BME 2105 Introduction to Biomedical Engineering

Molecular and Cellular Fundamentals
Lecture 10 - Diffusion

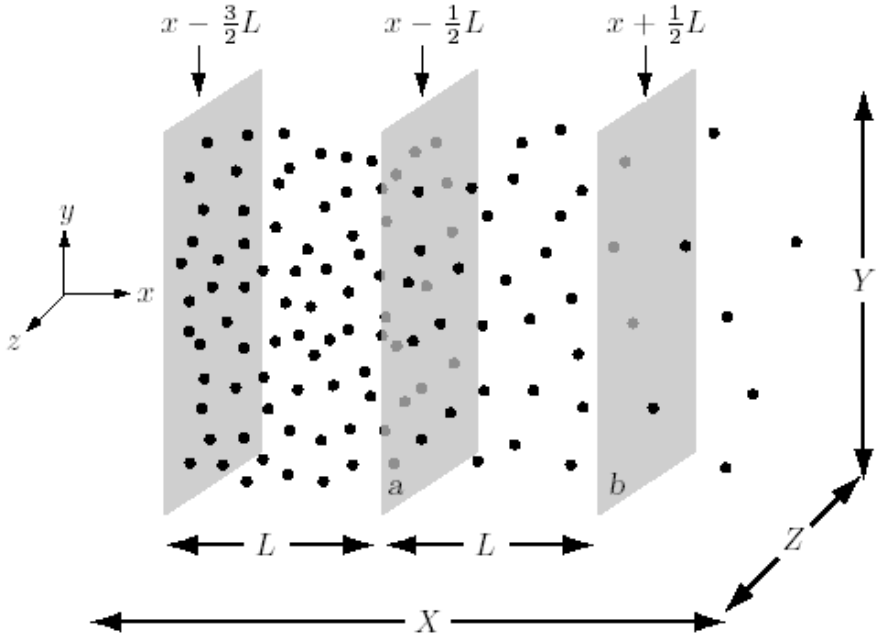
Diffusion dominates at molecular and cellular scales:

Reynold's number

$$\mathrm{Re} = \frac{vL\rho}{\eta}$$

v=average velocity
L=characteristic length
ρ=media density
η=dynamic viscosity (1 poise = $1 g*cm^{-1}*sec^{-1}$; Pascal second = $1 kg*m^{-1}*s^{-1}$)
For a fish (10 cm long) swimming in water (100 cm/sec) $Re=10^5$
For a bacterium (10^{-4} cm long) in water (10^{-3} cm/sec) $Re=10^{-5}$
So, at molecular and cellular scales, turbulence does not significantly assist diffusion (conduction) in transport. Similarly, convection and radiation are not too important.

1D Fick's law



Break the space up into slabs of width L, sides Y and Z, each centered on x=0,L,2L,3L,....

For a given time interval Δt, all the particles in the slab move either left or right, with equal probability (free diffusion). Choose Δt such that we only need to examine one crossing, and no particles cross over two boundaries. In the diagram, more particles move from x=0 to x=L than from x=L to x=0, simply because there are more particles in x=0 than in x=Lf. Mathematically, for a situation in which N(x) = number of particles in a box of size L * Y * Z = c(x) * L * Y * Z

Total transfer over the given time interval across any given barrier from x to x+L is

$$flux = \frac{1}{2*\Delta t}\left(N(x) - N(x+L)\right)$$

note that as L→0,

$$\left(\mathrm{N(x) - N(x+L)}\right) \rightarrow -\mathrm{L}\frac{\mathrm{dN(x)}}{\mathrm{dx}}$$

(go right to Fick's law unless interested)

Substituting back in,

$$\text{flux per area } = \frac{flux}{Y*Z} = j = \frac{1}{Y*Z*\Delta t}*\frac{1}{2}*L*\left(-\frac{d}{dx}\left[N(x) = LYZc(x)\right]\right) = -\frac{1}{\Delta t}\frac{L^2}{2}\frac{dc(x)}{dx}$$

rewrite, using the definition we had from the discrete case, as

$$\mathrm{j = -D*\frac{dC(x)}{dx}; D = \frac{L^2}{2\Delta t}}\text{; Fick's Law}$$

