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Equations describing passive transport through vesicular membranes

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Abstract

A theoretical description of the kinetics of the passive transport of both lipophobic and lipophilic nonelectrolytes, weak acids, and weak bases through membranes of large unilamellar vesicles is discussed. Equations are derived which may be used to obtain permeability coefficients and predict the extent of LUV entrapment of permeant molecules. Theoretical curves are generated to illustrate the difference between lipophobic and lipophilic permeation. By applying a diffusional approach rather than a simple first order kinetic approach to the problem of passive transport, some of the inconsistencies observed in other works are corrected. © 1998 Elsevier Science B.V.

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1. Introduction

The kinetics of passive membrane permeation is of great importance in pharmacological, environmental, and biophysical chemistry. The study of passive transport through biological membranes has been ongoing for decades. Forty five years ago, Zwolinski et al. presented one of the first detailed kinetic approaches to biological diffusion [1]. Since then, numerous studies have been performed in an effort to present a clear, simple, mathematical model for the kinetics of the passive diffusion process. One of the difficulties, as discussed by Cussler [2], in deriving such a model arises from the inability to apply first-order, reversible chemical reaction kinetics to the problem. Hence, alternative approaches must be pursued.

The general methods in which the kinetics of membrane permeation are traditionally approached are well discussed [2–5]. However, a detailed approach taking into account such factors as membrane asymmetry, differences in inner and outer membrane surface areas and volumes, varying permeant lipophobicity, permeation of weak acids/bases etc. has not been reported. Previous studies [6–19] have dealt with some of these phenomena, but none have combined all into a comprehensive set of equations that would permit the analysis of any general passive diffusion system.

Cramer and Prestegard [6], Viscio and Prestegard [9], and Prestegard et al. [8,13], derived a set of coupled differential equations describing the initial flow of weak acids between aqueous compartments of small vesicles. Using numerical iteration, these

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equations were fitted to data obtained from nuclear magnetic resonance spectroscopy (NMR) to provide permeability coefficients. Since reproducibility in vesicle preparation was a concern, assumptions regarding the uniformity of vesicle dimensions had to be made. With the development of extrusion techniques to consistently create large unilamellar vesicles (LUV's) of uniform size [11,12], these assumptions are no longer necessary. However, a closed form solution to the equations describing permeation is desirable as it would alleviate the need for numerical iteration.

A study by Kamp and Hamilton [16] discusses the movement of lipophilic acids across phospholipid bilayers in response to pH gradients. Equations derived in this study provide a method of calculating rates constants from initial rates of acidification.

The work of Cafiso and Hubbell [7,10] on the investigation of membrane electrical properties has provided a closed form solution for passive diffusion of a lipophilic permeant. These equations have subsequently been employed in describing the LUV uptake of lipophilic drugs and model peptides in response to pH [17,18]. However, these equations do not accurately predict the results expected for lipophobic permeants. A recent study by Herring et al. [15] and Cullen et al. [19] on bioaccumulation and bioconcentration of environmentally sensitive molecules has outlined the kinetics of permeation of highly lipophobic molecules through the membranes of LUV's. This study contains a closed form solution, similar to that given by Kirk [14], to a set of coupled differential equations. However, since these equations were derived to describe only lipophobic molecules which do not accumulate to any large extent within the lipid membrane, they are not applicable to all permeants.

The above discussion indicates the need for a set of equations to describe passive diffusion through the membranes of LUV's in response to a concentration or pH gradient. Furthermore, this set of equations should be general enough to include permeants of various degrees of lipophilicity passing into and out of spherical vesicles of all sizes. In the present study we derive sets of equations that address these concerns. In addition, theoretical curves are generated to illustrate the differences between permeation of highly lipophobic and lipophilic molecules.

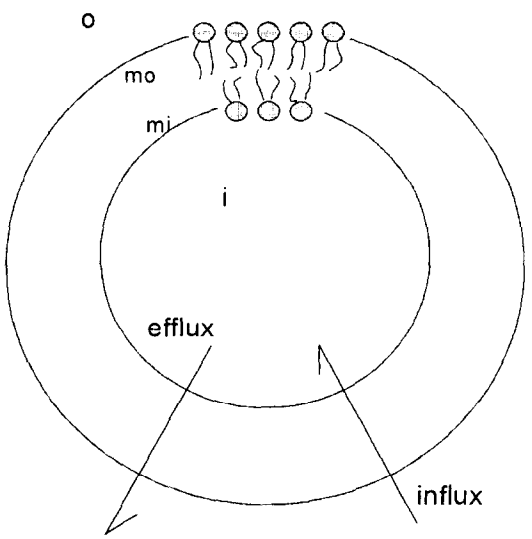


Fig. 1. Large unilamellar vesicle (LUV) showing the four regions occupied by the molecules: the outer aqueous solution (o), the external membranous region (mo), the internal membranous region (mi), and the inner aqueous solution (i), respectively. The arrow labeled efflux depicts an experiment with the bulk of permeant trapped inside the vesicle at time, $t = 0$. The arrow labeled influx depicts an experiment with the bulk of permeant outside the vesicle at $t = 0$.

