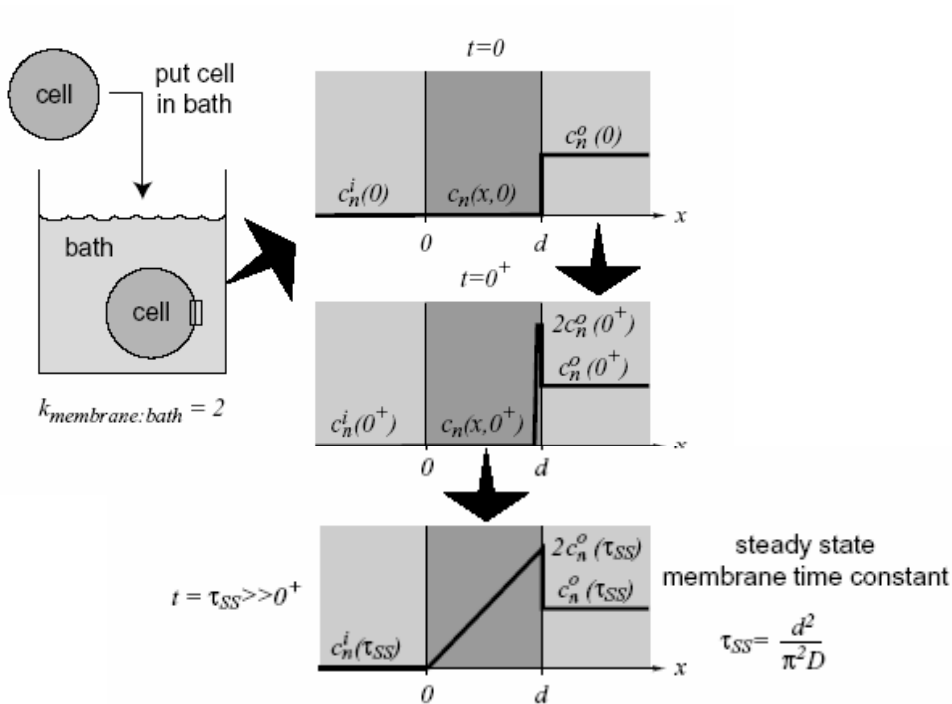


Recitation #3 6.021J

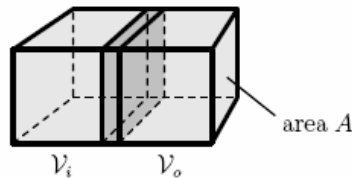


$$\Phi_n(t) = P_n (c_n^i(t) - c_n^o(t)) ; P_n = \frac{D_n k_n}{d}$$

Fick's law for membranes

P_n = permeability of membrane to solute n

Concentration in cell changes: two-compartment diffusion

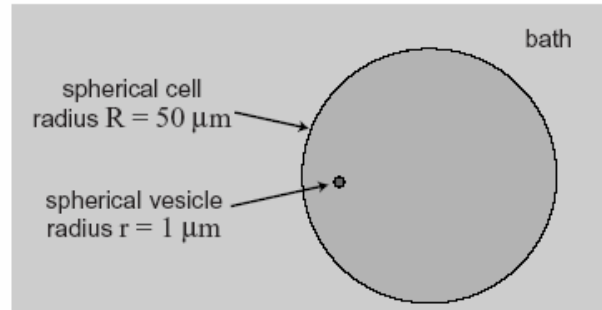


$$c_n^i(t) = c_n^i(\infty) + [c_n^i(0) - c_n^i(\infty)]e^{-t/\tau_{EQ}}$$

$$c_n^i(\infty) = \frac{N_n}{V_i + V_o}$$

$$\tau_{EQ} = \frac{1}{AP_n(\frac{1}{V_i} + \frac{1}{V_o})}$$

Problem 1. All cells are surrounded by a cell membrane. The cytoplasm of most cells contains a variety of organelles that are also enclosed within membranes. Assume that a spherical cell with radius $R = 50\mu\text{m}$ contains a spherical organelle called a vesicle, with radius $r = 1\mu\text{m}$, as shown in the following figure.



