Lipid Transfer Between Vesicles: Effect of High Vesicle Concentration

Paulo F. F. Almeida
Departamento de Química, Universidade de Coimbra, 3049 Coimbra Codex, Portugal

ABSTRACT The problem of the desorption of a lipid molecule from a lipid vesicle (donor) and its incorporation into another vesicle (acceptor) at high acceptor concentrations, which has been investigated experimentally (Jones, J. D. and Thompson, T. E., 1990. *Biochemistry*, 29:1593–1600), is analyzed here from a theoretical point of view, formulated in terms of the diffusion equation with appropriate boundary conditions. The goal is to determine whether or not the observed acceleration of the off-rate from a donor is caused by interaction with an acceptor vesicle at short range, or is simply the result of statistical effects due the proximity of the acceptor and its influence on the probability of the test lipid returning to the donor. We establish a correspondence between the theoretical parameters and the experimental, thermodynamic and dynamic variables entering the problem. The solution shows that, because of the extremely high Gibbs activation energy for desorption of a phospholipid, the process would always be first-order, even at very high vesicle concentrations. This means that acceleration of the off-rate must be due to donor-acceptor interactions at short distances, as proposed in the experimental work.

INTRODUCTION

Biological problems are usually complex, by their nature, because of the number and interdependence of the variables involved. Consequently, the space of possible configurations is very large, and their analysis and modeling often require the use of computer simulations. However, if the problems can be somehow simplified and rendered amenable to treatment with analytical mathematical methods, the information obtained is often more precise and can be cast in simpler terms. The present article is an attempt at presenting such a solution for a simplified problem in biochemistry.

The problem we wish to consider is the desorption of a lipid molecule from a lipid vesicle (donor) and its incorporation into another vesicle (acceptor), which has been investigated experimentally by following the time dependence of a population of fluorescent (Roseman and Thompson, 1980) and, more recently, radioactively labeled phospholipids initially located in the donor vesicles (McLean and Phillips, 1981). This requires that the donor and acceptor vesicles be separated for analysis of radio-label content, which is normally achieved by using donor and acceptor vesicles with a different charge (McLean and Phillips, 1981) or size (Wimley and Thompson, 1991). It was found that at small vesicle concentrations, the decay of radio-label in the donors is first-order, that is, independent of the concentration of acceptor (Roseman and Thompson, 1980; McLean and Phillips, 1981), but dependent only on the off-rate from the donor vesicle. At high vesicle concentrations the process also has a second-order component (Jones and Thompson, 1989; Jones and Thompson, 1990), which is to say that it becomes faster as the concentration of acceptor vesicles is increased. It was proposed that this second-order component results from the interaction of two vesicles, a donor and an acceptor, at short range, giving rise to an acceleration of the intrinsic off-rate from the donor (Jones and Thompson, 1990). However, one possibility mentioned by those authors but not quantitatively addressed is that this acceleration could result simply from a statistical effect: at low concentrations of vesicles the most probable fate of the lipid molecule that comes off the donor is to go back into it before finding an acceptor vesicle, but at very high acceptor concentrations this probability is altered because now there is usually an acceptor vesicle in the vicinity. If this alternative explanation were correct, then perhaps there would be no need to invoke donor-acceptor vesicle interactions to explain the increased off-rate from the donors at large acceptor concentrations. Here, we analyze this problem to determine whether or not statistical effects could be responsible for the acceleration of the intrinsic off-rate, and conclude that they could not—but the qualitative answer depends on the magnitude of the variables entering the problem in a decisive way.

We first define a simplified representation of the experimental situation considered and formulate the mathematical problem in terms of the diffusion equation with appropriate boundary conditions. We then establish a correspondence between the parameters in the mathematical model and the experimental, thermodynamic, and dynamic variables from a comparative analysis of the low acceptor concentration regime in the theory and experiment. Finally, we use the values thus obtained to analyze the theoretical prediction for the high acceptor concentration regime. Fig. 1 is a scheme of the real situation and of the model used in the present analysis. The radius of the donor lipid vesicle is a and the average distance to the next vesicle (acceptor) is L. Strictly, because we will also take a to be the length of the lipid molecule, we are modeling the donor vesicle as a

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Dedicated to Dr. T. E. Thompson.

Address reprint requests to Dr. Paulo F. F. Almeida, Dept. de Química, Universidade de Coimbra, 3049 Coimbra codex, Portugal. Tel.: 351-39-852080; Fax: 351-39-827703; E-mail: pferrand@ci.uc.pt.

