Random Walks in Biology

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Chapter 1

Diffusion: Microscopic Theory

Diffusion is the random migration of molecules or small particles arising from motion due to thermal energy. A particle at absolute temperature T has, on the average, a kinetic energy associated with movement along each axis of $kT/2$, where k is Boltzmann's constant. Einstein showed in 1905 that this is true regardless of the size of the particle, even for particles large enough to be seen under a microscope, i.e., particles that exhibit Brownian movement. A particle of mass m and velocity v_x on the x axis has a kinetic energy $mv_x^2/2$. This quantity fluctuates, but on the average $\langle mv_x^2/2 \rangle = kT/2$, where $\langle \ \rangle$ denotes an average over time or over an ensemble of similar particles. From this relationship we compute the mean-square velocity,

$$\langle v_x^2 \rangle = kT/m, \tag{1.1}$$

and the root-mean-square velocity,

$$\langle v_x^2 \rangle^{1/2} = (kT/m)^{1/2}. \tag{1.2}$$

We can use Eq.1.2 to estimate the instantaneous velocity of a small particle, for example, a molecule of the protein lysozyme. Lysozyme has a molecular weight 1.4×10^4 g. This is the mass of one mole, or 6.0×10^{23} molecules; the mass of one molecule is $m = 2.3 \times 10^{-20}$ g. The value of kT at 300°K (27°C) is 4.14×10^{-14} g cm^2/sec^2. Therefore, $\langle v_x^2 \rangle^{1/2} = 1.3 \times 10^3$ cm/sec. This is a sizeable speed. If there were no obstructions, the molecule would cross a typical classroom in about 1 second. Since the protein is not in a vacuum but is immersed in an aqueous medium, it does not go very far before it bumps into molecules of
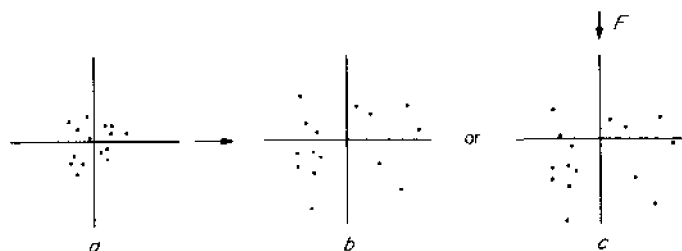
Fig. 1.1. Particles confined initially in a small region of space (*a*) diffuse symmetrically outward (*b*) or outward and downward (*c*) if subjected to an externally applied force, *F*.

water. As a result, it is forced to wander around: to execute a random walk. If a number of such particles were confined initially in a small region of space, as shown in Fig. 1.1a, they would wander about in all directions and spread out, as shown in Fig. 1.1b. This is simple diffusion. If a force were applied externally, such as that due to gravity, the particles would spread out and move downward, as shown in Fig. 1.1c. This is diffusion with drift. In this chapter, we analyze simple diffusion from a microscopic point of view. We look at the subject more broadly in Chapters 2 and 3. Diffusion with drift is considered in Chapter 4.

One-dimensional random walk

In order to characterize diffusive spreading, it is convenient to reduce the problem to its barest essentials, and to consider the motion of particles along one axis only, say the x axis, as shown in Fig. 1.2. The particles start at time $t = 0$ at position $x = 0$ and execute a random walk according to the following rules:

1) Each particle steps to the right or to the left once every τ seconds, moving at velocity $\pm v_x$ a distance
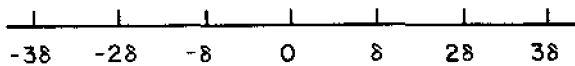
Fig. 1.2. Particles executing a one-dimensional random walk start at the origin, 0, and move in steps of length δ, occupying positions 0, $\pm \delta$, $\pm 2\delta$, $\pm 3\delta$, ...

$\delta = \pm v_x \tau$. For simplicity, we treat τ and δ as constants. In practice, they will depend on the size of the particle, the structure of the liquid, and the absolute temperature T.