Assume that the membranes surrounding the cell and vesicle are uniform lipid bilayers with identical compositions and the same thickness $d = 10\text{ nm}$. Assume that solute X is transported across both the cell and vesicle membrane via the dissolve and diffuse mechanism. Assume that X dissolves equally well in the bath and in the aqueous interiors of the vesicle and cell. Assume that the solute X dissolves 100 times less readily in the membrane (i.e., the partitioning coefficient is 0.01). Assume the diffusivity of X in the membranes is $10^{-7}\text{ cm}^2/\text{s}$.

Initially, the concentration of X is zero inside the cell and inside the vesicle. At time $t = 0$, the cell is plunged into a bath that contains X with concentration 1 mmol/L .

- a) Estimate the time that is required for the concentration of X in the cell to reach 0.5 mmol/L . Find a numerical value or explain why it is not possible to obtain a numerical value with the information that is given.
- b) Estimate the time that is required for the concentration of X in the vesicle to reach 0.5 mmol/L . Find a numerical value or explain why it is not possible to obtain a numerical value with the information that is given.

Solutions to Problem 1

Problem 1. There are three interesting time constants. The first is the steady-state time constant for the membrane (which is the same for both the vesicle and cell. It is given by

$$\tau_{SS} = \frac{d^2}{\pi^2 D}$$

where D is the diffusivity of the solute in the membrane and d is the thickness of the membrane. Substituting,

$$\tau_{SS} = \frac{(10\text{nm})^2}{\pi^2 \times 10^{-7}\text{cm}^2/\text{s}} \approx 1\mu\text{s}.$$

The second is the equilibrium time constant for the cell,

$$\tau_{EQ}|_{\text{cell}} = \frac{d\mathcal{V}_C}{AkD}$$

where \mathcal{V}_C is the volume of the cell and k is the partition coefficient (here we have ignored the effect of the volume of the bath, because it is so large, and the volume of the vesicle, because it is so small). Thus

$$\tau_{EQ}|_{\text{cell}} = \frac{10\text{nm} \times \frac{4}{3}\pi R^3}{4\pi R^2 \times 0.01 \times 10^{-7}\text{cm}^2/\text{s}} = \frac{10\text{nm} \times \frac{R}{3}}{0.01 \times 10^{-7}\text{cm}^2/\text{s}} = \frac{10\text{nm} \times 50\mu\text{m}/3}{0.01 \times 10^{-7}\text{cm}^2/\text{s}} \approx 1.7\text{s}.$$

The third is the equilibrium time constant for the vesicle,

$$\tau_{EQ}|_{\text{vesicle}} = \frac{d\mathcal{V}_V}{AkD}$$

where \mathcal{V}_V is the volume of the vesicle (here we have ignored the effect of the volume of the cell because it is so large). Thus

$$\tau_{EQ}|_{\text{vesicle}} = \frac{10\text{nm} \times \frac{4}{3}\pi r^3}{4\pi r^2 \times 0.01 \times 10^{-7}\text{cm}^2/\text{s}} = \frac{10\text{nm} \times \frac{r}{3}}{0.01 \times 10^{-7}\text{cm}^2/\text{s}} = \frac{10\text{nm} \times 1\mu\text{m}/3}{0.01 \times 10^{-7}\text{cm}^2/\text{s}} \approx 33\text{ms}.$$

- a) Since $\tau_{EQ}|_{\text{cell}} \gg \tau_{SS}$, the concentration $c_c(t)$ in the cell will increase nearly exponentially with time with a time constant equal to $\tau_{EQ}|_{\text{cell}}$,

$$c_c(t) \approx (1\text{mmol/L}) \times (1 - e^{-t/\tau_{EQ}|_{\text{cell}}}).$$