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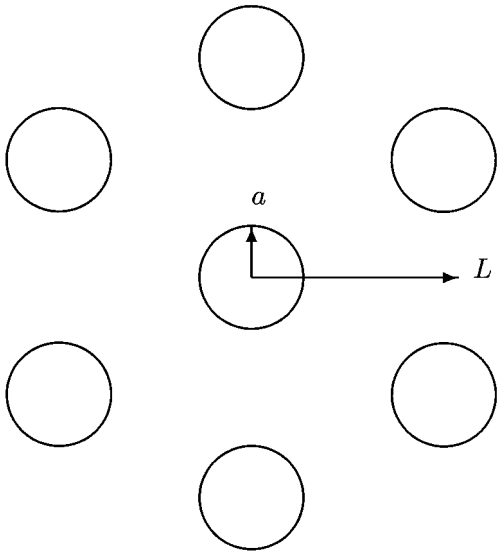

FIGURE 1 Schematics of the model.

micelle. For the present calculation, however, this is correct because the flip-flop movement of the phospholipids is very slow and does not enter the experimental problem either, because only initial rates are measured in the experiments discussed here. Including the two leaflets of the membrane in the mathematical model would require introducing another concentric sphere, and would complicate the solution enormously and unnecessarily. In a typical experiment, the acceptor concentration is much larger than the donor concentration. Therefore, the probability that another donor-type vesicle will be the recipient of the desorbing, test lipid molecule (radio-labeled) is practically zero. The set of acceptor vesicles thus behaves as a sink of infinite capacity.

THEORY

The mathematical model consists of two concentric spheres of radii a and L (Fig. 1). Inside the small sphere ($r < a$) the concentration is u_0 and the diffusion coefficient is D_0. Outside ($a < r < L$), the concentration is u_1 and the diffusion coefficient is D_1.

In each region (subscripts 0 and 1 are used as appropriate) we must solve the diffusion equation

$$\frac{\partial u(r, t)}{\partial t} = D\nabla^2 u(r, t), \tag{1}$$

with the boundary conditions

$$r = 0, \quad u_0(0, t) \neq \infty \tag{2}$$

$$r = a, \begin{cases} D_0 \dfrac{\partial u_0(a, t)}{\partial r} = D_1 \dfrac{\partial u_1(a, t)}{\partial r} \\ \dfrac{\partial u_0(a, t)}{\partial r} = -H/D_0[u_0(a, t) - qu_1(a, t)] \end{cases} \tag{3}$$

and

$$r = L, \quad u_1(L, t) = 0, \tag{4}$$

where H is the coefficient of surface transfer at the vesicle/water interface. Because the set of acceptor vesicles functions as a sink of infinite capacity we can use a perfectly absorbing (Dirichlet) boundary condition at $r = L$. The second boundary condition, at $r = a$ (Eq. 3), deserves some comment. Essentially it is a modified version of the radiation boundary condition type (Carslaw and Jaeger, 1959). Its meaning is easiest to see if we consider what happens in a solution containing only donor vesicles. When equilibrium is reached ($t \to \infty$), there is no net transfer of lipid from the vesicle to the solution. In this case the concentration in solution (u_1) is the equilibrium concentration of lipid, that is, the monomer solubility in water. In equilibrium, the net flux across the vesicle surface is zero, $\partial u_0(a, \infty)/\partial r = 0$, and we obtain

$$u_0(a, \infty) = qu_1(a, \infty).$$

Therefore q is seen to be the equilibrium constant, that is, the partition coefficient of the lipid between vesicle and water,

$$q = \frac{[u_0]_{\text{eq.}}}{[u_1]_{\text{eq.}}},$$

which has an approximate value of $q = 10^{10}$ (Tanford, 1980). Another way of looking at it is to think of q as the exponential of a potential difference between water and vesicle (higher in water), corresponding to the higher free energy of a lipid molecule in water.