2) The probability of going to the right at each step is 1/2, and the probability of going to the left at each step is 1/2. The particles, by interacting with the molecules of water, forget what they did on the previous leg of their journey. Successive steps are statistically independent. The walk is not biased.

3) Each particle moves independently of all the other particles. The particles do not interact with one another. In practice, this will be true provided that the suspension of particles is reasonably dilute.

These rules have two striking consequences. The first is that the particles go nowhere on the average. The second is that their root-mean-square displacement is proportional not to the time, but to the square-root of the time. It is possible to establish these propositions by using an iterative procedure. Consider an ensemble of N particles. Let $x_i(n)$ be the position of the ith particle after the nth step. According to rule 1, the position of a particle after the nth step differs from its position after the $(n - 1)$th step by $\pm \delta$:

$$x_i(n) = x_i(n - 1) \pm \delta. \qquad (1.3)$$

According to rules 2 and 3, the $+$ sign will apply to roughly half of the particles, the $-$ sign to the other half. The mean displacement of the particles after the nth step can be found by summing over the particle index i and

dividing by N:

$$\langle x(n) \rangle = \frac{1}{N} \sum_{i=1}^{N} x_i(n). \qquad (1.4)$$

On expressing $x_i(n)$ in terms of $x_i(n-1)$, Eq. 1.3, we find

$$\langle x(n) \rangle = \frac{1}{N} \sum_{i=1}^{N} [x_i(n-1) \pm \delta]$$

$$= \frac{1}{N} \sum_{i=1}^{N} x_i(n-1) = \langle x(n-1) \rangle. \qquad (1.5)$$

The second term in the brackets averages to zero, because its sign is positive for roughly half of the particles, negative for the other half. Eq. 1.5 tells us that the mean position of the particles does not change from step to step. Since the particles all start at the origin, where the mean position is zero, the mean position remains zero. This is the first proposition. The spreading of the particles is symmetrical about the origin, as shown in Fig. 1.3.
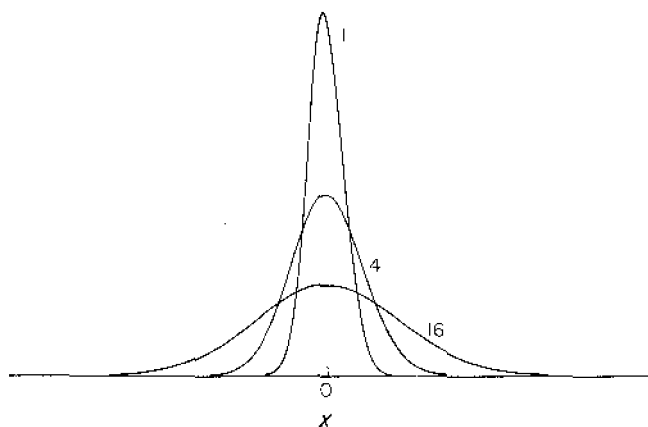
Fig. 1.3. The probability of finding particles at different points x at times $t = 1, 4$, and 16. The particles start out at position $x = 0$ at time $t = 0$. The standard deviations (root-mean-square widths) of the distributions increase with the square-root of the time. Their peak heights decrease with the square-root of the time. See Eq. 1.22.

How much do the particles spread? A convenient measure of spreading is the root-mean-square displacement $\langle x^2(n) \rangle^{1/2}$. Here we average the square of the displacement rather than the displacement itself. Since the square of a negative number is positive, the result must be finite; it cannot be zero. To find $\langle x^2(n) \rangle$, we write $x_i(n)$ in terms of $x_i(n-1)$, as in Eq. 1.3, and take the square:

$$x_i^2(n) = x_i^2(n-1) \pm 2\delta x_i(n-1) + \delta^2. \qquad (1.6)$$

Then we compute the mean,

$$\langle x^2(n) \rangle = \frac{1}{N} \sum_{i=1}^{N} x_i^2(n), \qquad (1.7)$$

which is

$$\langle x^2(n) \rangle = \frac{1}{N} \sum_{i=1}^{N} [x_i^2(n-1) \pm 2\delta x_i(n-1) + \delta^2]$$