Continuity equation:

$$\frac{\partial \mathrm{C(x,t)}}{\partial \mathrm{t}} = -\frac{\partial \mathrm{j}}{\partial \mathrm{x}}$$

which is simply a statement that for any region, the change in stuff as a function of time is the balance of stuff flowing in against that flowing out. Together, we get

$$\frac{\mathrm{dC(x,t)}}{\mathrm{dt}} = \mathrm{D*\frac{d^2C(x,t)}{dx^2}}\text{; Diffusion equation}$$

or more simply

$$C_t = D*C_{xx}\text{; Diffusion equation}$$

This can be extended into higher dimensions. The Diffusion equation becomes

$$C_t = D\nabla^2 C$$

Impulse response

The most fundamental solution to this type of equation is the Gaussian distribution, which described the evolution of a delta function input with time.

$$\mathrm{C(x,t) = \sqrt{\frac{1}{4Dt\pi}}\exp\left(-\frac{x^2}{4Dt}\right)}$$

note that $\mathrm{P(x) = \frac{1}{\sigma\sqrt{2\pi}}\exp\left(-(x-x_0)^2/(2\sigma^2)\right)}$; Gaussian

$$\int_{-\infty}^{\infty}\exp(-y^2)dy = \sqrt{\pi}$$

Things to point out:

- σ^2 goes as 2Dt, similar to that from the discrete case
- Once again, for simple diffusion, D is a constant that can be measured, not dependent on L or Δt, but a combination in which the details vanish.
- So, why two different ways at getting at the same equation? Both are approximations of reality. For example, the Gaussian solution to the diffusion equation does imply that some particles move in excess of the speed of light. On the other hand, the

idea that particles exhibit a set jumping point in lockstep Δt behavior, and a detailed look at how the jumps actually happen, are unrealistic.

All spatial directions are independent. So, by separation of variables:

$$C(x,y,z,t) = C(x,t) * C(y,t) * C(z,t)$$

That is, each direction contributes either a Gaussian or a factor of 1 since integration over the Gaussian=1. So, the general form is

$$C(r,t) = \frac{1}{(4Dt\pi)^{n/2}} \prod_{i=1..n} \exp\left(-\frac{r_i^2}{4Dt}\right) = \frac{1}{(4Dt\pi)^{n/2}} \exp\left(-\frac{\vec{\mathbf{r}}^2}{4Dt}\right)$$; n-degrees of freedom

So, is diffusion down an axon truly a 1D system? To be accurate, no, it is a three-dimensional problem. However, if you assume a single concentration at any cross section, 1D diffusion applies. That is, a particular dimension can be separated if:

- Symmetry imposes the condition that there is no net flux in that dimension, or
- The system is such that diffusion is not allowed in that dimension

  Subtle, but important difference.

Estimation of the diffusion coefficient:

See Nelson section 4.1.4 for more complete discussion. It is important to see how the discrete model and continuous model interact to get these relations.

The Einstein relation for diffusion is:

$$D = \mu k_B T \text{ ; } \mu = \text{mobility} = v/f$$

k_B = Boltzmann constant = 1.38×10^{-23} J/K

T = temperature

Where f is the force needed to maintain a steady velocity of v.

For a ***sphere*** of radius, R, moving through a medium with dynamic viscosity, η:

$$\mu = \frac{1}{6\pi\eta R}$$ Stokes formula for creeping flow

Thus, the Stokes-Einstein relation gives us:

$$D = \frac{k_B T}{6\pi\eta R}$$

Good predictor for things with spherical symmetry, moderate success for things of lower dimensional symmetry. The problem is that there is no analytical solution to Stokes flow in 2 dimensions.

- As stated before, small particles in water, D ~ 1 μm^2/msec
- For proteins, $MW/A_0 = (4/3)\pi a^3 \rho$; $\rho \sim 1.3$ g/cm^3 for proteins, A_0=Avogadros #.

    - Myoglobin, MW 17kDa, η_{water}=0.764 cp=7.64E-4 Ns/m -> a=1.7 nm, D=.16 $\mu m^2/ms$
- molecules of gas, D ~ 10 $\mu m^2/\mu s$
- freely diffusing molecule in cell membrane, D ~ 1 $\mu m^2/sec$

What are some implications

For 1D diffusion of a small molecule in water (D~1 $\mu m^2/sec$) across characteristic distances:

cell of diameter 10μm; t=50 msec.

large cell of diameter 100 μm; t=5 sec

axon of length 10mm; t=50,000 sec = 14 hours

So, diffusion is great for most cell-level transport distances, and is energy-free, but not good for longer distances. In those cases, some other transport mechanism is needed.

