such as gramicidin (Anderson, 1983). A realistic treatment of such an effect would require the inclusion of the angular derivatives of the potential and concentration in the Nernst–Planck equation in spherical coordinates.

7.3.1b. More Complicated ψ s. Our group is currently in the process of characterizing the relationship between the structure of the potential profile in the channel and bath and the features of the current–voltage–concentration surface (Gates, 1988). This program can be carried out through the use of piecewise linear potentials that furnish analytical results that can be used to obtain asymptotic properties. These results can then be compared with numerical evaluations of the integral solution of smooth potentials. In Fig. 15 are the current–voltage–concentration curves associated with the potential profiles of Fig. 12. An interesting feature of these plots is that they are virtually indistinguishable in the low-voltage range even though the profiles are radically different. It would therefore be necessary to obtain data at large voltages to determine even the grossest features of the potential profile in such channels.

7.3.1c. Multiple Permeant Species to Include Block. The one-ion model can be generalized easily to allow for more than one permeant species (see Section 6). A special case of some interest are channel-blocking agents. In the case of large organic blockers of the SR potassium channel such as hexamethonium and decamethonium, nonlinearities observed in the logarithm of the conductance ratio (J. Tang, personal communication) can be accounted for in a natural way.

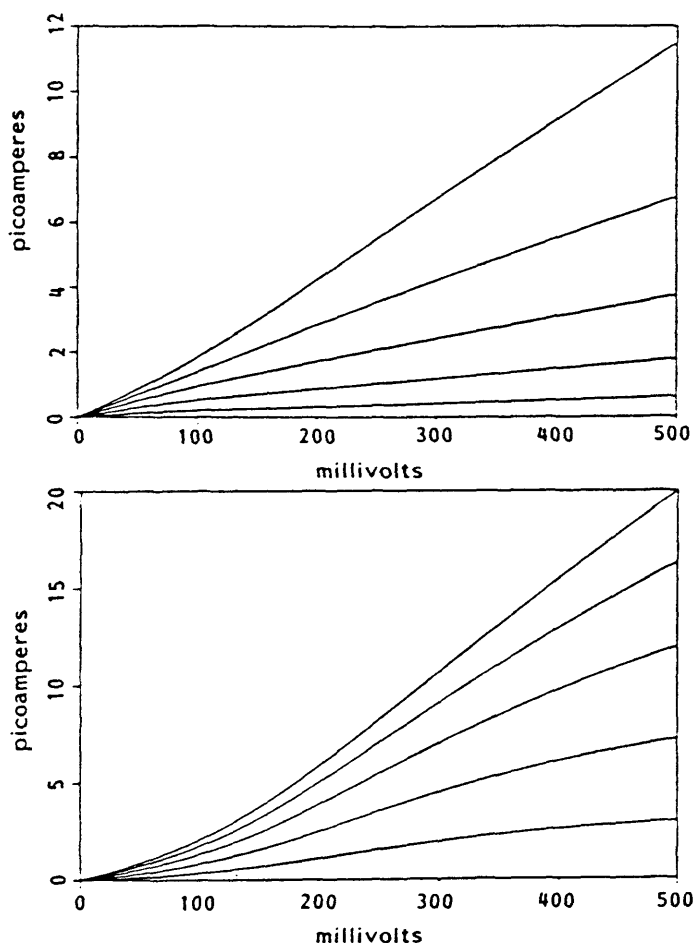


FIGURE 15. Current–voltage–concentration curves for the potential profiles given in Fig. 12. The upper panel is the current associated with the well potential and the lower panel is the current associated with the barrier potential. The concentrations and diffusion constants are the same as for the current in Fig. 3, associated with the constant-field potential. The saturating small signal conductance for these two cases is 20 picosiemens.

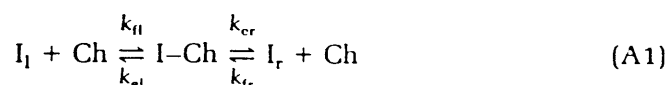
7.3.1d. Nonstationary ϕ . Lastly, nonstationary potential functions can be modeled through the introduction of additional states in a way analogous to generalizations of the Eyring rate theory (Lauger, 1987; Eisenman and Dani, 1987). In such a formalism, a series of potential functions, each associated with a different conformation of the protein, are introduced. Transitions of the protein between these conformational states can be coupled to other processes to yield coupled and active transport. In the case where the conformational transitions are coupled to occupancy of the channel by the permeant ion, nonlinear Edie–Hoffstie plots of the small signal conductance can arise (Hladky and Haydon, 1984), a characteristic that is usually taken as evidence of multiple occupancy.