$$= \langle x^2(n-1) \rangle + \delta^2. \qquad (1.8)$$

As before, the second term in the brackets averages to zero; its sign is positive for roughly half of the particles, negative for the other half. Since $x_i(0) = 0$ for all particles i, $\langle x^2(0) \rangle = 0$. Thus, $\langle x^2(1) \rangle = \delta^2$, $\langle x^2(2) \rangle = 2\delta^2$, ..., and $\langle x^2(n) \rangle = n\delta^2$. We conclude that the mean-square displacement increases with the step number n, the root-mean-square displacement with the square-root of n. According to rule 1, the particles execute n steps in a time $t = n\tau$; n is proportional to t. It follows that the mean-square displacement is proportional to t, the root-mean-square displacement to the square-root of t. This is the second proposition. The spreading increases as the square-root of the time, as shown in Fig. 1.3.

To see this more explicity, note that $n = t/\tau$, so that

$$\langle x^2(t) \rangle = (t/\tau)\delta^2 = (\delta^2/\tau)t, \qquad (1.9)$$

where we write $x(t)$ rather than $x(n)$ to denote the fact that x now is being considered as a function of t. For convenience, we define a diffusion coefficient, $D = \delta^2/2\tau$, in units cm²/sec. The reason for the factor 1/2 will become clear in Chapter 2. This gives us

$$\langle x^2 \rangle = 2Dt \qquad (1.10)$$

and

$$\langle x^2 \rangle^{1/2} = (2Dt)^{1/2}, \qquad (1.11)$$

where, for simplicity, we drop the explicit functional reference (t). The diffusion coefficient, D, characterizes the migration of particles of a given kind in a given medium at a given temperature. In general, it depends on the size of the particle, the structure of the medium, and the absolute temperature. For a small molecule in water at room temperature, $D \approx 10^{-5}$ cm²/sec.

A particle with a diffusion coefficient of this order of magnitude diffuses a distance $x \approx 10^{-4}$ cm (the width of a bacterium) in a time $t \approx x^2/2D \approx 5 \times 10^{-4}$ sec, or about half a millisecond. It diffuses a distance $x = 1$ cm (the width of a test tube) in a time $t \approx x^2/2D \approx 5 \times 10^4$ sec, or about 14 hours. The difference is dramatic. In order for a particle to wander twice as far, it takes 4 times as long. In order for it to wander 10 times as far, it takes 100 times as long. Therefore, there is no such thing as a diffusion velocity; displacement is not proportional to time but rather to the square-root of the time. What happens if we try to define a diffusion velocity by dividing the root-mean-square displacement by the time? The result is an explicit function of the time. Dividing both sides of Eq. 1.11 by t, we find

$$\frac{\langle x^2 \rangle^{1/2}}{t} = \left(\frac{2D}{t} \right)^{1/2}. \qquad (1.12)$$

Thus, the shorter the period of observation, t, the larger the apparent velocity. For values of t smaller than τ, the apparent velocity is larger than $\delta/\tau = v_x$, the instantaneous velocity of the particle. This is an absurd result.

In Chapter 2 we will speak of adsorption rates or diffusion currents. These expressions refer to the number of particles that are adsorbed at, or cross, a given boundary in unit time. They are bulk properties of an ensemble of particles, proportional to their number. They are not rates that tell us how long it takes a particle, by diffusion, to go from here to there. This time depends on the square of the distance, as defined by Eq. 1.10. When next you come across the expression "diffusion rate," think twice! This phrase is ambiguous, at best, and often used incorrectly.