2. Analysis

A four-compartment model similar to that used by Cafiso and Hubbell [10] is depicted in Fig. 1 in which there are external and internal membranous regions represented by mo and mi respectively, and the inner (i) and outer (o) aqueous compartments. A scheme for the passive diffusion of a non electrolyte, I, may be represented as:

$$I_o \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I_{mo} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} I_{mi} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} I_i$$

k_1, k_{-1}, k_2, and k_{-2} represents constants for permeation across the vesicle/solution interface. k_f and k_r are constants for transmembrane diffusion. The equations which describe the flow of particles from each region are:

$$\frac{dn_o}{dt} = k_{-1}[I_{mo}] - k_1[I_o] \quad (1a)$$

$$\frac{dn_i}{dt} = k_{-2}[I_{mi}] - k_2[I_i] \quad (1b)$$

$$\frac{dn_{mo}}{dt} = k_1[I_o] - k_{-1}[I_{mo}] + k_r[I_{mi}] - k_f[I_{mo}] \quad (1c)$$

$$\frac{dn_{mi}}{dt} = k_2[I_i] - k_{-2}[I_{mi}] + k_f[I_{mo}] - k_r[I_{mi}]. \quad (1d)$$

n_x is the number of molecules of I in region x and $[I_x]$ is the concentration of molecules I in region x. It should be noted here that, as a result of their dependence on membrane surface area and thickness (see below), the rate constants, k_1, k_{-1}, k_2 and k_{-2} carry the units $m^3\ s^{-1}$.

The closed form solution of this set of coupled differential equations depends on the nature of the system of interest. We will discuss two cases of special interest. The first case involves permeants which are highly lipophobic (i.e., $K \ll 1$ where K is the membrane/water partition coefficient) and do not accumulate to any large extent in the membranous regions mo and mi. The second involves lipophilic permeants, in which case the extent to which molecules accumulate in the membranous regions depends on the value of their partition coefficient.

3. Lipophobic permeants

If a permeant is highly lipophobic then at any time during the systems response a pH or concentration gradient, the number of particles in the vesicle membrane will be very small. If transmembrane diffusion is rate limiting, the number of membrane bound particles will be approximately constant and a steady state approximation may be applied in the regions mo and mi. With Eqs. (1c) and (1d) set to zero, solving the resultant equations for $[I_{mo}]$ and $[I_{mi}]$ yields:

$$[I_{mo}] = \left(\frac{k_{-1} + k_f}{k_r} - \frac{k_f}{k_{-2} + k_r}\right)^{-1} \times \left(\frac{k_1}{k_r}[I_o] + \frac{k_2}{k_{-2} + k_r}[I_i]\right) \quad (2a)$$

$$[I_{mi}] = \left(\frac{k_{-2} + k_r}{k_f} - \frac{k_r}{k_{-1} + k_f}\right)^{-1} \times \left(\frac{k_2}{k_f}[I_i] + \frac{k_1}{k_{-1} + k_f}[I_o]\right) \quad (2b)$$

Eqs. (2a) and (2b) may be substituted into Eqs. (1a) and (1b) to give closed form solutions to the differential equations as outlined in Appendix A. However, because transmembrane diffusion is rate limiting, diffusion away from the interface will occur much faster than diffusion through the bilayer, i.e., $k_{-2} \gg k_r$ and $k_{-1} \gg k_f$. Equations derived under these conditions provide a more useful set of equations than those in Appendix A.

Thus, using the conditions of rapid diffusion to and from the membrane surface, Eq. (A1) of Appendix A becomes:

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = -K_1 k_f[I_o] + K_2 k_r[I_i] \quad (3)$$

$K_1 = k_1/k_{-1} = [I_{mo}^{eq}]/[I_o^{eq}]$ and $K_2 = k_2/k_{-2} = [I_{mi}^{eq}]/[I_i^{eq}]$ are the partition coefficients at the outer and inner membrane interfaces respectively, and $[I_x^{eq}]$ is the concentration of molecules I in region x when the system reaches equilibrium. As required by conservation of mass, the flow of n_o and n_i are equal and opposite, for in the steady state the loss of a molecule in one aqueous compartment must result in the gain of a molecule in the other. This symmetry in n_o and n_i is not obtained for lipophilic permeants (vide infra).