Steady State equations:

In terms of diffusion-based transport, this is an important counterpart to the transient solutions we just worked through..

Again, the diffusion equation can be generalized as:

$$C_t = D\nabla^2 C$$

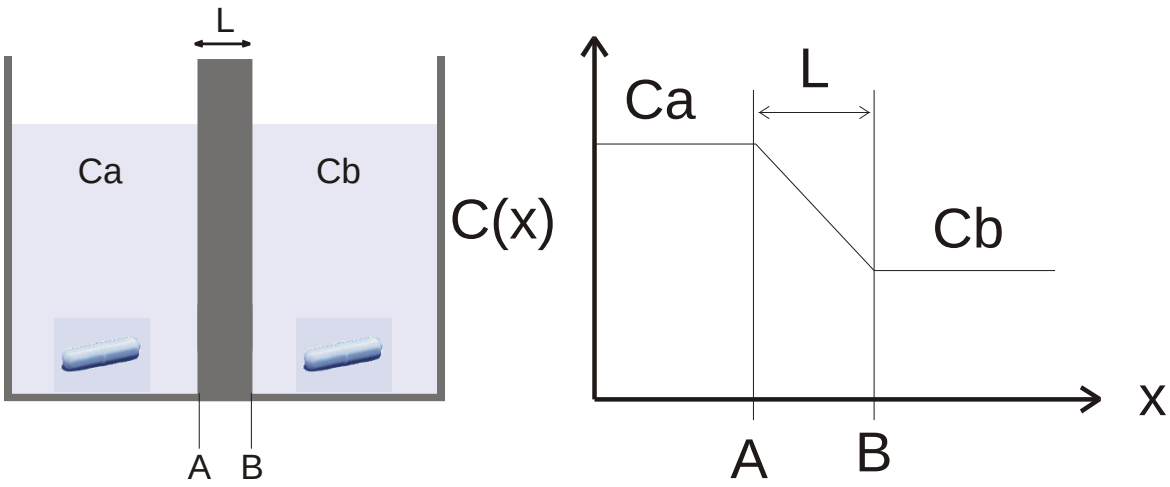
And in steady state, this can be rewritten as:

$$C_t = D\nabla^2 C = 0$$

1D diffusion through a region of defined diffusion coefficient – steady state equation

The starting point for this discussion is to consider transport through a single slab. For the moment, assume this slab represents a region of fluid or other structure which is unstirred, nonmoving, and only diffusion can transport molecules. For simplicity, assume that the regions next to these are extremely well-stirred, such that the concentration in each can be defined as a single, constant number. We will know that this configuration is not possible, but for sake of analysis of the middle layer, assume that this is true.

In rectangular coordinates, the diffusion equation, in short, says that the concentration profile must be piecewise linear. For the following situation, in which the region between A and B is composed of a medium of diffusion coefficient $D_{AB.}$ Forget how we get Ca and Cb experimentally, just focus on what's going on between A and B



$$C(x) = \begin{cases} Ca & x < A \\ Ca + \dfrac{Cb - Ca}{L}(x - A) & A < x < B \\ Cb & x > B \end{cases}$$

Note that the flux in region between A and B is

$$j = -D_{AB}\left(\frac{Cb - Ca}{L}\right) = D_{AB}\left(\frac{Ca - Cb}{L}\right)$$

Apparent paradox: the earlier discussion said that transport happens on $t^{-1/2}$ time, but the steady state solution seems to contradict this; it is, in fact constant with time. If we follow a specific molecule, we do see that $\langle x^2 \rangle$ goes as Dt. However, bulk transport independent of time (i.e., steady state)

Electrical Analogy

Electrical circuit: I=1/R * V
diffusion: J=DA/L*ΔC; R=L/(DA)
more convenient to think in terms of conductance per area, or conductivity, k=D/L

These act as resistors, conductors, can be assembled as you encountered in electrical engineering:

For two slabs (a) and (b) in series (different notation than above), the flux resultant from a concentration difference ΔC is:

$$J = J_a = J_b = \frac{1}{R_a}\Delta C_a = \frac{1}{R_b}\Delta C_b$$

$$\Delta C = \Delta C_a + \Delta C_b$$

$$J = \frac{1}{(R_a + R_b)} * \Delta C = \frac{1}{R_{ser}}\Delta C; R_{ser} = R_a + R_b$$

(or, for conductances)

$$J = J_a = J_b = k_a A_a \Delta C_a = k_b A_b \Delta C_b$$
$$\Delta C = \Delta C_a + \Delta C_b$$
$$J = \frac{1}{\frac{1}{k_a A_a} + \frac{1}{k_b A_b}} * \Delta C = k_{ser} A_{a=b} \Delta C; \frac{1}{k_{ser}} = \frac{1}{k_a} + \frac{1}{k_b}$$

And for two slabs (a) and (b) in parallel

$$\Delta C = \Delta C_a = \Delta C_b$$
$$J = J_a + J_b$$
$$J = (k_a A_a + k_b A_b) * \Delta C = k_{par} A_{a=b} \Delta C; k_{par} = k_a + k_b$$

(or, for resistances)

$$\Delta C = \Delta C_a = \Delta C_b$$
$$J = J_a + J_b$$
$$J = (\frac{1}{R_a} + \frac{1}{R_b}) * \Delta C = \frac{1}{R_{para}} \Delta C; \frac{1}{R_{para}} = \frac{1}{R_a} + \frac{1}{R_b}$$

Each representation is equally valid, and each will be useful in different contexts.

Finally, this electrical analogy should drive home the point that conservation of mass must hold. In a slab geometry, flux is constant at all points. Thus, with no gradient in the regions outside this single slab, no transport could take place. We hand-wave this by invoking the well-stirred condition, which relies on inertial and forced flow to dominate. In fact, in a small region near the slab, these additional factors cannot function, and there is a deviation from a constant concentration right near the slab.

Other steady state solutions

Diffusion to adsorbing sphere

Consider an absorbing sphere of radius s in a bath of far field concentration C_0. This means $C(r=s)=0$, $C(\infty)=C_0$

Steady state solution,

$$0 = D * C_{xx} \text{ or } D\nabla^2 C = 0$$

For spherical symmetry

$$\frac{1}{r^2} \frac{d}{dr} \left(r^2 \frac{dC(r)}{dr} \right) = 0$$

so,

$$r^2 \frac{dC(r)}{dr} = C_1 \text{ then } \frac{dC(r)}{dr} = r^{-2} C_1 \text{ and thus } C(r) = C_2 - r^{-1} C_1$$

now, since $C(\infty) = C_0$, then $C_2 = C_0$

since $C(s)=0$, $C_1 = sC_0$

and thus,

$$C(r) = C_0(1 - \frac{s}{r})$$

also note that the flux per unit area is

$$j(r) = -D\nabla C(r) = -DC_0 \frac{s}{r^2}$$

which if integrated over a shell of radius r (and surface area $4\pi r^2$) is constant! This is the equivalent as stating that flow through many slabs in series is constant, and dictated by the size of the sphere!

$$J = -D4\pi C_0 s$$

note that you can think of this as $J=\Delta C/R$ where $R=1/(4\pi Ds)$

Diffusion to a disk adsorber

Consider an adsorbing disk of radius s in a semi-infinite bath of far field concentration C_0. This means $C(r=s)=0$

Steady state solution (the solution is ugly),

$$0 = D * C_{xx} \text{ or } D\nabla^2 C = 0$$

From cylindrical symmetry, the transport through the disk is:

$$J = 4DsC_0$$

which can be thought of as $J=\Delta C/R$, where $R=1/(4Ds)$

What else influences diffusion through layers?

Partition coefficient

Remember Henry's Law: $\sigma = \frac{[x]}{P_x}$

Partition coefficient is similar: $\beta = [x]_{solvent1} / [x]_{solvent2}$

Usually, this will be in reference to water, so: $\beta = [x]_{solvent} / [x]_{water} = C_{solvent} / C_{water}$

This also applies to materials, such as polymers in which a drug is dissolved. In that case, the material is equivalent to the solvent.