The concentration $c_c(t)$ will reach half its initial value when

$$(0.5\text{mmol/L}) = (1\text{mmol/L}) \times (1 - e^{-t/\tau_{EQ}|_{\text{cell}}})$$

so

$$t = -\ln 0.5 \tau_{EQ}|_{\text{cell}} = 0.69 \times 1.7\text{s} \approx 1.2\text{s}.$$

- b) Since $\tau_{EQ}|_{\text{vesicle}} \ll \tau_{EQ}|_{\text{cell}}$ the concentration inside the vesicle is nearly equal to that in the cell. Therefore, the answer to part b is the same as the answer to part a,

$$t \approx 1.2\text{s}.$$

Problem 2. A rigid, homogeneous membrane separates two well-stirred compartments with rigid walls which contain aqueous solutions of a solute. The cross-sectional area of the membrane and compartments is $A = 64 \text{ cm}^2$. The solute is transported through the membrane by diffusion only. The membrane:solution partition coefficient for the solute is 1.0, but the value of the diffusion coefficient for the solute in the membrane is unknown except that it is known that $D \geq 0$. The widths are: 10 cm for compartment 1, 1 cm for compartment 2, and 0.1 cm for the membrane. The geometry is shown in Figure 1. The concentration of the solute in compartments 1 and 2 are $c^1(t)$

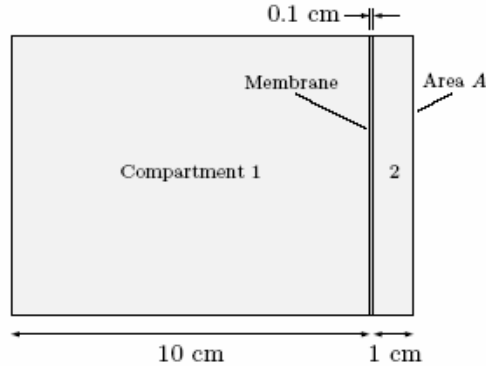


Figure 1: Geometry for two-compartment diffusion between compartment 1 and 2 through a membrane.

and $c^2(t)$, respectively. The concentration of the solute in the membrane depends on position in the membrane and is $c(x, t)$.

At $t = 0$ the concentration of the solute in compartment 1, $c^1(0) = 100 \text{ mmol/cm}^3$, and the concentrations in the membrane and in compartment 2 are zero, $c^2(0) = c(x, 0) = 0$. The resulting initial spatial dependence of the concentration is shown in Figure 2.

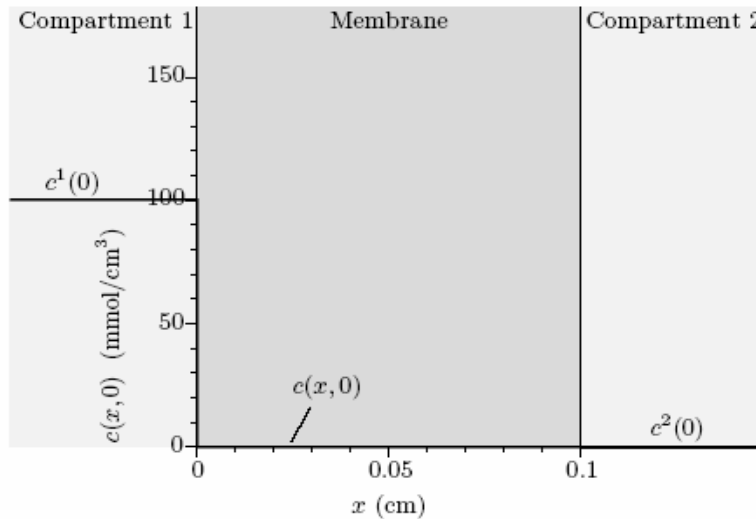


Figure 2: The initial concentration of solute in the two compartments and in the membrane are shown at $t = 0$. The figure illustrates the concentration in the membrane and in a portion of each compartment only. The concentrations in the compartments are uniform in space.