The initial conditions are

$$r < a, \quad u_0(r, 0) = f_0 \tag{5}$$

$$a < r < L, \quad u_1(r, 0) = 0 \tag{6}$$

For our present purposes, we do not need the full solution of this problem but only the time-dependence of the lipid concentration in the donor vesicle, that is, in the region $r < a$, which is given by

$$U(t) = 4\pi \int_0^a u_0(r, t) r^2 dr/(4\pi a^3/3). \tag{7}$$

Let us define:

$$\gamma = \sqrt{D_0/D_1},$$

$$b = L/a,$$

and

$$\epsilon = Ha/D_0.$$

In the Appendix we derive the solution of this problem with the Laplace transform method (Carslaw and Jaeger,

1959) and show that the expression for $u_0(r, t)$ in Eq. 7 is

$$u_0(r, t) = f_0 \sum_{n=1}^{\infty} R_n(0) e^{-\mu_n^2 D_0 t/a^2} \quad (8)$$

where $R_n(0)e^{-\mu_n^2 D_0/a^2 t}$ are the residues at the nth singularities of the solution in Laplace space and, in the summation, the residue at $\mu_0 = 0$ is excluded. This completes the solution.

RESULTS AND DISCUSSION

Let us first consider the constants entering our problem and their numerical values. The parameter q is the equilibrium partition coefficient of the phospholipid between vesicle and water, $q = [u_0]_{eq.}/[u_1]_{eq.}$. It can be obtained from the solubility of the lipid in water, which is $s_w \approx 10^{-10}$ M (Tanford, 1980), corresponding to a value of $q = 10^{10}$ after conversion to units of molecules/cm^3.

D_1 is the diffusion coefficient of a lipid molecule in water and is typically of the order of 5×10^{-6} cm^2/s (Jones and Thompson, 1990). D_0 is not so easy to estimate. Lateral diffusion along the plane of the membrane in the donor vesicle has little relevance to this process because we are interested here in movement that brings the lipid to the surface, leading to desorption. Moreover, what are measured experimentally are initial rates of desorption: thus the flip-flop movement is not relevant either. D_0 could then correspond essentially to the wobbling of a lipid molecule in and out of its cage in the lipid bilayer, as if this were a small volume in the gas phase (interactions of the lipid tails will hamper this movement, but we include this effect in ϵ, below). If we treat the situation as if the lipid would jump with a rate given by the corresponding velocity in the gas phase under identical conditions, for a lipid with a mass of $M_w \approx 600$ daltons ($m \approx 10^{-21}$ g), a temperature of about 300 K, and a characteristic distance $a \simeq 20$ Å (the length of the lipid molecule), we would get $D_0 \approx (a/2)(kT/m)^{1/2} \approx 10^{-3}$ cm^2/s. This is of course only an upper bound, because interaction of the lipid with water, as it comes out of the bilayer, will render the process slower than if it were in the gas phase. The lower bound is the lipid diffusion coefficient in water, about 5×10^{-6} cm^2/s. Jones and Thompson (1990) used this value and we shall do that as well.

Consider now the expression for the label concentration given by Eqs. 7 and 8. If the value of μ_1 is much smaller than all other μ_n, the corresponding rate will dominate the entire process. Experimentally we know that, at low acceptor concentrations, the desorption process is described by a single exponential law, $\exp(-k_{off}t)$, controlled only by the off-rate constant, k_{off}. We show in the Appendix that, in fact, $\mu_1 \ll \mu_n$ for all $n > 1$, but let us for the moment follow the consequences of this condition because they lead to a better understanding of the physical meaning of the terms in the solution. In this case, then, the total label concentration in the donors is $U(t) = f_0 e^{-\mu_1^2 D_0 t/a^2}$, from which we see that the off-rate constant is

$$k_{off} = \mu_1^2 D_0/a^2 \quad (9)$$

The expression for the rate constant in the activated state theory (Eyring, 1935) for a process of the type considered is of the form

$$k_{off} = D_0/a^2 e^{-\Delta G^{\ddagger}/kT} \quad (10)$$

(see for example Hill, 1960), which has a simple and intuitive meaning: a^2/D_0 is the time it would take for Brownian diffusion to bring a lipid out of the membrane over the distance a, considering only frictional interaction with water; and $\Delta G^{\ddagger}$ is the Gibbs activation energy barrier, which contains the difference in interactions of the lipid with water and the membrane, including lipid-lipid interactions and the hydrophobic effect. The activated state for this process corresponds to a situation in which the desorbing lipid is almost entirely out of the bilayer (Nichols, 1985). With the values for D_0, a, and the off-rate constant for POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) of $k_{off} = 2.5 \times 10^{-6}$ s^{-1}, corresponding to a relaxation time of about 100 hours (Jones and Thompson, 1990) at 300 K, we can calculate a Gibbs activation energy of $\Delta G^{\ddagger} = 19$ kcal/mol.