The partition coefficient is a function of hydrophobicity/hydophilicity, among other things

What does this do to transport?

$$J = A\frac{D}{L}(\beta C_A - \beta C_b) = A\frac{D}{L}\beta\Delta C$$

So, a change in partition coefficient increases or decreases flux, independent of diffusion coefficient!

Note that the change in concentration is measured as if the material/solvent was immersed in water, and the concentrations were measured in the water right next to the surface of the material/region. This is the typical situation, in which a barrier is separating two aqueous buffers. It is relatively easy to measure the concentration in the buffers, but the concentration internal to the barrier must be inferred.

Diffusion under drift, against a barrier.

What happens when I apply a force to the particles being followed, but not the media? Additionally, impose the condition that at some position there is a barrier to further diffusion of particles. This is the case of centrifugation in a test tube, for example. The flux equation becomes:

$$j(x) = -D\frac{dC(x)}{dx} + vC(x)$$

To impose that j(x)=0 at a barrier further implies that j(x)=0 throughout the space for steady state to be true; what goes in, must go out. The flux equation becomes:

$$D\frac{dC(x)}{dx} = vC(x) \text{ then gives us } \frac{dC(x)}{dx} = \frac{v}{D}C(x)$$

and thus

$$C(x) = C_0 \exp\left(\frac{v}{D}x\right)$$

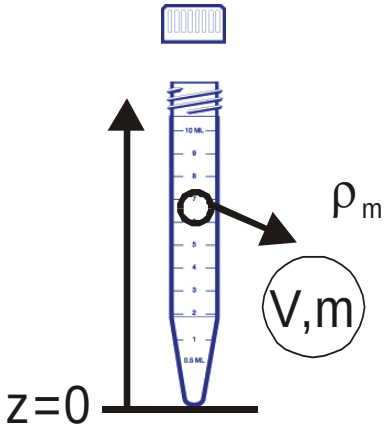
Not too informative in itself, let's give it a context.

Sedimentation

Consider a tall container of fluid in a gravitational field, and we follow a set of particles that are of a different density than the liquid.

The force inducing drift is buoyancy (density and gravity working together), and the bottom of the glass or top of the fluid are the no-flux boundaries of this system, depending on buoyancy; denser items sink, less dense items float.

g is gravity, acting downwards, but let's leave the directionality to the force calculation (g > 0, and not a vector itself).



$F=g*(V\rho_m-m)= - m_{net}g$; where ρ_m=density of media, V=volume of particle, m=mass of particle, $m_{net}=m-V\rho_m$. $m > V*\rho_m$, F < 0, downwards

From this force, we get a drift velocity of $v=-\mu*g*m_{net}$, where μ is mobility. Using the Einstein relation, we get:

$$v = - D*m_{net}*g/(k_BT)$$

Put this in, and for sake of thinking in vertical coordinates, put in terms of z:

$$c(z) = C_0 \exp\left(-\frac{m_{net}gz}{k_BT}\right) = C_0 \exp\left(-\frac{z}{z^*}\right)$$

Put some numbers into it:

Myoglobin, 17kDa. Protein has a general density of about 1.3 g/ml. This protein has a rough diameter of 4 nm. The term z* works out to be about 42 m.

Thus, in a typical 4 cm tube, at equilibrium in a 1 X gravitational field, the ratio of concentration at top compared to bottom is 99.9%. Never settles out!

However, centrifuge it long enough at 10,000 X g, z^* is about 4 mm, you'll get it out of solution.

Binding interactions:

Returning to the fundamental binding interaction:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

Many biological interactions are diffusion limited. That is, when A encounters B, they interact, so the reaction rate is limited by how often these molecules interact and that is limited by diffusion.

This gives a typical forward rate reaction of k_1~1 x 10^6 M-1s-1

This leads to a measure of how quickly the complex dissociates. Looking at only the dissociation reaction, thus ignoring the forward reaction, we note that

$$C(t)=C(0)*\exp(-k_{-1}*t)$$

So, we can take the value $1/k_{-1}$ as a measure of how long the complex endures.

How to get k_{-1}? Remember that

$$K_D = \frac{[A]*[B]}{[C]} = \frac{k_{-1}}{k_1}$$

Thus,

$$k_{-1} = K_D * k_1$$

Note: this is derived from the association reaction above, but is relating to the reverse reaction. Be careful on how k_1 and k_{-1} are defined.