Two- and three-dimensional random walks

Rules 1 to 3 apply in each dimension. In addition, we assert that motions in the x, y, and z directions are statistically independent. If $\langle x^2 \rangle = 2Dt$, then $\langle y^2 \rangle = 2Dt$ and $\langle z^2 \rangle = 2Dt$. In two dimensions, the square of the distance from the origin to the point (x,y) is $r^2 = x^2 + y^2$; therefore,

$$\langle r^2 \rangle = 4Dt. \qquad (1.13)$$

In three dimensions, $r^2 = x^2 + y^2 + z^2$, and

$$\langle r^2 \rangle = 6Dt. \qquad (1.14)$$

A computer simulation of a two-dimensional random walk is shown in Fig. 1.4. Steps in the x and y directions were made at the same times, so the particle always moved diagonally. The simulation makes graphic a remarkable feature of the random walk, discussed further in Chapter 3. Since explorations over short distances can be made in much shorter times than explorations over long
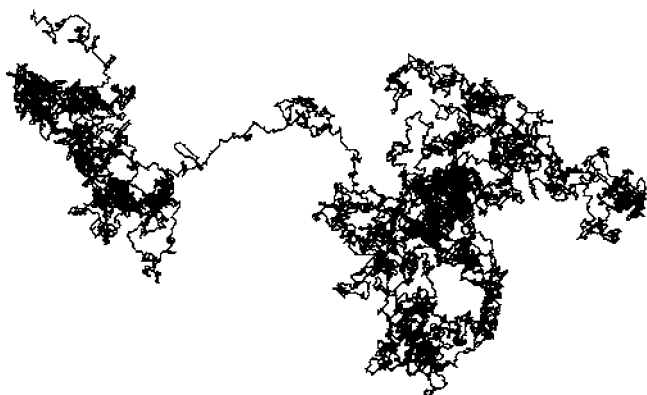
Fig. 1.4. An x, y plot of a two-dimensional random walk of $n = 18,050$ steps. The computer pen started at the upper left corner of the track and worked its way to the upper right edge of the track. It repeatedly traversed regions that are completely black. It moved, as the crow flies, 196 step lengths. The expected root-mean-square displacement is $(2n)^{1/2} = 190$ step lengths.

distances, the particle tends to explore a given region of space rather thoroughly. It tends to return to the same point many times before finally wandering away. When it does wander away, it chooses new regions to explore blindly. A particle moving at random has no tendency to move toward regions of space that it has not occupied before; it has absolutely no inkling of the past. Its track does not fill up the space uniformly.

The binomial distribution

We have learned so far that particles undergoing free diffusion have a zero mean displacement and a root-mean-square displacement that is proportional to the square-root of the time. What else can we say about the shape of the distribution of particles? To find out, we have to work out the probabilities that the particles step different distances to the right or to the left. While doing

so, it is convenient to generalize the one-dimensional random walk and suppose that a particle steps to the right with a probability p and to the left with a probability q. Since the probability of stepping one way or the other is 1, $q = 1 - p$. The probability that such a particle steps exactly k times to the right in n trials is given by the binomial distribution

$$P(k;n,p) = \frac{n!}{k!(n-k)!} p^k q^{n-k}. \qquad (1.15)$$

This equation is derived in Appendix A; see Eqs. A.17, A.18. The displacement of the particle in n trials, $x(n)$, is equal to the number of steps to the right less the number of steps to the left times the step length, δ:

$$x(n) = [k - (n - k)]\delta = (2k - n)\delta. \qquad (1.16)$$

Since we know the distribution of k, we know the distribution of x. The two distributions have the same shapes. The probability machine shown in Fig. A.3 converts one into the other.

The mean displacement of the particle is

$$\langle x(n) \rangle = (2\langle k \rangle - n)\delta, \qquad (1.17)$$

where

$$\langle k \rangle = np; \qquad (1.18)$$

see Eq. A.22. The mean-square displacement is

$$\langle x^2(n) \rangle = \langle [(2k - n)\delta]^2 \rangle$$
$$= (4\langle k^2 \rangle - 4\langle k \rangle n + n^2)\delta^2, \qquad (1.19)$$

where

$$\langle k^2 \rangle = (np)^2 + npq; \qquad (1.20)$$

see Eq. A.23. For the case $p = q = 1/2$, Eqs. 1.17 and 1.19 yield $\langle x(n) \rangle = 0$ and $\langle x^2(n) \rangle = n\delta^2$, as expected.