- a) Sketch the solute concentration distribution in the membrane and in the two compartments at equilibrium. Make a sketch similar to Figure 2 and label important values on the curve.
- b) For $t = 100$ seconds, the concentration $c(x, 100)$ and flux $\phi(x, 100)$ in the membrane and the concentrations in the two baths, $c^1(100)$ and $c^2(100)$, are shown in Figure 3.

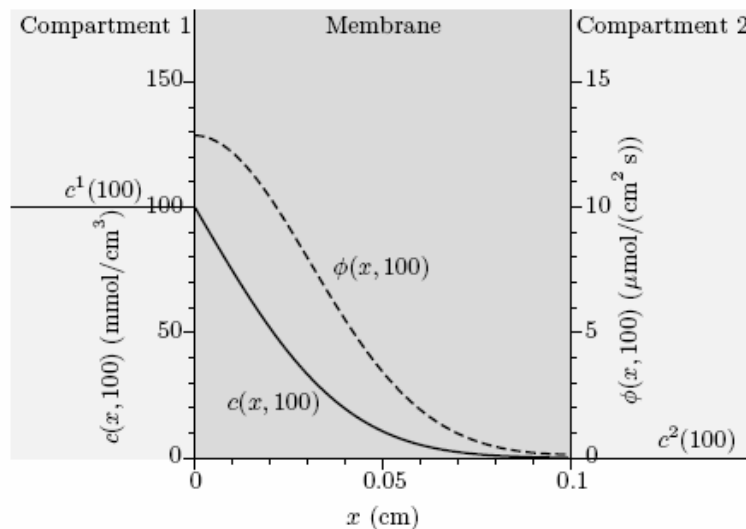


Figure 3: The concentration of solute (solid line) in the two compartments and in the membrane are shown at $t = 100$ seconds. The figure illustrates the concentration in the membrane and in a portion of each compartment only. The concentrations in the compartments are uniform in space. The flux of solute is shown (dashed line) in the membrane only.

- i) Is the concentration in the membrane at its steady-state distribution? Explain.
- ii) Estimate the diffusion coefficient for the solute in the membrane, D , from the data in Figure 3. Clearly indicate your method.
- c) For $t = 1000$ seconds, the concentration in the membrane, $c(x, 1000)$, and the concentrations in the bath are shown in Figure 4. In addition, Figure 4 also shows the dependence of concentration on time at two locations: one in the membrane, $c(0.04, t)$; and the other in compartment 2, $c^2(t)$.
- i) Is the concentration in the membrane at its steady-state distribution? Explain.
- ii) Estimate the equilibrium time constant for equilibration of the solute in the two compartments, τ_{eq} , from the data in Figure 4. Clearly indicate your method.
- iii) Estimate the steady-state time constant for the solute in the membrane, τ_{ss} , from the data in Figure 4. Clearly indicate your method.
- iv) Estimate the diffusion coefficient of the solute in the membrane D from the data in Figure 4. Clearly indicate your method.
- v) Estimate the quantity $\int_0^{1000} \phi(0.1, t) dt$ from the data in Figure 4. Clearly indicate your method.
- vi) Sketch $c^2(t)$ and $c(0.04, t)$ versus t from $t = 0$ to $t \approx 5\tau_{eq}$.