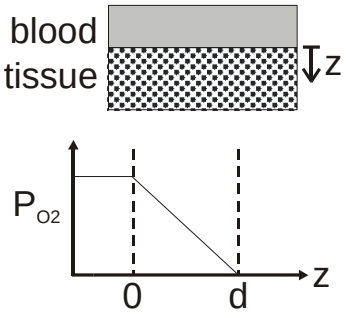
For a single dissociation reaction with K_m=25 mM=k_{-1}(dissoc)/k_1(assoc)

k_{-1}=25mM*1E6($M^{-1}s^{-1}$)=2.5 x 10^4 s^{-1}

$\tau = 1/k_{-1}$ = 4 x 10^{-5} sec = 40 μsec.

Examples:

Oxygen delivery to tissues



The partial pressure of oxygen in tissues at rest is 40 mmHg, and can drop to 20 mmHg with exercise. Here, we examine a converse problem, how deep into tissues oxygen can be delivered under these minimally oxygenated, under equilibrium conditions.

Most exchange between oxygen in red blood cells and tissues happens in capillaries, the small-diameter, final branches of the vasculature. For simplicity, here this system is modeled as a 1-D slab diffusion geometry; certainly, a cylindrical system centered on the capillaries would better represent real tissues.

Assume a layer of well-stirred, oxygenated blood at $P_{O2,blood}$ of 100 mmHg overlying a slab that represents living tissue. Metabolism in this tissue exerts an oxygen demand, which must be furnished by transport from the blood.

We're trying to find the maximum depth, d, at which oxygen demand can be supplied in this configuration. For simplicity, we'll assume that if we're looking at a specific depth d, all of the oxygen demand required by the slab of tissue between the blood and this depth is localized to a plane at this depth:

Total demand (d) = A*d*j_{O2}; j_{O2} = demand per volume of tissue, A = cross sectional area.

Since no demand is satisfied between the blood-tissue interface and that depth, there are no sources-sinks of O_2 until depth d, and the profile must thus be linear (see figure).

The greatest transport will be when P_{O2} at the depth d is 0 (why?).

From these ideas, we can readily derive the following relation for the maximum depth of oxygenation:

$$d = \sqrt{\frac{D_{O2} * k_{O2} * P_{O2,blood}}{j_{O2}}}$$

get this from:

j = demand per area = $(D_{O2}/d)k_{O2}P_{O2,tissue} = d*j_{O2}$, Solve for d.

Some useful numbers:

- k_{O2} = solubility of oxygen in aqueous solution (tissues) = 1.4 x 10^{-6} M/mmHg
- j_{O2} = oxygen requirement of tissues = 1 x 10^{-8} mole/(sec*cm^3)
- D_{O2} = diffusion coefficient for oxygen in tissues = 1 x 10^{-5} cm^2/sec

So, for $P_{O2,blood}$=100 mm Hg and assuming that the corresponding concentration is representative of the concentration in tissue immediately adjacent to blood,

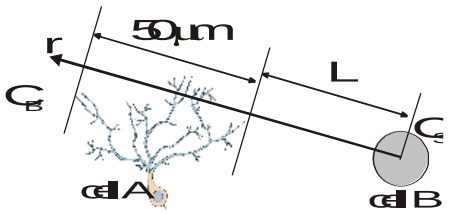
$$d = \sqrt{\frac{1E-5 cm^2/sec * 1.4E-6 mole/(L*mmHg) * 100 mmHg}{j1E-8 mole/(sec*cm^3)}\left(1E-3\frac{cm^3}{L}\right)} = .0118cm = 118\mu m$$

not a bad approximation, since the distance between capillaries tends to be in the 100 μm range, and this must supply times of high demand. Also note that it is not good for P_{O2} in tissues at

any depth to equal 0; if this is the concentration outside a cell, it would be drawing whatever O_2 there is inside the cell out.