The Gaussian distribution

A small particle, such as lysozyme, steps an enormous number of times every second. Given the instantaneous velocity estimated from Eq. 1.2, $v_x = \delta/\tau \simeq 10^3$ cm/sec, and a diffusion constant, $D \simeq \delta^2/2\tau \simeq 10^{-6}$ cm^2/sec, we can compute the step length, δ, and the step rate, $1/\tau$. The step length is $2D/v_x \simeq (10^{-6}$ cm^2/sec$)/(10^3$ cm/sec$) = 10^{-9}$ cm, and the step rate is $v_x/\delta \simeq (10^3$ cm/sec$)/(10^{-9}$ cm$) = 10^{12}$ sec^{-1}. Of these $n = 10^{12}$ steps taken each second, $np = 0.5 \times 10^{12}$ are taken to the right. The standard deviation in this number is $(npq)^{1/2} = 0.5 \times 10^6$; see Eq. A.25. So, to a precision of about a part in a million, half of the steps taken each second are made to the right and half to the left. What happens to the distribution of x in this limit? As stated in Appendix A, when n and np are both very large, the binomial distribution, $P(k;n,p)$, is equivalent to

$$P(k)dk = \frac{1}{(2\pi\sigma^2)^{1/2}} e^{-(k-\mu)^2/2\sigma^2} dk, \qquad (1.21)$$

where $P(k)dk$ is the probability of finding a value of k between $k + dk$, $\mu = \langle k \rangle = np$, and $\sigma^2 = npq$; see Eq. A.27. This is the Gaussian or normal distribution. By substituting $x = (2k - n)\delta$, $dx = 2\delta dk$, $p = q = 1/2$, $t = n/\tau$, and $D = \delta^2/2\tau$, we obtain

$$P(x)dx = \frac{1}{(4\pi Dt)^{1/2}} e^{-x^2/4Dt} dx, \qquad (1.22)$$

where $P(x)dx$ is the probability of finding a particle between x and $x + dx$. This is the function plotted in Fig. 1.3. The variance of this distribution is $\sigma_x^2 = 2Dt$; its standard deviation is $\sigma_x = (2Dt)^{1/2}$.

The Gaussian or normal distribution is the distribution encountered most frequently in discussions of propagation of errors. It is tabulated, for example, in the *Hand-*

book of Chemistry and Physics, as the "normal curve of error"; see Fig. A.5. About 68% of the area of the curve is within one standard deviation of the origin. Thus, if the root-mean-square displacement of the particles is $(2Dt)^{1/2}$, the chances are 0.32 that a particle has wandered that far or farther. The chances are 0.045 that it has wandered twice as far or farther and 0.0026 that it has wandered three times as far or farther. These numbers are the areas under the curve for $|x| \geq \sigma_x$, $2\sigma_x$, and $3\sigma_x$, respectively.

Visualizing the Gaussian distribution: It is instructive to generate the distributions shown in Fig. 1.3 experimentally. This can be done by layering aqueous solutions of a dye, such as fluorescein or methylene blue, into water. For a first try, layer the dye at the center of a vertical column of water in a graduated cylinder. The dye promptly sinks to the bottom! It does so because it has a higher specific gravity than the surrounding medium. For a second try, match the specific gravity of the medium to the dye by adding sucrose to the water. Now the dye drifts about and becomes uniformly dispersed in a matter of minutes or hours. It does so because there is nothing to stabilize the system against convective flow. Any variation in temperature that increases the specific gravity of regions of the fluid that are higher in the column relative to those that are lower drives this flow. For a final try, layer the dye into a column of water containing more sucrose at the bottom than at the top, i.e., into a sucrose density gradient; a 0-to-2% w/v solution will do. Match the specific gravity of the solution of the dye to that at the midpoint of the gradient and layer it there. Now, patterns of the sort shown in Fig. 1.3 will evolve over a period of many days. The diffusion coefficients of fluorescein, methylene blue, and sucrose are all about

5×10^{-6} cm²/sec. A sucrose gradient $x = 10$ cm high will survive for a period of time of order $t = x^2/2D = 10^7$ sec, or about 4 months. The dye will generate a Gaussian distribution with a standard deviation $\sigma_x = 2.5$ cm in a time $t = \sigma_x^2/2D \simeq 6 \times 10^5$ sec, or in about 1 week. Try it!