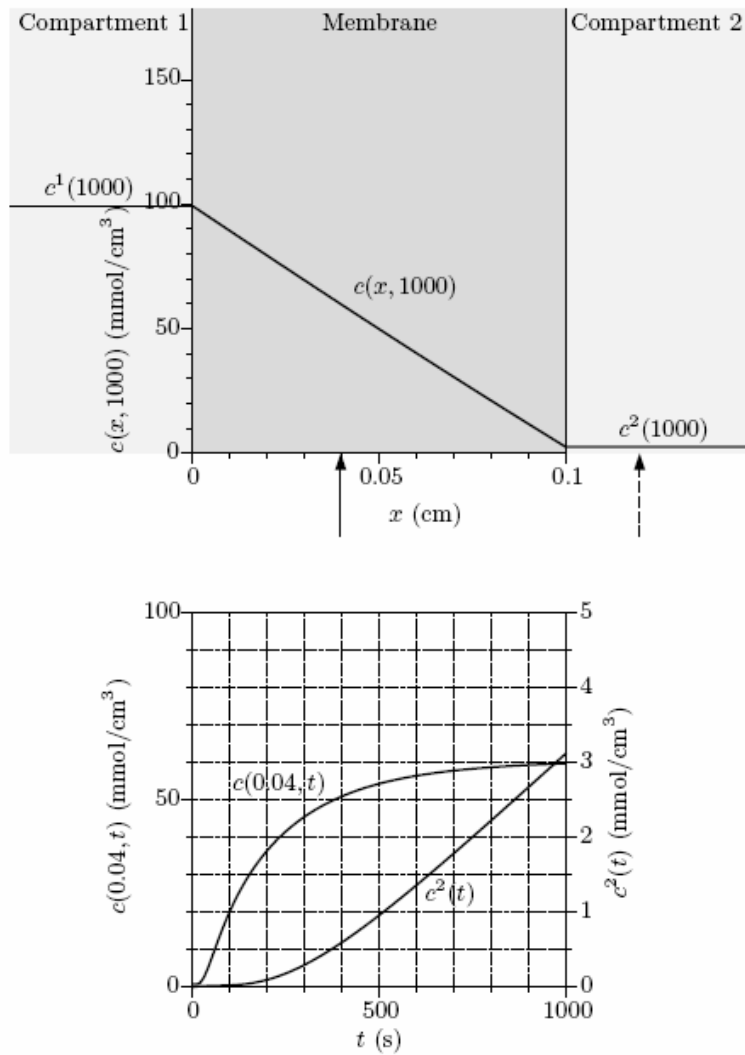


Figure 4: The concentration of solute in the two compartments and in the membrane are shown at $t = 1000$ seconds (upper panel) The figure illustrates the concentration in the membrane and in a portion of each compartment only. The concentrations in the compartments are uniform in space. Also shown (lower panel) are the dependence of concentration on time for the time interval $(0, 1000)$ for a location in the membrane, $c(0.04, t)$ (indicated in the upper panel with a solid arrow) and in compartment 2, $c^2(t)$ (indicated in the upper panel with a dashed arrow).

Problem 2. An important caveat in this problem is that, where it is so stated, the parameters are to be estimated from the measurements presented in the Figures.

- a. Since the membrane:solution partition coefficient is 1, at equilibrium the concentration in the two compartments and in the membrane will be the same. Thus, the total quantity of solute at $t = 0$ can be found and then distributed uniformly over the total volume of the system. The initial quantity of solute is $100 \text{ mmol/cm}^3 \cdot 10 \cdot A$. This is distributed over the total volume of the system which is $(10 + 1 + 0.1) \cdot A \text{ cm}^3$. Therefore, the final concentration is

$$\frac{1000A}{11.1A} = 90.09 \text{ mmol/cm}^3,$$

which is shown in Figure 1.