(*Note:* Jones and Thompson (1990), following Nichols (1985), used a model due to Aniansson et al. (1976) that is based on a derivation by Kramers (1940), and calculated the Gibbs activation energy to be 23 kcal/mol. That model, which obtains the off-rate using a formalism alternative to the activated state method, leads to an expression that is formally identical to that used here in Eq. 10. The difference is that a characteristic distance δ appears instead of a: $k_{off} = D_0/\delta^2 e^{-\Delta G^{\ddagger}/kT}$; δ is the width of the free energy barrier about kT units below the maximum, which is of the order of 1 Å: $\delta \approx a\, kT/\Delta G^{\ddagger}$, $a \simeq 20$ Å being the length of the lipid moiety in the bilayer. From an operational point of view, over a temperature range that is not too broad, the two formulas are equivalent: use of their expression simply results in a slightly larger activation barrier (4 kcal/mol more) and in an additional factor of $a^2/\delta^2 = O(10^3)$ that, together, give a factor that has the same value as that obtained using 19 kcal/mol for the activation barrier, as we do here. We prefer to use the activated state formalism because it leads to a simpler expression and the interpretation of the results becomes clearer.)

The meaning of the parameter ϵ is interesting and deserves some discussion. From the 3rd boundary condition in Eq. 3, we see that $\epsilon = Ha/D_0$ is a dimensionless parameter, proportional to the coefficient of surface transfer, H, at the interface between vesicle and water. Thus, ϵ is essentially the probability of crossing the activation barrier of width a for desorption, $\epsilon \approx e^{-\Delta G^{\ddagger}/kT}$. Actually, anticipating a result derived in the Appendix, let us take

$$\epsilon = 1/3 e^{-\Delta G^{\ddagger}/kT}$$

which, with $\Delta G^{\ddagger} = 19$ kcal/mol, has the value $\epsilon = 6.66 \times 10^{-15}$.

We can now obtain the solution of our problem for the case of low acceptor concentration, using these parameter values: $q = 10^{10}$, $\gamma^2 = D_0/D_1 = 1$, $b = 100$ (dilute regime, $b = L/a \gg 1$), and $\epsilon = 6.66 \times 10^{-15}$. We find that, using Eq. 8, $\mu_1 \ll \mu_n$ for all $n > 1$ (Appendix). Therefore, indeed, the smallest rate completely dominates the process and all we need to take into account is this first pole, at μ_1. The time dependence of the total lipid concentration in the donor vesicle is

$$U(t) = f_0 e^{-\mu_1^2 D_0 t/a^2} \tag{11}$$

and the process is a single exponential decay, consistent with the experimental observation in the dilute regime (Jones and Thompson, 1990), with a rate constant for desorption, $k_{off} = \mu_1^2 D_0/a^2$. Also, as shown in the Appendix, $\mu_1^2 = 3\epsilon$, giving $\mu_1^2 = e^{-\Delta G^{\ddagger}/kT}$.

Now, for the case of very high acceptor vesicle concentration we let the parameter $b = L/a$ become small, corresponding to a small average intervesicle distance L, keeping all other parameters fixed. But we find that, if $b = 10$ or even $b = 1$, we still obtain $\mu_1 \ll \mu_n$ for all $n > 1$, and $\mu_1^2 = 3\epsilon$, given any reasonable choice for the experimental constants. This means that μ_1^2 is independent of L. The answer to our problem is therefore clear: with the experimental values for the constants entering the problem, the off-rate is independent of vesicle concentration. In principle this would not have to be so, judging only from the functional dependence of the solution on b. But the very small value of ϵ, which arises from the very large activation barrier for desorption, has that consequence. This conclusion is qualitatively independent of the particular values assigned to the parameters in the model. Even if the other parameters, namely D_0, D_1, and q, were somewhat off, the value of ϵ is so small that it determines the result. In particular for D_0 (the parameter that probably has the largest uncertainty), use of a different value would lead to a different, though still very small, ϵ. Notice also that $D_0\mu_1$ appears as a product in Eq. 9, and thus cannot be varied independently while remaining consistent with the experimental values of the off-rate constants at low acceptor concentration.