The constants k_f and k_r (units $m^3\ s^{-1}$) are functions of the width of the membrane (δr), the diffusion coefficient within the membrane (D_{mem}), and the outer and inner surface areas of the vesicle (A_o and A_i) [5], such that:

$$k_f = \frac{D_{mem} A_o}{\delta r} \quad k_r = \frac{D_{mem} A_i}{\delta r}$$

Combined with the definition of the permeability coefficient ($P = KD_{mem}/\delta r$), $K_1 k_f = P_1 A_o$ and $K_2 k_r = P_2 A_i$. Defining N_T as the total number of molecules in the system and N_{aq} as the number of molecules in the aqueous compartments, the conservation of mass equation is:

$$n_o + n_i = N_T - (n_{mo} + n_{mi}) = N_{aq} \quad (4)$$

N_{aq} is a constant under the steady state approximation. Solving Eq. (4) for n_i, Eq. (3) may now be written as:

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = -\gamma_{ss} n_o + \frac{P_2 A_i N_{aq}}{V_i} \quad (5)$$

$$\text{where } \gamma_{ss}(s^{-1}) = \frac{P_1 A_o}{V_o} + \frac{P_2 A_i}{V_i} \quad (6)$$

γ_{ss} is the apparent rate constant for the permeation process under the steady state approximation.

Under equilibrium conditions where net flow ceases ($dn/dt = 0$) it can be shown that:

$$n_o^{eq} = \frac{P_2 A_i N_{aq}}{\gamma_{ss} V_i} \quad (7a)$$

$$n_i^{eq} = \frac{P_1 A_o N_{aq}}{\gamma_{ss} V_o} \quad (7b)$$

n_o^{eq} and n_i^{eq} are the number of molecules in the outer and inner aqueous compartments respectively, at equilibrium. Hence Eq. (5) becomes:

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = -\gamma_{ss}(n_o - n_o^{eq}) \quad (8)$$

The solution to the above differential equation is simply:

$$n_o(t) = n_o^{eq} + (n_o^0 - n_o^{eq})\exp[-\gamma_{ss}(t - t_o)] \quad (9a)$$

$$n_i(t) = n_i^{eq} + (n_i^0 - n_i^{eq})\exp[-\gamma_{ss}(t - t_o)] \quad (9b)$$

n_o^0 and n_i^0 are the respective number of molecules in the outer and inner aqueous compartments at $t = t_0$. In the case of LUV's with a diameter > 100 nm, $A_o \approx A_i = A$. If the composition of the inner and outer leaflets of the membrane bilayer are the same then $K_1 = K_2$, hence $P_1 = P_2 = P$ and $\gamma_{ss} = PA(1 + V_i/V_o)/V_i$. Eqs. (9a), (9b) and (6) are similar in form to the equations described by Kirk [14] and those employed by Herring et al. [15] and Chakrabarti et al. [18] for passive diffusion of lipophobic permeants through membranes of LUV's.

4. Lipophilic permeants

In the case of lipophilic ($K > 1$) permeants, a substantial number of particles may partition into the membrane. Under these circumstances, the time rate of change of n_{mo} and n_{mi} (Eqs. (1c) and (1d)) may not be equated to zero, hence the steady state approximation will no longer be valid and a different approach must be taken. Since the steps leading to partitioning into the membrane occur much faster than the transmembrane steps [20] it is reasonable to assume that the molecules at the interface will stay at equilibrium as the system responds to the pH and/or concentration gradient. Therefore, throughout the systems approach to final equilibrium (at which time $dn/dt = 0$ in all compartments), the aqueous and membrane bound particles at each interface will have a constant ratio given by the partition coefficients, $K_1 = [I_{mo}]/[I_o] = k_1/k_{-1}$ and $K_2 = [I_{mi}]/[I_i] = k_2/k_{-2}$. However, this is only an approximation and these partition coefficients must not be substituted into Eqs. (1a) and (1b): the results $dn_o/dt = dn_i/dt = 0$ making n_o = constant and n_i = constant are obviously erroneous.

By substituting Eq. (1a) into Eq. (1c), Eq. (1b) into Eq. (1d), and making use of K_1 and K_2, we can write:

$$\frac{dn_o}{dt} = \frac{1}{R_o}(-K_1 k_f[I_o] + K_2 k_r[I_i]) \quad (10a)$$

$$\frac{dn_i}{dt} = -\frac{1}{R_i}(-K_1 k_f[I_o] + K_2 k_r[I_i]) \quad (10b)$$

where $R_o = 1 + K_1 V_{mo}/V_o$ and $R_i = 1 + K_2 V_{mi}/V_i$. An important consequence of this approach is that the concentration of permeant in the membrane is time dependent, hence, the rates of change of n_o and n_i are unequal (i.e., $dn_o/dt \neq dn_i/dt$).