It is evident from this experiment that diffusive transport takes a long time when distances are large. Here is another example: The diffusion coefficient of a small molecule in air is about 10^{-1} cm²/sec. If one relied on diffusion to carry molecules of perfume across a crowded room, delays of the order of 1 month would be required. Evidently, the makers of scent owe their livelihood to close encounters, wind, and/or convective flow.

Chapter 2

Diffusion: Macroscopic Theory

Fick's equations

Most discussions of diffusion start with Fick's equations, differential equations that describe the spatial and temporal variation of nonuniform distributions of particles. I find it more illuminating to derive these equations from the model of the random walk. Suppose we know the number of particles at each point along the x axis at time t, as shown in Fig. 2.1. How many particles will move across unit area in unit time from the point x to the point $x + \delta$? What is the net flux in the x direction, J_x? At time $t + \tau$, i.e., after the next step, half the particles at x will have stepped across the dashed line from left to right, and half the particles at $x + \delta$ will have stepped across the dashed line from right to left. The net number crossing to the right will be

$$-\frac{1}{2}[N(x + \delta) - N(x)].$$

To obtain the net flux, we divide by the area normal to the
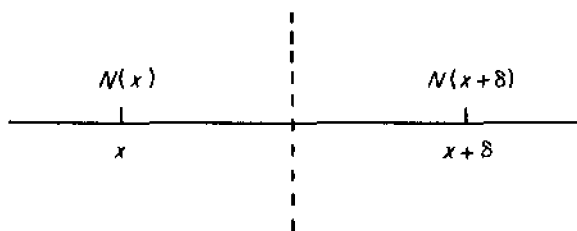
Fig. 2.1. At time t, there are $N(x)$ particles at position x, $N(x + \delta)$ particles at position $x + \delta$. At time $t + \tau$, half of each set will have stepped to the right and half to the left.

x axis, A, and by the time interval, τ,

$$J_x = -\frac{1}{2} [N(x + \delta) - N(x)]/A\tau.$$

Multiplying by δ^2/δ^2 and rearranging, we obtain

$$J_x = -\frac{\delta^2}{2\tau}\frac{1}{\delta}\left[\frac{N(x + \delta)}{A\delta} - \frac{N(x)}{A\delta}\right].$$

The quantity $\delta^2/2\tau$ is the diffusion coefficient, D. $N(x + \delta)/A\delta$ is the number of particles per unit volume at the point $x + \delta$, i.e., the concentration $C(x + \delta)$. $N(x)/A\delta$ is the concentration $C(x)$. Therefore,

$$J_x = -D\frac{1}{\delta}[C(x + \delta) - C(x)].$$

But δ is very small. In the limit $\delta \rightarrow 0$, by the definition of a partial derivative, as explained in Appendix B, we obtain

$$J_x = -D\frac{\partial C}{\partial x}. \qquad (2.1)$$

This is Fick's first equation. It states that the net flux (at x and t) is proportional to the slope of the concentration function (at x and t); the constant of proportionality is $-D$. If the particles are uniformly distributed, the slope is 0, i.e., $\partial C/\partial x = 0$, and $J_x = 0$. If J_x is 0, the distribution will not change with time; the system is at equilibrium. If the slope is constant, i.e., if $\partial C/\partial x$ is constant, J_x is constant. This occurs when C is a linear function of x, as shown in Fig. 2.2. In practice, a gradient of this kind can be maintained only if there is a source for particles at one point and an adsorber for particles at another, e.g., in a pipe connecting reservoirs held at fixed concentrations C_1 and C_2.
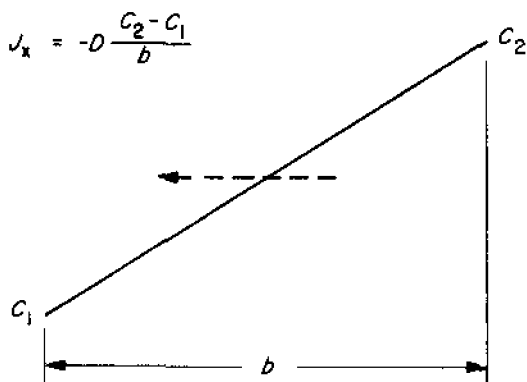
$$J_x = -D \frac{C_2 - C_1}{b}$$

Fig. 2.2. The flux due to a linear concentration gradient $(C_2 - C_1)/b$. There is a net movement of particles from right to left, solely because there are more particles at the right than at the left.