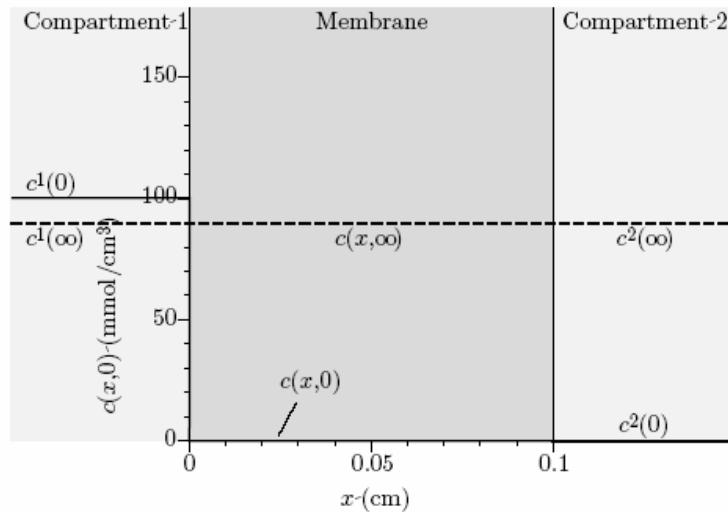


Figure 1: The final (dashed line) and the initial concentration (solid line) of solute in the two compartments and the membrane are shown. The figure illustrates the concentration in the membrane and in only a portion of each compartment. The concentrations in the compartments are uniform in space.

- b. This section applies for $t = 100$ seconds.
- The concentration profile in the membrane is not a linear function of position nor is the flux constant. Hence, diffusion in the membrane is not in steady state.
 - Because the diffusion regime is not in steady state in the membrane, it is not valid to use results that depend upon steady-state conditions, such as Fick's law for membranes. However, the diffusion coefficient for the solute in the membrane can be obtained from more fundamental considerations by using Fick's first law evaluated at $t = 100$

$$\phi(x, 100) = -D \frac{\partial c(x, 100)}{\partial x}.$$

As indicated in Figure 2, a convenient place to measure the slope of $c(x, 100)$ is at $x = 0+$, i.e., just inside the membrane. From the figure,

$$\left(\frac{\partial c(x, 100)}{\partial x} \right)_{x=0+} \approx -\frac{100 \times 10^{-3}}{0.04} = -2.5 \text{ mol/cm}^4.$$

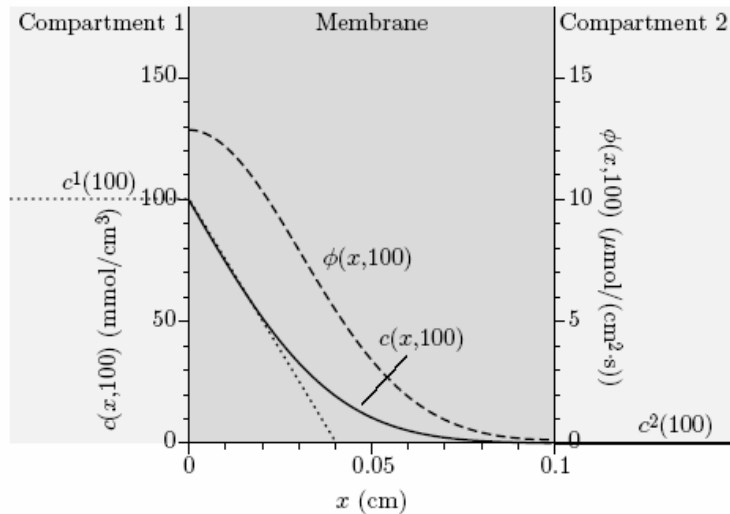


Figure 2: The concentration of solute in the two compartments and the membrane (solid line) are shown at $t = 100$. The figure illustrates the concentration in the membrane and in a portion of each compartment only. The concentrations in the compartments are uniform in space. The flux of solute is shown (dashed line) in the membrane only. A line tangent to $c(x, 100)$ at $x = 0$ is also shown (dotted).

At $x = 0+$, $\phi(0+, 100) \approx 12.8 \times 10^{-6} \text{ mol}/(\text{cm}^2 \cdot \text{s})$. Therefore,

$$D \approx -\frac{12.8 \times 10^{-6}}{-2.5} = 5.1 \times 10^{-6} \text{ cm}^2/\text{s}.$$

c. This section applies for $t = 1000$ seconds.