Defining N_T as the total number of I molecules, the conservation of mass requires that:

$$n_o + n_{mo} + n_{mi} + n_i = N_T \quad (11)$$

By using the partition coefficients this equation may be solved for n_o and n_i and substituted into Eqs. (10a) and (10b). Making use of the definition of the permeability coefficients, we now have:

$$\frac{dn_o}{dt} = -\gamma_{fe} n_o + \frac{P_2 A_i N_T}{R_o R_i V_i} \quad (12a)$$

$$\frac{dn_i}{dt} = -\gamma_{fe} n_i + \frac{P_1 A_o N_T}{R_o R_i V_o} \quad (12b)$$

$$\text{where } \gamma_{fe}(s^{-1}) = \frac{P_1 A_o}{R_o V_o} + \frac{P_2 A_i}{R_i V_i} \quad (13)$$

The apparent rate constant for the permeation process under this 'fast equilibrium' approximation is γ_{fe}.

Using the equilibrium conditions we have:

$$n_o^{eq} = \frac{P_2 A_i N_T}{\gamma_{fe} R_o R_i V_i} \quad (14a)$$

$$n_i^{eq} = \frac{P_1 A_o N_T}{\gamma_{fe} R_o R_i V_o} \quad (14b)$$

and the solution to the differential equations is:

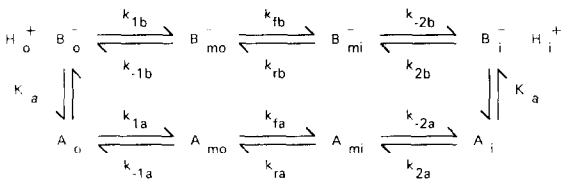
$$n_o(t) = n_o^{eq} + (n_o^0 - n_o^{eq})\exp[-\gamma_{fe}(t - t_o)] \quad (15a)$$

$$n_i(t) = n_i^{eq} + (n_i^0 - n_i^{eq})\exp[-\gamma_{fe}(t - t_o)] \quad (15b)$$

Under the conditions of membrane symmetry where $K_1 = K_2$ and $A_i \approx A_o = A$, $\gamma_{fe} = PA(1 + R_i V_i / R_o V_o)/R_i V_i$. It should be noted that γ_{fe} and γ_{ss} (Eq. (6)) differ only through the constants, R_i and R_o, and as R_i and R_o approach unity, γ_{fe} approaches γ_{ss}. In addition, it is observed that Eqs. (9a), (9b), (15a) and (15b) are deceptively similar, but nonetheless Eqs. (15a) and (15b) give the result $dn_o/dt \neq dn_i/dt$.

5. Weak electrolytes as permeants

For weak acids and bases the scheme for passive diffusion may be represented as:



where A represents the acid and B is its conjugate base. The dissociation constant of the acid in aqueous solution is K_a. This model assumes H^+ leakage is minimal and that no H^+ carrier exists in the membrane. Acid dissociation within the membrane is negligible compared to that in aqueous phases [21] and has therefore been neglected. A set of eight equations similar to Eqs. (1a), (1b), (1c) and (1d) describing the flow of a_x and b_x, where x is the specified region and a and b represent numbers of molecules of A and B respectively, may be deduced. Since most forms of detection cannot distinguish between the acid and its conjugate base, it is reasonable to write the equations in terms of $N_x = a_x + b_x$, the number of molecules of both A and B in region x. When the system is sufficiently buffered so that the pH inside and outside the liposomes is constant throughout the systems approach to equilibrium, a closed form solution may be found by applying the steady state or fast equilibrium approximations. However, if the system is not adequately buffered the pH becomes time dependent and the equations must be solved numerically [8,9] or extended to include pH effects discussed by Kamp and Hamilton [16].

If both the acid and base are lipophobic the steady state approach will be valid, that is, a_{mo}, b_{mo}, a_{mi}, and b_{mi} are all approximately constant with time, then, following the approach taken for a non electrolyte, the flow of molecules inside and outside the vesicles becomes:

$$\frac{dN_o}{dt} = -\frac{dN_i}{dt} = -\gamma_{ss}(N_o - N_o^{eq}) \quad (16)$$

γ_{ss} is the apparent rate constant as in Eq. (6) and N_o^{eq} is the number of molecules in the outside aqueous compartment at equilibrium. However, now:

$$P_1 = P_{1a}\alpha_o + P_{1b}(1 - \alpha_o) \quad (17a)$$

$$P_2 = P_{2a}\alpha_i + P_{2b}(1 - \alpha_i) \quad (17b)$$

$P_{1a} = K_{1a} D_{mem,a}/\delta r$ and similarly for P_{1b}, P_{2a}, and P_{2b}. $D_{mem,a}$ and $D_{mem,b}$ are the membrane diffusion coefficients for the acid and base respectively.