7.3.2. Two-Ion Case

We now turn to the question of multiple occupancy. Experimental evidence suggests, with the possible exception of the SR potassium channel, that multiple occupancy must be included in a channel model. In the case of very narrow channels such as the gramicidin A channel, the distance between ions in the channel can be assumed fixed (Levitt, 1982). Hybrid forms of diffusion and discrete state space binding models can also be used to allow for multiple occupancy. For example, Levitt (1982) generated a model where an ion could bind at either end of the channel while a second ion diffused within the channel. More recently, Levitt (1987) reported numerical results for a double-occupancy channel model where the distance between the two ions in the channel was free to vary. To date, a thorough understanding of the properties of these models has been limited by the fact that only numerical solutions have been available. In situations where more than two ions can enter the channel and the motions of all the ions are on similar time scales, stochastic Brownian dynamics can be used to build pair correlation functions. These correlation functions can be used to correct the potential function for ion-ion interactions in a one-dimensional diffusion model where the potential profile in the channel will now be both concentration and voltage dependent.

8. APPENDIX: COMPARISON OF ONE-ION DIFFUSION MODEL WITH TRADITIONAL CHEMICAL KINETICS

In this appendix we relate the state model described in Eqs. (30) through (36) with traditional chemical kinetic models. Ion association with the channel can be thought of as a bimolecular diffusion reaction problem. In the same way, the ion exiting from the channel can be thought of as a unimolecular breakup reaction. Ion transport through a channel is analogous to enzyme catalysis. In this formalism the reactant is an ion on one side of the membrane, the product is an ion on the other side of the membrane, and the reaction coordinate is the distance the ion has moved through the pore. Ion channels have the additional feature that the potential energy of the reaction coordinate depends on the applied transmembrane voltage. Thus, we have



Here, k_{fl} and k_{lr} are second-order association rate constants describing the rate at which ions fill the left and right ends of the channel, respectively. k_{el} and k_{er} are the voltage-dependent first-order dissociation rate constants describing the rate at which ions exit the left and right ends of the channel, respectively. Equation (A1) gives rise to two equations analogous to Eq. (30) through (32):

$$(k_{fl}[I]_l + k_{lr}[I]_r)[Ch] - (k_{el} + k_{er})[I-Ch] = \frac{d}{dt} [I-Ch] \quad (A2)$$

$$[\text{Ch}] + [\text{I-Ch}] = [\text{Ch}]_t \quad (\text{A3})$$

where $[\text{Ch}]_t$ is the total concentration of channels. We can again make the steady-state assumption and write a matrix equation corresponding to Eq. (33):

$$\begin{bmatrix} k_{fl}[\text{I}]_l + k_{fr}[\text{I}]_r & -(k_{el} + k_{er}) \\ 1 & 1 \end{bmatrix} \begin{bmatrix} [\text{Ch}] \\ [\text{I-Ch}] \end{bmatrix} = \begin{bmatrix} 0 \\ [\text{Ch}]_t \end{bmatrix} \quad (\text{A4})$$

We can now solve for the concentration of occupied and unoccupied channels:

$$\frac{[\text{I-Ch}]}{[\text{Ch}]_t} = \frac{k_{fl}[\text{I}]_l + k_{fr}[\text{I}]_r}{k_{fl}[\text{I}]_l + k_{fr}[\text{I}]_r + k_{er} + k_{el}} \quad (\text{A5})$$

$$\frac{[\text{Ch}]}{[\text{Ch}]_t} = \frac{k_{er} + k_{el}}{k_{fl}[\text{I}]_l + k_{fr}[\text{I}]_r + k_{er} + k_{el}} \quad (\text{A6})$$