- i. The concentration profile in the membrane is linear. Hence, the concentration in the membrane is in steady state.
- ii. Note that $c^2(t)$ shows a delay and then a portion that has a linear increase in concentration over a time duration when $c(0.04, t)$ has saturated. Thus, during this time interval, the concentration in the membrane has reached steady state and, therefore, the thin-membrane approximation is a good approximation. Hence, during this time interval, $c^2(t)$ rises exponentially and has the form $C(1 - e^{-t/\tau_{eq}})$. But the time is clearly much less than the equilibrium time, i.e., $t \ll \tau_{eq}$. Therefore, $C(1 - e^{-t/\tau_{eq}}) \approx C(1 - (1 - t/\tau_{eq})) = Ct/\tau_{eq}$ where C is the final value or equilibrium value of the concentration which equals $90.09 \text{ mmol}/\text{cm}^3$. Thus, slope of the concentration must be found from Figure 3 during a time interval when steady state has been reached in the membrane and after the initial transient, i.e., $t > 500 \text{ ms}$. The slope is approximately $3.1 \times 10^{-3}/700 = 4.4 \times 10^{-6} \text{ mol}/\text{cm}^3 \cdot \text{s}$. Therefore, $\tau_{eq} = 90.09 \times 10^{-3}/(4.4 \times 10^{-6}) = 2.1 \times 10^4 \text{ s}$.
- iii. From the beginning of the experiment, concentration profile within the membrane will quickly reach the membrane steady state, which is characterized by the linear concentration profile. At $t = 1000$, it is clear that the membrane already reached the steady state. From the profile of $c(0.04, t)$, it does have a fast transition at the onset to the steady state value, which changes very slowly from that point on. Therefore, the

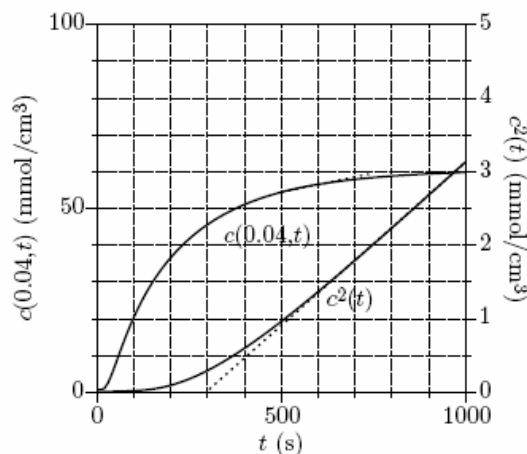


Figure 3: The dependence of concentration on time is shown for the time interval $(0, 1000)$ for a location in the membrane, $c(0.04, t)$ and in compartment 2, $c^2(t)$. The slope of the line tangent to $c^2(t)$ is shown with a dotted line. A line tangent to $c(0.04, t)$ beginning at $t = 500$ s is also shown.

timescale of the initial transition toward the membrane steady state is indeed τ_{ss} , which is estimated to be about 250 s. Hence, $\tau_{ss} \approx 250$ s.

- iv. *Method #1.* At $t = 1000$, the concentration in the membrane is approximately at steady state and $\partial c(x, 1000)/\partial x \approx -100/0.1 = -1000 \text{ mmol/cm}^4$. Thus, $\phi(x, 1000) = -D\partial c(x, 1000)/\partial x \approx 1000D$. But by continuity, the flux into compartment 2 must equal the rate of increase of solute in compartment 2. Therefore, $\phi(x, 1000) = 1000D = (dc^2(t)/dt)_{t=1000} \cdot 1$. The derivative can be evaluated directly from the lower figure as follows

$$(dc^2(t)/dt)_{t=1000} \approx 3.1/0.7 \times 10^{-3} \text{ mmol/cm}^3 \cdot \text{s}.$$

Combining these relations yields $D \approx 4.5 \times 10^{-6} \text{ cm}^2/\text{s}$.