$K_{1a} = k_{1a}/k_{-1a} = [A_{mo}^{eq}]/[A_o^{eq}]$ and K_{1b} are the respective partition coefficients of the acid and base at the outside interface. K_{2a} and K_{2b} are the partition coefficients at the inside interface. $\alpha_o = [H_o^+]/([H_o^+] + K_a)$ is the extent of acid dissociation in the outer aqueous compartment and a similar equation may be written for α_i. If the membrane is symmetric such that $K_{1a} = K_{2a}$, $K_{1b} = K_{2b}$, and $A_i \approx A_o = A$, $P_1 = P_2$ only when $\alpha_i = \alpha_o$ (i.e., the pH in both compartments is the same).

The solutions to the differential equations are:

$$N_o(t) = N_o^{eq} + \left(N_o^0 - N_o^{eq}\right)\exp\left[-\gamma_{ss}(t - t_o)\right] \quad (18a)$$

$$N_i(t) = N_i^{eq} + \left(N_i^0 - N_i^{eq}\right)\exp\left[-\gamma_{ss}(t - t_o)\right] \quad (18b)$$

N_o^0 and N_i^0 are the number of molecules at $t = t_0$ in the outer and inner aqueous compartments, respectively. As in Eqs. (7a) and (7b), equilibrium values of the number of particles can be predicted to be:

$$N_o^{eq} = \frac{P_2 A_i N_{aq}}{\gamma_{ss} V_i} \quad (19a)$$

$$N_i^{eq} = \frac{P_1 A_o N_{aq}}{\gamma_{ss} V_o} \quad (19b)$$

P_1 and P_2 are given in Eqs. (17a) and (17b). $N_{aq} = N_o = N_i$ is the total number of molecules in both aqueous phases.

If either the acid or base are lipophilic, then a steady state approximation is no longer valid and the rapid established equilibrium approach must be taken. In this case, it is assumed that the acid and base are at equilibrium between the aqueous and membranous phases at all times. Following the approach taken for the non-electrolytic permeant:

$$N_o(t) = N_o^{eq} + \left(N_o^0 - N_o^{eq}\right)\exp\left[-\gamma_{fe}(t - t_o)\right] \quad (20a)$$

$$N_i(t) = N_i^{eq} + \left(N_i^0 - N_i^{eq}\right)\exp\left[-\gamma_{fe}(t - t_o)\right] \quad (20b)$$

γ_{fe} is the apparent rate constant as in Eq. (13). P_1 and P_2 are given in Eq. (16) and R_o and R_i are now:

$$R_o = 1 + \frac{K_1^* V_{mo}}{V_o} \quad (21a)$$

$$R_i = 1 + \frac{K_2^* V_{mi}}{V_i} \quad (21b)$$

where $K_1^* = K_{1a}\alpha_o + K_{1b}(1 - \alpha_o)$ (22a)

$$K_2^* = K_{2a}\alpha_i + K_{2b}(1 - \alpha_i) \quad (22b)$$

$K_{1a} = [A_{mo}]/[A_o]$ is the partition coefficient of the acid at the outside interface and similarly for K_{1b}, K_{2a}, and K_{2b}. The fractions of undissociated species, α_o and α_i are as defined earlier.

6. Discussion

We have defined sets of equations that describe the kinetics of passive diffusion of non electrolytes and weak acids/bases through the membranes of LUV's. Although only weak acids/bases with one dissociable group were discussed, the approaches taken could easily be extended to permeants with multiple dissociable groups. In order to make the equations general to many applications we have derived these equations using as few assumptions as possible.

As briefly discussed in this analysis, the equations and their solutions may be simplified further in many cases. In most experiments, the membranes formed will be symmetric, thus the partition coefficients will be the same at both membrane interfaces so the permeability coefficients, P_1 and P_2, will be equal providing no pH gradient exists. Also, for large vesicles, A_o and A_i will differ by very small amounts. For example, if LUV's are prepared with an outer diameter of 150 nm and we assume a membrane width of 50 Å, A_o/A_i is approximately 1.1, hence A_o and A_i may be approximated as equal.