In general, we would expect that the rate at which ions appear at a given channel would depend on the flux through nearby channels. As mentioned in the Introduction, we restrict our treatment to the limit of low-channel concentration. Taking this into account yields the following relationship between the transition rates previously described and the more traditional rate coefficients:

$$F(l) = \lim_{[\text{Ch}]_t \rightarrow 0} [\text{I}]_l k_{fl} \quad (\text{A7})$$

$$F(r) = \lim_{[\text{Ch}]_t \rightarrow 0} [\text{I}]_r k_{fr} \quad (\text{A8})$$

$$E(l|l)P(l) + E(r|l)P(r) = \lim_{[\text{Ch}]_t \rightarrow 0} k_{el}[\text{Ch-I}]/[\text{Ch}]_t \quad (\text{A9})$$

$$E(l|r)P(l) + E(r|r)P(r) = \lim_{[\text{Ch}]_t \rightarrow 0} k_{er}[\text{Ch-I}]/[\text{Ch}]_t \quad (\text{A10})$$

To verify this algebraically, first note that in the traditional kinetic scheme the probability that a channel is empty is given by the ratio of the concentration of unoccupied channels and the total channel concentration, i.e., Eq. (A6). The identifications in Eqs. (A7) through (A10) can then be used to show that Eqs. (32) and (A6) are equivalent results for the probability that the channel is empty.

9. SYMBOLS

Greek

α	Dimensionless electrical distance that defines the location of a binding site, first introduced in Section 7.2.1, Eq. (151)
β	Reciprocal of the mean thermal kinetic energy [$\beta = (k_b T)^{-1}$] used in Section 7.1.1, Eq. (124)
γ	Used as a dimensionless concentration in Section 7.1.1, Eq. (126)
δ	Length of the channel and membrane thickness, introduced in Section 2.1, Eq. (2)
$\Delta\varphi$	Difference in dimensionless potential across the membrane introduced in Section 2.1, Eq. (4)
$\Delta t_{i,j,k}$	Durations associated with trajectories of an ion in a channel introduced in Section 5.3, Eqs. (74) and (75)
ϵ_0	Permittivity of vacuum ($\epsilon_0 = 8.854 \times 10^{-12}$ farad/meter)
η	Characteristic length defined in Section 7.1.2, Eqs. (132) and (133)
κ	Dielectric constant
λ	Debye length used in defining the characteristic length η of Eqs. (132) and (133)
μ	Chemical potential defined in Section 7.1.2, Eq. (129) (units, joules)
ξ	Dimensionless length defined in Section 7.1.1, Eq. (126)
π	Ratio of the circumference of a circle to its radius (≈ 3.1416)
ρ	Used for the capture radius introduced in Section 2.1, Eqs. (8) and (9)
σ	Used for the specific conductivity in Section 7.1.3, Eq. (135)
τ	Mean first passage time, notation defined in Fig. 8
$\tau(0 \delta)$	Mean first passage time with an initial position and reflecting boundary at $x = 0$ and an absorbing boundary at $x = \delta$ defined in Section 5.2, Eqs. (67) and (82)
$\tau(\delta 0)$	Mean first passage time with an initial position and reflecting boundary at $x = \delta$ and an absorbing boundary at $x = 0$ defined in Section 5.2, Eq. (83)
φ	Dimensionless potential energy ($\varphi = \beta U$), see Section 2.1, Eq. (1)
ψ	Electrostatic Helmholtz free energy (units, volts) defined in Section 7.1.1, Eq. (124)

English

A	Cross-sectional area of the channel
B	Quantity defined in Eq. (25) which has units of time