We conclude that a statistical effect arising from changes in probabilities of return to the donor vesicle caused by a shorter average acceptor-donor distance (high vesicle concentrations) cannot explain the acceleration of the off-rate in those conditions. If this were the only effect, the process would always be first-order, as indicated by the present calculation. Another explanation is therefore needed, such as that presented by Jones and Thompson (1990), according to which a nearby acceptor induces a perturbation of the donor vesicle, resulting in an acceleration of the normal desorption process.

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APPENDIX

With the substitution $u = v/r$, the diffusion equation (Eq. 1) becomes

$$\frac{\partial v(r,t)}{\partial t} = D\frac{\partial^2 v(r,t)}{\partial r^2}. \tag{A1}$$

Applying the Laplace transform,

$$\hat{V}(r,s) = \int_0^\infty v(r,t)e^{-st}dt, \tag{A2}$$

we obtain the subsidiary equation:

$$D\frac{\partial^2 v(r,t)}{\partial r^2} = s\hat{V} - rf_0. \tag{A3}$$

The solution for the region $r < a$ is

$$\hat{V}_0(r,s) = A_0\cosh(\lambda_0 r) + B_0\sinh(\lambda_0 r) + rf_0/s, \tag{A4}$$

where $\lambda_0 = \sqrt{s/D_0}$. Now reverting back to the u-notation, with $\hat{V}_0 = \hat{U}_0 r$, we obtain

$$\hat{U}_0(r,s) = A_0\frac{\cosh(\lambda_0 r)}{r} + B_0\frac{\sinh(\lambda_0 r)}{r} + \frac{f_0}{s}. \tag{A5}$$

Applying the boundary condition (Eq. 2) requires that $A_0 = 0$, so we have

$$\hat{U}_0(r,s) = B_0\frac{\sinh(\lambda_0 r)}{r} + \frac{f_0}{s}. \tag{A6}$$

For the region $a < r < L$ the solution is

$$\hat{U}_1(r,s) = A_1\frac{\cosh(\lambda_1 r)}{r} + B_1\frac{\sinh(\lambda_1 r)}{r}. \tag{A7}$$

Using the boundary condition at $r = L$ (Eq. 4) this gives

$$\hat{U}_1 = \frac{B_1(\sinh(\lambda_1 r) - \tanh(\lambda_1 L)\cosh(\lambda_1 r))}{r}, \tag{A8}$$

where $\lambda_1 = \sqrt{s/D_1}$ and $\hat{U}_1 = \hat{V}_1/r$. Now we use the boundary conditions at $r = a$ (Eq. 3); the first one, $D_0\,(\partial/\partial r)\,\hat{U}_0 = D_1\,(\partial/\partial r)\,\hat{U}_1$, gives for B_1:

$$B_1 = \frac{-D_0B_0(\cosh(\lambda_0 a)\lambda_0 a - \sinh(\lambda_0 a))}{D_1(\sinh(\lambda_1 a) - \tanh(\lambda_1 L)\cosh(\lambda_1 a) - \lambda_1 a\cosh(\lambda_1 a) + \lambda_1 a\tanh(\lambda_1 L)\sinh(\lambda_1 a))}$$

Using the second BC at $r = a$ (Eq. 3),

$$\frac{\partial \hat{U}_0}{\partial r} = -\frac{H(\hat{U}_0 - q\hat{U}_1)}{D_0},$$

we obtain for B_0,

$$B_0 = -Ha^2 f_0 D_1 (\sinh(\lambda_1 a) - \tanh(\lambda_1 L)\cosh(\lambda_1 a) - \lambda_1 a\cosh(\lambda_1 a) + \lambda_1 a\tanh(\lambda_1 L)\sinh(\lambda_1 a))/(s(\cosh(\lambda_0 a)\lambda_0 a D_0 D_1\sinh(\lambda_1 a) - \cosh(\lambda_0 a)\lambda_0 a D_0 D_1\tanh(\lambda_1 L)\cosh(\lambda_1 a)$$