To describe permeation of molecules of varying lipophilicity, two separate approaches were taken to derive closed form solutions to the sets of coupled differential equations. In the case of extremely lipophobic molecules, a steady state approach to the permeant in the regions mo and mi correctly predicts that the rate of change of molecules in one aqueous compartment is equal and opposite to the rate of change in the other. The closed form of the solution does not explicitly contain any partition coefficients although they are implicit in the permeability coefficients since $P = KD/\delta r$. Since lipophilic molecules may accumulate to an appreciable extent within the membrane the symmetry in the rate of change of the number of molecules between the two aqueous compartments is not observed when the 'fast equilibrium' approach is used.

To illustrate the effect that increasing lipophilicity has on the derived equations, curves have been

generated using Eqs. (20a) and (20b) as well as Eqs. (18a) and (18b) and the definitions of the partition coefficients. These curves are displayed in Fig. 2 for permeants of varying K^* but identical apparent rate constants, γ. These graphs illustrate results of efflux experiments with no pH gradient. It is observed that as K^* becomes increasingly smaller, the curves representing the number of molecules in the inner and outer aqueous compartments become increasingly symmetrical and the number of particles partitioned into the membrane decreases. In Fig. 2 III, with $K^* = 0.1$, the curves representing the number of molecules in the inner and outer compartments are virtually indistinguishable from the symmetrical curves of Fig. 2IV which were calculated from the steady state Eqs. (18a) and (18b). This is predicted since in Eqs. (21a) and (21b) it is observed that as K^* decreases, R_o and R_i approach unity, hence γ_{fe} given by Eq. (13) becomes equal to γ_{ss} in Eq. (6). The value of K^* at which R_i becomes unity is independent of vesicle concentration unlike R_o where the term V_{mo}/V_o will be affected. Therefore the conditions at which the fast equilibrium and steady state approaches converge is dependent on both K^* and vesicle concentration. It is also important to point out that although the apparent rate constant, γ_{fe} may be obtained without knowledge of any partition coefficients, the permeability coefficients may not. If K^* can be easily determined from existing data, then the partition coefficients for the acid and base may be calculated from determining K^* at different pH's. However, if K^* can not be determined experi-