When we derived Eq. 1.10, we defined $D = \delta^2/2\tau$. The reason for the $1/2$ is now clear; it makes Fick's first equation more tidy. Note that if C is expressed in particles/cm³, J_x is in particles/cm²sec. If C is expressed in moles/cm³, J_x is in moles/cm²sec.

Fick's second equation follows from the first, provided that the total number of particles is conserved, i.e., that the particles are neither created nor destroyed. Consider the box shown in Fig. 2.3. In a period of time τ, $J_x(x)A\tau$ particles will enter from the left and $J_x(x + \delta)A\tau$ particles will leave from the right. The volume of the box is $A\delta$. If particles are neither created nor destroyed, the number of particles per unit volume in the box must increase at the rate

$$\frac{1}{\tau}[C(t + \tau) - C(t)] = -\frac{1}{\tau}[J_x(x + \delta) - J_x(x)]A\tau/A\delta$$

$$= -\frac{1}{\delta}[J_x(x + \delta) - J_x(x)].$$
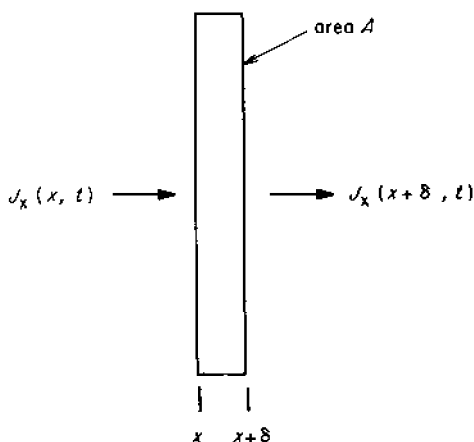
Fig. 2.3. Fluxes through the faces of a thin box extending from position x to position $x + \delta$. The area of each face is A. The faces are normal to the x axis.

In the limit $\tau \to 0$ and $\delta \to 0$, this means that

$$\frac{\partial C}{\partial t} = -\frac{\partial J_x}{\partial x}, \qquad (2.2)$$

or, given Eq. 2.1, that

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial x^2}. \qquad (2.3)$$

This is Fick's second equation. It states that the time rate of change in concentration (at x and t) is proportional to the curvature of the concentration function (at x and t); the constant of proportionality is D. If the slope is constant, $\partial^2 C/\partial x^2 = 0$, and the concentration is stationary: just as many particles diffuse in from the region of higher concentration as diffuse out to the region of lower concentration. Eq. 2.3 tells us how a nonuniform distribution of particles will redistribute itself in time. If we know the initial distribution and other boundary conditions, we can figure out all later distributions.

In three dimensions we have $J_x = -D\,\partial C/\partial x$, $J_y = -D\,\partial C/\partial y$, and $J_z = -D\,\partial C/\partial z$. These are components of a flux vector,

$$\mathbf{J} = -D \text{ grad } C. \qquad (2.4)$$

The concentration changes with time as

$$\frac{\partial C}{\partial t} = D\,\nabla^2 C, \qquad (2.5)$$

where ∇^2 is the three-dimensional Laplacian, $\partial^2/\partial x^2 + \partial^2/\partial y^2 + \partial^2/\partial z^2$.