D	Diffusion coefficient (units, meters ² /seconds)
D_b	Diffusion coefficient in the bath outside of the channel
$E(l l)$	The steady-state rate at which ions (having trajectories that start and end in the left bath) exit the left end of the channel defined in Section 3.2 (units, number/second)
$E(l r)$	The steady-state rate at which ions (having trajectories that start in the left bath and end in the right bath) exit the right end of the channel defined in Section 3.2 (units, number/second)
$E(r l)$	The steady-state rate at which ions (having trajectories that start in the right bath and end in the left bath) exit the left end of the channel defined in Section 3.2 (units, number/second)
$E(r r)$	The steady-state rate at which ions (having trajectories that start and end in the right bath) exit the right end of the channel defined in Section 3.2 (units, number/second)
E_l	Sum of $E(l l)$ and $E(l r)$
E_r	Sum of $E(r l)$ and $E(r r)$
$F(l)$	The steady-state rate at which ions (that have trajectories starting in the left bath) encounter the capture radius defining the left end of the channel defined in Section 3.2 (units, number/second)
$F(r)$	The steady-state rate at which ions (that have trajectories starting in the right bath) encounter the capture radius defining the right end of the channel defined in Section 3.2 (units, number/second)
G_{ss}	Small signal conductance of a channel (units, siemens or ohms ⁻¹) introduced in Section 2.2, Eq. (29)
I	Current (units, amperes)
I_i	Current under the assumption of independence defined in Section 2.1, Eq. (5)
I	Rate at which ions are injected in a system when calculating mean first passage times and mean occupancy times introduced in Section 5.1, Eq. (61)
J	Flux through a channel (units, number/second)
J_i	Flux through a channel under the assumption of independence defined in Section 2.1, Eqs. (2), (3), and (11)
$J(a b)$	Unidirectional flux from a source at $x = a$ to an absorbing boundary at $x = b$ defined in Section 4.2, Eq. (39)
\hat{K}_a	Affinity vector defined in Section 6.1, Eq. (112)
k_b	Boltzmann's constant ($k_b = 1.381 \times 10^{-23}$ joule/kelvin)
k_{el}	First-order rate coefficient describing the rate at which ions enter the left end of the channel introduced in Section 8, Eq. (A1)
k_{er}	First-order rate coefficient describing the rate at which ions enter the right end of the channel introduced in Section 8, Eq. (A1)

k_{fl}	Second-order rate coefficient describing the rate at which ions enter the left end of the channel introduced in Section 8, Eq. (A1)
k_{fr}	Second-order rate coefficient describing the rate at which ions enter the right end of the channel introduced in Section 8, Eq. (A1)
L_a^b	Integral of the exponential of the dimensionless potential from $x = a$ to $x = b$ defined in Section 4.1, Eq. (37) (units, meters)
n	Density of ions (units, number/meter ³) introduced in Section 2.1, Eq. (1)
\bar{n}	Concentration vector defined in Section 6.1, Eq. (111)
P_0	Probability that the channel is empty, i.e., contains zero ions, introduced in Section 2.2, Eq. (14) and defined in Sections 2.2, Eq. (24), 3.2, Eq. (34), 6.1, Eq. (109)
$P(l)$	Probability that the channel contains an ion whose trajectory initiated on the left side of the channel defined in Section 3.2, Eqs. (35) and (53)
$p(N;x)$	Grand canonical ensemble density function defined in Section 7.1.2, Eq. (129)
$P(r)$	Probability that the channel contains an ion whose trajectory initiated on the right side of the channel defined in Section 3.2, Eqs. (36) and (54)
P_{cis}	Probability that an ion will be absorbed on the same side of the channel that it originated from defined in Section 5.3, Eq. (72)
P_{trans}	Probability that an ion will be absorbed on the opposite side of the channel that it originated from defined in Section 5.3, Eq. (71)
P_{ll}	Cis probability for the left end of the channel, see Section 5.3, Eq. (72)
P_{lr}	Trans probability for the left end of the channel, see Section 5.3, Eq. (71)
P_{rl}	Trans probability for the right end of the channel, see Section 5.3, Eq. (71)
P_{rr}	Cis-probability for the right end of the channel, see Section 5.3, Eq. (72)
R_a^b	Electrodiffusive length defined in Section 4.1, Eq. (38) (units, seconds/meters ³)
R_0	Ratio of the mean filling time to the characteristic relaxation time under the assumption of a constant field in the bath defined in Section 7.1.3, Eq. (139)
t	Mean occupancy time introduced in Section 5.3, for complete notation see Fig. 8
T	Dimensionless time given by the ratio of the mean first passage time of an ion from a constant-field channel and zero transmembrane voltage and the average time an ion takes to find the channel defined in Section 7.2, Eq. (148)

t_l	Average time spent in the channel by an ion whose initial position was at the left boundary of the channel defined in Section 5.4, Eq. (104)
t_r	Average time spent in the channel by an ion whose initial position was at the right boundary of the channel defined in Section 5.4, Eq. (106)
t_r	Characteristic time for relaxation of the distribution of ions in the bath due to a sudden change in the potential field defined in Section 7.1.3, Eq. (136)
U	Potential energy of a system (units, joules) used in Section 2.1, Eq. (1) and Section 7.1.2, Eq. (129)
x	Spatial coordinate
x_i	Point at which a trajectory originates, see Fig. 5
z	Valence of an ion

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