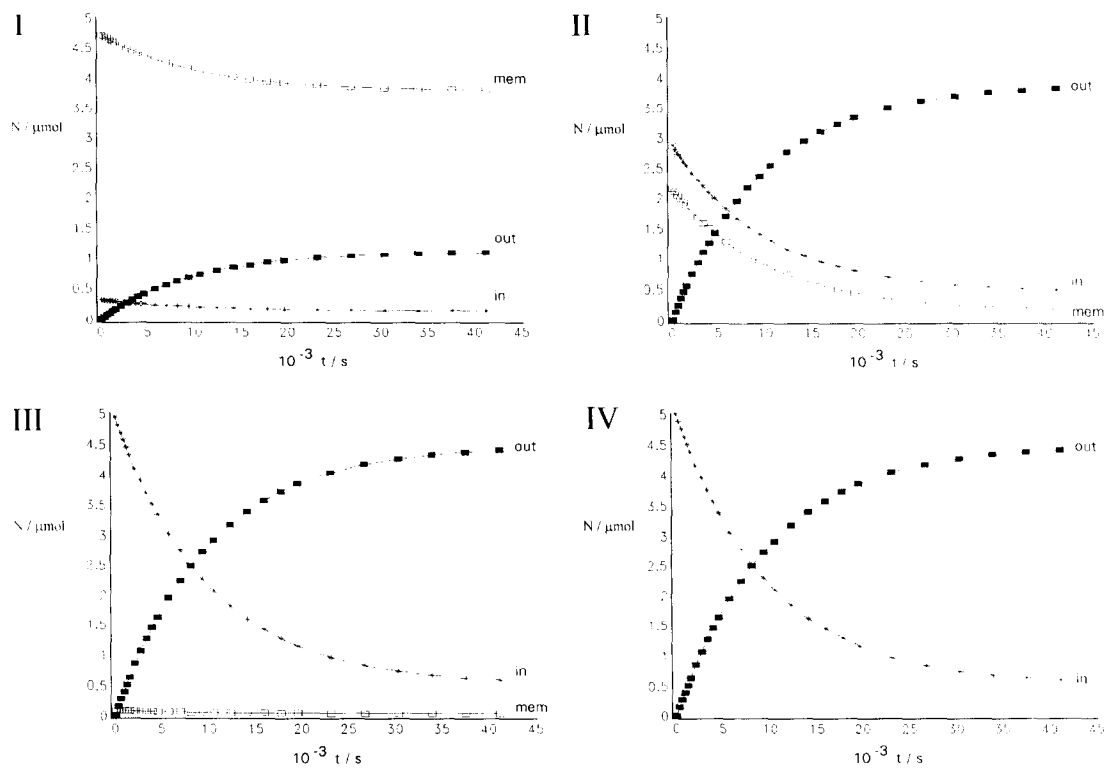


Fig. 2. Graphs depicting the effect of the apparent partition coefficient, K^*, on the theoretical results of efflux experiments with similar apparent rate constants, γ. Graph (I) $K^* = 100$, (II) $K^* = 5$, (III) $K^* = 0.1$, and (IV) steady state results. The number of molecules on the inside of the vesicles (N_i) are represented by the asterix (*), the molecules on the outside of the vesicles (N_o) by the filled squares (■), and the membrane bound molecules ($N_{mo} + N_{mi}$) by the open squares (□). $V_i = 0.1$ mL, $V_{mo} \approx V_{mi} = 0.1V_i$, and $V_o = 0.88$ mL. $N_o(0) + N_{mo}(0) = 0$, $N_i(0) + N_{mi}(0) = 5$ µmol. $\gamma = 1 \times 10^{-4} s^{-1}$.

mentally, then an estimate must be made from octanol/water, lecithin/water, etc. measurements [22,23].

From Eqs. (17a) and (17b) it is observed that the apparent permeabilities, P_1 and P_2, are functions of proton concentration in the aqueous compartments. Therefore, if an acid and its conjugate base permeate at different rates, P_1 and P_2 as well as γ will be pH dependent. It has been well discussed that in most cases, permeation of the bilayer occurs predominantly via the neutral form of an acid or base [15,17–19,24,25]. Differences in permeabilities between neutral and ionic species result in linear plots of P_1 vs. α_o and P_2 vs. α_i. From the slopes of these curves, the permeabilities of the acid (P_{1a} and P_{2a}) and conjugate base (P_{1b} and P_{2b}) may be calculated.

An important aspect of liposome use is the entrapment of molecules for drug delivery. The approaches taken to provide closed form solutions of the differential equations allow calculation of equilibrium values for the amount of permeant in any region of the LUV system and may be used to predict the extent of entrapment at equilibrium. Using Eqs. (14a) and (14b) in the fast equilibrium model for a weak acid or base, the ratio of trapped molecules to free molecules in LUV's with bilayers having identical composition and $A_i \approx A_o$ will be:

$$\frac{N_{mo}^{eq} + N_{mi}^{eq} + N_i^{eq}}{N_o^{eq}} = \frac{P_1}{P_2}\left(\frac{V_i}{V_o} + \frac{K^* V_{mi}}{V_o}\right) + \frac{K^* V_{mo}}{V_o} \tag{23}$$

The ratio P_1/P_2 depends on the extent of acid dissociation (α) in the aqueous compartments if a pH gradient is applied (if no pH gradient is applied $P_1/P_2 = 1$). As shown by Harrigan et al. [17], Chakrabarti et al. [18], and Madden et al. [26], in the presence of a pH gradient, the number of trapped molecules may be enhanced. Under the conditions used in their experiments, $[H_o^+]$, $[H_i^+] \gg K_a$, and permeation of the neutral base occurred much more rapidly than permeation of the ionized conjugate acid (therefore Eq. (17a) reduces to $P_1 = P_{1b}(1 - \alpha_o)$ and similarly for P_2), so $P_1/P_2 \approx [H_i^+]/H_o^+]$. Therefore by lowering the pH of the inner aqueous compartment, they were able to entrap a greater number of particles in the LUV's than would be possible without a pH gradient. Similarly, it is straightforward to show that for a neutral acid, $P_1/P_2 \approx [H_o^+]/[H_i^+]$, and therefore raising the pH of the inner compartment would result in greater entrapment. Thus, to enhance entrapment, the gradient imposed will depend upon whether the permeant molecule is basic or acidic.

Cussler [2] has indicated that kinetic problems involving systems, such as lipid membranes, must be analyzed by either mass transfer or diffusion and not by using simple first order kinetics. In this work, the approach taken in solving the kinetic problems of diffusion is based on particle flow (i.e., the diffusion method). The first order kinetic approach taken by Cafiso and Hubbell [10] treated the membrane/interface step of the permeation mechanism as elementary. Therefore they write the disappearance of permeant from the region mo as:

$$-\frac{dn_{mo}}{dt} = k'_f n_{mo} - k'_r n_{mi} \tag{24}$$

In their notation k'_f and k'_r have the units s^{-1}, hence $k'_f V_{mo} = k_f$ and $k'_r V_{mi} = k_r$ where k_f and k_r are as in Eqs. (1a), (1b), (1c) and (1d). Eq. (24) results in an apparent rate constant in Eqs. (9a) and (9b) of:

$$\gamma = k'_f + \frac{\varepsilon_o}{\varepsilon_i}\frac{K_i}{K_o}\frac{V_{mi}}{V_{mo}}\frac{V_o}{V_i}k'_r \tag{25}$$

where ε_o and ε_i are equal to R_o and R_i, respectively, in Eqs. (10a) and (10b). However, deriving an apparent rate constant beginning with the appearance of permeant at the region mi:

$$\frac{dn_{mi}}{dt} = -k'_r n_{mi} + k'_f n_{mo} \tag{26}$$

leads to an apparent rate constant:

$$\gamma = k'_r + \frac{\varepsilon_i}{\varepsilon_o}\frac{K_o}{K_i}\frac{V_{mo}}{V_{mi}}\frac{V_i}{V_o}k'_f \tag{27}$$

Therefore an inconsistency is present in the value of γ. This inconsistency may also be found in previous studies [15,19]. By treating the membrane/interface step of the permeation scheme as elementary, the

flow of particles from the region mo (Eq. (24)) does not contain the terms $k_1[I_o]$ and $k_{-1}[I_{mo}]$, representing permeant transport from region mo to region o as in Eq. (1c). The inclusion of these terms makes Eq. (24):

$$-\frac{dn_o}{dt} - \frac{dn_{mo}}{dt} = k_1' n_{mo} - k_1' n_{mi}$$

and the use of this expression subsequently removes the inconsistency in γ. Similarly, the terms for transport across the inner surface, $k_2[I_i]$ and $k_{-2}[I_{mi}]$ are omitted in Eq. (26) but are present in Eq. (1d).

7. Conclusion

In this study, we have presented a comprehensive set of equations that permits the analysis of many systems responding to pH and/or concentration gradients via passive diffusion. Many of the previous inconsistencies arising from membrane asymmetry, vesicle concentration, and weak acid/base permeation have been dealt with. An accurate equation, requiring no assumptions, for the entrapment of lipophilic drug molecules is also presented. We have shown the effect varying lipophilicity has on the equations in order to illustrate why the steady state approach is not always appropriate. In a study to be published we will outline the protocol for using these equations to deduce permeability coefficients for environmentally sensitive lipophobic permeants.

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Appendix A

To derive the most general form of Eqs. (9a) and (9b) we substitute Eqs. (2a) and (2b) into Eqs. (1a) and (1b) giving

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = C_1 k_1[I_o] + C_2 k_2[I_i] \quad \text{(A1)}$$

$$\text{where } C_1 = \frac{k_{-2}}{k_{-1} + k_f}\left(\frac{k_f}{k_{-1} + k_f} - \frac{k_{-2} + k_f}{k_f}\right) + 1 \quad \text{(A2)}$$

$$\text{and } C_2 = 1 + \frac{k_{-2}}{k_f}\left(\frac{k_f}{k_{-1} + k_f} - \frac{k_{-2} + k_f}{k_f}\right) + 1 \quad \text{(A3)}$$

Using the mass balance equation (Eq. (4)) from the text, Eq. (A1) may be written

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = \left(\frac{C_1 k_1}{V_o} - \frac{C_2 k_2}{V_i}\right) n_o + \frac{C_2 k_2 N_{aq}}{V_i} \quad \text{(A4)}$$

Under equilibrium conditions where $dn_o/dt = dn_i/dt = 0$,

$$n_o^{eq} = \frac{C_2 k_2 N_{aq}}{\gamma V_i} \quad \text{(A4a)}$$

$$n_i^{eq} = \frac{C_1 k_1 N_{aq}}{\gamma V_o} \quad \text{(A4b)}$$

$$\text{where } \gamma = \frac{C_2 k_2}{V_i} - \frac{C_1 k_1}{V_o} \quad \text{(A5)}$$

where n_o^{eq} and n_i^{eq} are the number of molecules in the outer and inner aqueous compartments respectively at equilibrium. Hence Eq. (A3) becomes

$$\frac{dn_o}{dt} = -\frac{dn_i}{dt} = -\gamma(n_o - n_o^{eq}) \quad \text{(A6)}$$

The solution to this equation is simply,

$$n_o(t) = n_o^{eq} + (n_o^0 - n_o^{eq})\exp[-\gamma(t - t_o)] \quad \text{(A47a)}$$

$$n_i(t) = n_i^{eq} + (n_i^0 - n_i^{eq})\exp[-\gamma(t - t_o)] \quad \text{(A7b)}$$

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