Chemotaxis

Cells are very sensitive to gradients of certain chemicals; a difference of 2-5% of some receptor ligands in the local cell environment can direct cell migration. As suggested in the diagram, consider the sensing by one cell (A) of a chemical being secreted by cell (B). Assume the following:



- Cell A measures 50 μm from the end closest to cell B to the end farthest from cell B
- Cell B measures 5 μm in radius
- Assume steady state condition.
- Spherical geometry of this system, with cell B at the origin.
- Concentration is thus a function of r only; ignore geometry of cell A
- The chemical compound in question exhibits a diffusion coefficient of 1 μm^2/msec
- There is a bulk concentration C_B of this compound ($C(r=\infty)=C_B$)
- There is a concentration of C_s of this compound at the surface of cell B ($C(r=s)=C_s$)

In the discussion on diffusion, we found the steady state solution for concentration of a perfectly absorbing sphere, starting with the spherical geometry version of Fick's law:

$$C_t = -D\nabla^2 C(r) = -D\left[\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC(r)}{dr}\right)\right]$$

What is the steady-state solution to this system?

Set C_t to zero. This also implies that the terms D and the $1/r^2$ don't mean anything.

So,

$$\frac{d}{dr}\left(r^2\frac{dC(r)}{dr}\right)=0$$

Integrate once,

$$\frac{dC(r)}{dr}=\frac{1}{r^2}*C_1$$

Integrate again,

$$C(r)=-\frac{1}{C_1}\left(\frac{1}{r}\right)+C_2$$

Now, apply boundary conditions ($C(r=s)=Cs$ and $C(r=\infty)=C_B$), we get to

$$C(r)=\frac{s}{r}(C_S-C_B)+C_B$$

It is not dependent on diffusion coefficient; the diffusion coefficient influences overall flux, but not concentration profile in any of the systems we studied.

Now, assuming that C_B=0, and that cell A must experience a 5% difference in concentration of the chemical across it's 50 μm extent to respond to cell B, describe as quantitatively as possible the dependence of this response on the distance between cells A and B, denoted as L in the diagram.

Setting C_B=0,

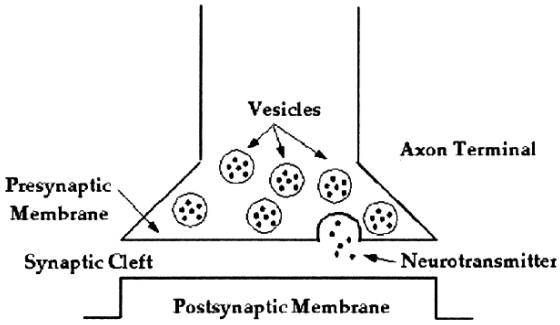
$$\frac{C(r)}{C(r+\Delta r)} = \frac{r+\Delta r}{r}$$

Where Δr is the distance across cell A. r->L. As L gets bigger, this ratio drops to 1, so the change across a 50 μm distance drops. Specifically, for Δr=50μm, the cutoff for a 5% difference is 1.05=(L+50μm)/L=> L=1000μm=1mm

(5 points)

Diffusion across a synapse

The neuromuscular junction is defined as the interface between a motor neuron and muscle cell, and is depicted graphically to the right. The synaptic cleft is a roughly 20 nm gap between the neuron membrane and the postsynaptic surface of the muscle cell. A typical signal transmission event starts with the arrival of an electric signal at the end of the neuron, which sequentially initiates: 1) fusion of storage vesicles containing acetylcholine with the presynaptic membrane, 2) release of acetylcholine into the 20-nm thick synaptic cleft, 3) activation of acetylcholine receptors on the other cell, which leads to activation of signaling systems in the second cell. The diffusion coefficient for acetylcholine in the synaptic cleft is 0.9 μm^2/msec. Assume that the vesicle fusion process produces a uniform distribution of acetylcholine along the neuron face in the synaptic cleft.



2a) Modeling acetylcholine as a sphere and the extracellular fluid in the synaptic cleft as water, what is the radius of acetylcholine?

The Stokes-Einstein relation, which applies for a spherical molecule, is written as $D=k_BT/(6\pi\eta R)$. D yields R=2.4E-10m

2b) How long does it take acetylcholine to diffuse across the cleft? That is, assuming a Gaussian solution accurately describes diffusion, when does $\sigma=\text{sqrt}(\sigma^2)$=20nm

Given the symmetry along the synaptic cleft, this is a 1D solution. σ=sqrt(2Dt). D=0.9 μm^2/msec, σ=20 nm -> 2E-4 msec

2c) Synaptic transmission normally takes 1 msec. Is the rate of diffusion of acetylcholine the main determinant of the transmission delay?

no