$$
\begin{aligned}
&- \cosh(\lambda_0 a)\lambda_0 a^2 D_0 D_1 \lambda_1 \cosh(\lambda_1 a) \\
&+ \cosh(\lambda_0 a)\lambda_0 a^2 D_0 D_1 \lambda_1 \tanh(\lambda_1 L)\sinh(\lambda_1 a) \\
&- \sinh(\lambda_0 a) D_0 D_1 \sinh(\lambda_1 a) \\
&+ \sinh(\lambda_0 a) D_0 D_1 \tanh(\lambda_1 L)\cosh(\lambda_1 a) \\
&+ \sinh(\lambda_0 a) D_0 D_1 \lambda_1 a \cosh(\lambda_1 a) \\
&- \sinh(\lambda_0 a) D_0 D_1 \lambda_1 a \tanh(\lambda_1 L)\sinh(\lambda_1 a) \\
&+ Ha \sinh(\lambda_0 a) D_1 \sinh(\lambda_1 a) \\
&- Ha \sinh(\lambda_0 a) D_1 \tanh(\lambda_1 L)\cosh(\lambda_1 a) \\
&- Ha^2 \sinh(\lambda_0 a) D_1 \lambda_1 \cosh(\lambda_1 a) \\
&+ Ha^2 \sinh(\lambda_0 a) D_1 \lambda_1 \tanh(\lambda_1 L)\sinh(\lambda_1 a) \\
&+ Ha^2 q D_0 \cosh(\lambda_0 a)\lambda_0 \sinh(\lambda_1 a) \\
&- Ha^2 q D_0 \cosh(\lambda_0 a)\lambda_0 \tanh(\lambda_1 L)\cosh(\lambda_1 a) \\
&- Haq D_0 \sinh(\lambda_0 a)\sinh(\lambda_1 a) \\
&+ Haq D_0 \sinh(\lambda_0 a)\tanh(\lambda_1 L)\cosh(\lambda_1 a))),
\end{aligned}
$$

which, upon some rearrangement, and defining

$$\gamma = \sqrt{D_0/D_1},$$

$$b = L/a,$$

$$z = \lambda_0 a,$$

and

$$\epsilon = Ha/D_0,$$

gives

$$B_0 = -\frac{f_0 a}{s\left(\dfrac{z\cosh(z) - (1-\epsilon)\sinh(z)}{\epsilon} + \dfrac{\gamma^2 q(z\cosh(z) - \sinh(z))}{1 - \gamma z \coth(z(1-b))}\right)} \tag{A9}$$

What we need is the time-dependence of the amount of material inside the sphere of radius a, that is,

$$4\pi \int_0^a u_0(r, t) r^2 dr. \tag{A10}$$

Thus, all we require is the inverse transform of U_0,

$$u_0(r, t) = \frac{1}{2\pi i}\int_C \hat{U}_0(r, s) e^{st} ds, \tag{A11}$$

where C represents an appropriate contour of integration in the complex plane (Carslaw and Jaeger, 1959). The solution in Laplace space (Eq. A6) is thus

$$U_0(r, s) = f_0\left[\frac{1}{s} - \frac{a\epsilon \sinh(zr/a)}{rs\left(z\cosh(z) - (1-\epsilon)\sinh(z) + \dfrac{\epsilon\gamma^2 q[z\cosh(z) - \sinh(z)]}{1 - \gamma z \coth(z(1-b))}\right)}\right]. \tag{A12}$$

There are terms of the form $\sinh(\sqrt{s})$ (note that $z = \sqrt{s/D_0}a$) both in the numerator and in denominator of the fraction in Eq. A12, but, as long as $\sqrt{s}$ represents the same branch in both, the fraction is a single-valued function of s.

In order to use the residue theorem, we need the zeros of the denominator of the second term in the right-hand side of Eq. A12. There is a first-order pole at $s = 0$ ($z = 0$), which cancels out with that coming from the initial condition. (It appears that there is another factor of z in this denominator as $z \to 0$, but the numerator, $a\epsilon \sinh(zr/a)$, also contains this factor as $z \to 0$.) We are then left with the task of finding the values of z, other than $z = 0$, such that:

$$z\cosh(z) - (1-\epsilon)\sinh(z) + \frac{\epsilon\gamma^2 q[z\cosh(z) - \sinh(z)]}{1 - \gamma z \coth(z(1-b))} = 0. \tag{A13}$$

Eq. A13 has no real roots. We follow the standard procedure (Carslaw and Jaeger, 1959) and write

$$z = i\mu$$

$$\rho = r/a$$

and Eq. A12 becomes:

$$\hat{U}_0(r, s) = f_0\left[\frac{1}{s} - \frac{\epsilon \sin(\rho\mu)}{s\rho G(\mu)}\right], \tag{A14}$$

where

$$G(\mu) = \mu\cos(\mu) - (1-\epsilon)\sin(\mu) + \frac{\epsilon\gamma^2 q(\mu\cos(\mu) - \sin(\mu))}{1 - \gamma\mu\cot(\mu(1-b))}. \tag{A15}$$