Method #2. A second method involves estimating D from the equilibrium time constant. The equilibrium time constant can be estimated fairly accurately because the thin-membrane approximation holds for times much larger than the steady-state time constant. Therefore, $\tau_{eq} = V_e/(PA)$ for the equilibrium time constant for two-component diffusion through a thin membrane. In this equation, V_e is the equivalent volume of the two compartments, A is the surface area of the membrane, $P = D/d$ is the permeability of the membrane where d is the membrane thickness and D is the diffusion coefficient in the membrane. Therefore,

$$\begin{aligned} \tau_{eq} &= \frac{V_1V_2d}{(V_1 + V_2)DA} = \frac{L_1AL_2Ad}{(L_1A + L_2A)DA} = \frac{L_1L_2d}{(L_1 + L_2)D} \\ &= \frac{10 \cdot 1 \cdot 0.1}{11D} = \frac{0.09}{D} \text{ s}, \end{aligned}$$

so that $D = 0.09/2.1 \times 10^4 = 4.3 \times 10^{-6} \text{ cm}^2/\text{s}$. Note that this estimate is within 16% of the estimated value of D found in part b.

- v. The integral is simply the total number of moles of solute that flow through the plane at $x = 0.1$ per unit area in the time interval $(0, 1000)$. Thus, since the number of

moles of solutes in compartment 2 is 0 at $t = 0$, the number of moles of solute in this compartment at $t = 1000$ must equal the quantity transported into the compartment. Therefore, $A \int_0^{1000} \phi(0.1, t) dt = 1 \cdot A \cdot c^2(1000)$ which can be divided by A to yield $\int_0^{1000} \phi(0.1, t) dt = c^2(1000)$. From Figure 3 it is apparent that $c^2(1000) = 3.2$ mmol/cm³. Therefore, $\int_0^{1000} \phi(0.1, t) dt = 3.2$ mmol/cm².

- vi. In a time interval from $t = 0$ to $t = 5\tau_{eq}$, both the concentration in the membrane and in both compartments will be close to equilibrium. Therefore, both will approach a concentration of 90.09 mmol/cm³ with the equilibrium time constant which is 2.1×10^4 s as shown in Figure 4. Note that in Figure 3 $c(0.04, t)$ appears to saturate at a steady-

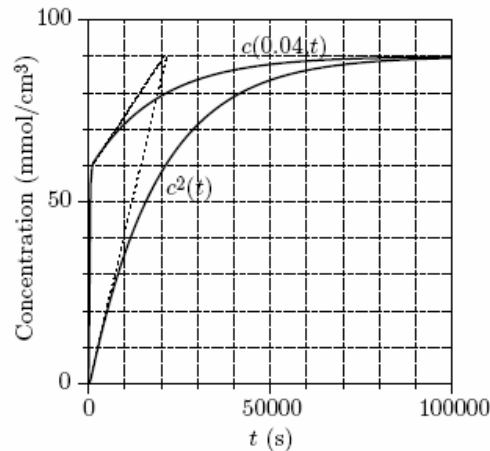


Figure 4: The dependence of concentration on time is shown for the time interval (0, 1000) for a location in the membrane, $c(0.04, t)$ and in compartment 2, $c^2(t)$. The slope of the line tangent to $c^2(t)$ is shown with a dotted line. A dashed line tangent to $c(0.04, t)$ beginning at $t = 500$ s is also shown.

state value which is about 60 mmol/cm³ in under 1000 seconds which represents about $4 \times \tau_{ss}$. However, this time is short compared to τ_{eq} . Thus, the steady-state value is not really saturated but changes slowly as the two volumes equilibrate. Figure 4 shows that the apparent saturation of $c(0.04, t)$ shown in Figure 3 is a mirage.