The solution is

$$
\begin{aligned}
u_0(r, t) &= \frac{1}{2\pi i}\int_C \hat{U}_0(r, s) e^{st} ds \\
&= \frac{f_0}{2\pi i}\int_C \left(\frac{1}{s} - \frac{\epsilon \sin(\rho\mu)}{s\rho G(\mu)}\right) e^{st} ds \\
&= f_0\left(1 - \sum_{n=0}^{\infty} R_n(t)\right),
\end{aligned} \tag{A16}
$$

where $R_n(t)$ is the residue at the nth singularity. The residue at $s = 0$ ($n = 0$) is 1 and cancels out the term coming from the initial condition, as already noted following Eq. A12, so we shall not need to consider this residue again. The expression

$$\frac{\epsilon \sin(\rho\mu)}{s\rho G(\mu)} e^{st}$$

has real and simple poles at the zeros (all real and simple) of $G(\mu)$. Using $\mu^2 = -sa^2/D_0$ and defining $\tau = D_0t/a^2$, the residue at each pole is given by

$$R_n(\tau) = R_n(0)e^{-\mu_n^2\tau}, \tag{A17}$$

where

$$R_n(0) = -\frac{2\epsilon \sin(\rho\mu_n)}{\rho\mu_n\left[\frac{\partial G(\mu)}{\partial \mu}\right]_{\mu=\mu_n}}.$$

The expression for our solution is then

$$u_0(\rho, \tau) = f_0 \sum_{n=1}^{\infty} R_n(0)e^{-\mu_n^2\tau}, \tag{A18}$$

where, in the summation, we already exclude the residue at $\mu = 0$ ($s = 0$), as noted above. Carrying out the differentiation yields

$$\frac{\partial G(\mu)}{\partial \mu} = \cos(\mu) - \mu \sin(\mu) - (1 - \epsilon)\cos(\mu) - \frac{\epsilon\gamma^2 q\mu \sin(\mu)}{1 - \gamma\mu \cot(\mu(1 - b))} - \frac{\epsilon\gamma^2 q(\mu \cos(\mu) - \sin(\mu))(-\gamma \cot(\mu(1 - b)) - \gamma\mu(-1 - \cot^2(\mu(1 - b)))(1 - b))}{(1 - \gamma\mu \cot(\mu(1 - b)))^2}$$

The residues are then given by

$$-2\epsilon \sin(\rho\mu_n)e^{-\mu_n^2\tau}/(\rho\, \mu_n(\cos(\mu_n) - \mu_n\sin(\mu_n) - (1 - \epsilon)\cos(\mu_n) - \frac{\epsilon\gamma^2 q\mu_n\sin(\mu_n)}{1 - \gamma\mu_n\cot(\mu_n(1 - b))} - \frac{\epsilon\gamma^2 q(\mu_n\cos(\mu_n) - \sin(\mu_n))(-\gamma \cot(\mu_n(1 - b)) - \gamma\mu_n(-1 - \cot^2(\mu_n(1 - b)))(1 - b))}{(1 - \gamma\mu_n\cot(\mu_n(1 - b)))^2}.$$

The concentration of lipid in the vesicle as a function of time is

$$U(\tau) = 4\pi\int_0^1 u_0(\rho, \tau)\rho^2 d\rho/(4\pi/3) = 3 \sum_{n=1}^{\infty} e^{-\mu_n^2\tau}\int_0^1 R_n(0)\rho^2 d\rho \tag{A19}$$

Experimentally (Jones and Thompson, 1990), in the dilute regime, the process is a single exponential decay with an off-rate constant $k_{off} = 2.5 \times 10^{-6}$ s^{-1} at 300 K (a relaxation time of about 100 hours). In this regime ($b \gg 1$, say $b = 100$), using D_0 and $D_1 = 5 \times 10^{-6}$ cm^2/s ($\gamma = D_0/D_1 = 1$), $a = 20$ Å, $q = 10^{10}$, and $\epsilon = 6.66 \times 10^{-15}$, we can plot the function $G(\mu)/\mu$. (Division by μ takes out the μ-factor in the numerator of Eq. A14; *cf.* comment preceding Eq. A13.) The plot (Fig. 2) shows that the first zero, $\mu_1 = 1.41 \times 10^{-7}$, is much smaller than any other. A magnification of the initial portion shows the location of the smallest zero (Fig. 3). Moreover, for the first pole, at $\mu = \mu_1$, the value of $3\int_0^1 R_n(0)\,\rho^2 d\rho$ (essentially = 1) is much larger than that for any other pole. Thus, all we need to take into account is this first pole, μ_1, and the time-dependence of the total lipid concentration in the donor vesicle is

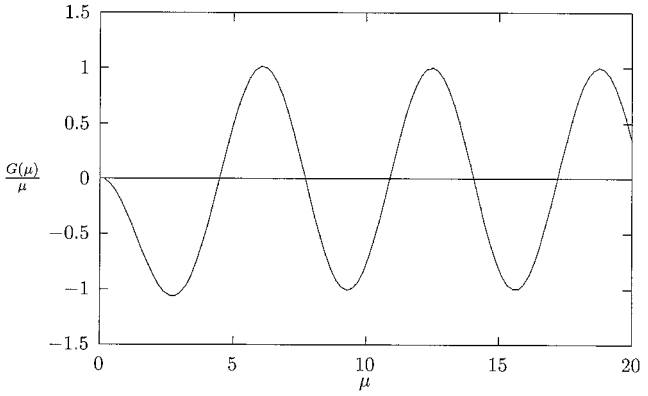

FIGURE 2 Location of the zeros of $G(\mu)$ (poles).

$$U(\tau) = f_0e^{-\mu_1^2\tau}. \tag{A20}$$

This is consistent with the experimental observation of a single exponential decay in the dilute regime. Notice that $\mu_1^2D_0/a^2 = k_{off}$.

The effect of vesicle concentration, that is, the average distance to the next vesicle, is represented by the parameter $b = L/a$ in our model. It could affect the mathematical problem in two ways: through the effect of b on the relative location of the zeros μ_n of $G(\mu)$ and through the effect of b on the values of μ_n that contribute most of the decay. It turns out that, with the values of q, γ, and ϵ given, the function $G(\mu)$ is independent of b (a plot with $b = 1.1$ looks exactly the same as that in Fig. 2). The lack of dependence of μ_1 on b can be understood from the following considerations. Some rearrangement of $G(\mu) = 0$ leads to:

$$\cot(\mu) = \frac{1}{\mu}\left[1 - \epsilon\frac{1 - \gamma\mu \cot(\mu(1 - b))}{1 - \gamma\mu \cot(\mu(1 - b)) + \beta}\right], \tag{A21}$$

where $\beta = \epsilon\gamma^2q$. Now, let

$$f(\mu) = \frac{1 - \gamma\mu \cot(\mu(1 - b))}{1 - \gamma\mu \cot(\mu(1 - b)) + \beta}, \tag{A22}$$

then

$$\lim_{\mu\to 0+} f(\mu) = \frac{(b - 1 + \gamma)}{(b - 1 + \gamma) + \beta(b - 1)} \cong 1$$

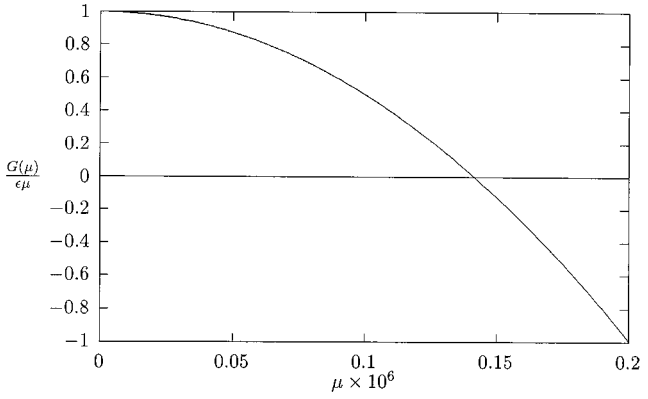

FIGURE 3 Location of the smallest zero.

for any b because $\beta = \gamma^2 q\epsilon$ is very small ($<10^{-4}$). For small μ, we can expand $\cot(\mu) = 1/\mu - \mu/3 + \ldots$ (which is justified because of the very small value of μ_1) in Eq. A21 and find:

$$\mu_1^2/(3\epsilon) = f(\mu_1) = 1.$$

Thus, there is no dependence of μ_1 on b.

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