If the problem is spherically symmetric, the flux is radial,

$$J_r = -D\,\partial C/\partial r, \qquad (2.6)$$

and

$$\frac{\partial C}{\partial t} = D\,\frac{1}{r^2}\,\frac{\partial}{\partial r}\left(r^2\,\frac{\partial C}{\partial r}\right). \qquad (2.7)$$

Time-dependent solutions to Fick's equations

One way to find solutions to Fick's equations is to look them up! An excellent source is Carslaw and Jaeger (1959), a book dealing with the conduction of heat in solids. The heat equation has the same form as the diffusion equation. In the notation of Carslaw and Jaeger,

$$\frac{\partial \nu}{\partial t} = \kappa\,\nabla^2\nu,$$

where ν is the temperature and κ is the thermal diffusivity. So, take their results and read C for ν and D for κ. Sources that do not require such translation include Crank (1975) and Jost (1960). But this strategy requires luck. If you happen to find a discussion of just the problem that you

are trying to solve, well and good. If not, you will soon be lost in a morass of complex equations. Here are some "trivial" examples.

Diffusion from a micropipette: A micropipette filled with an aqueous solution of a green fluorescent dye is inserted into a large body of water. At time $t = 0$, particles of the dye are injected into the water at the rate i per sec for an infinitesimal period of time dt. The total number of particles injected is $N = idt$. With these boundary conditions, Eq. 2.7 has the solution

$$C(r,t) = \frac{N}{(4\pi Dt)^{3/2}} e^{-r^2/4Dt}. \qquad (2.8)$$

This is a three-dimensional Gaussian distribution; compare Eq. 1.22. Looking through a microscope, one sees the sudden appearance of a green spot that spreads rapidly outward and fades away. The concentration remains highest at the tip of the pipette, but it decreases there as the three-halves power of the time. An observer at radius r sees a wave that peaks at $t = r^2/6D$ at a concentration $C = 0.0736\,N/r^3$. He finds that the concentration rises most rapidly at time $t = r^2/16.325D$ at a rate $\partial C/\partial t = 1.054\,ND/r^5$.

The wave due to a pulse of length t_0 can be found by integrating Eq. 2.8 with respect to time. For $t \leq t_0$,

$$C(r,t) = \frac{i}{4\pi Dr} \operatorname{erfc} \frac{r}{(4Dt)^{1/2}}, \qquad (2.9)$$

where erfc x is the error function complement, $1 - \operatorname{erf} x$, and erf x is the error function, defined by the integral

$$\operatorname{erf} x = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2}\, du. \qquad (2.10)$$

This function is tabulated, just like sin x or cos x; see, for

example, Chapter 7 of Abramowitz and Stegun (1972) or Appendix II of Carslaw and Jaeger (1959). Note that erf $0 = 0$, erf $\infty = 1$, and erf $(-x) = -$erf x. If the pulse is long enough, the concentration approaches the steady-state value

$$C(r,t) = \frac{i}{4\pi Dr}. \tag{2.11}$$

For $t > t_0$,

$$C(r,t) = \frac{i}{4\pi Dr}\left\{ \text{erfc}\,\frac{r}{(4Dt)^{1/2}} - \text{erfc}\,\frac{r}{[4D(t - t_0)]^{1/2}} \right\}. \tag{2.12}$$

Figure 2.4 shows the concentration observed at $r = 10^{-3}$ cm for a pulse of constant injection rate i and length $t_0 = 1$ sec for particles of diffusion coefficient $D = 10^{-5}$, 3×10^{-6}, 10^{-6}, 3×10^{-7}, 10^{-7}, and 3×10^{-8} cm^2/sec. If the diffusion coefficient is large, the particles diffuse beyond the observer while the pulse is still on, and the peak concentration is given by Eq. 2.11. If the diffusion coefficient is small, the events occur on a time scale that is long compared to the length of the pulse, and the peak concentration approaches $C = 0.0736\,it_0/r^3$, as required by Eq. 2.8. For other examples of diffusion from constrictions, see Jaeger (1965).

Diffusion in a pipe: In principle, one could measure diffusion coefficients with experiments of the kind illustrated in Fig. 2.4, but in practice it is easier to work in one dimension and to follow the spread of a narrow band of particles, as shown in Fig. 1.3. Alternatively, one can expose a column of solution at concentration C_0 to one at concentration 0 and watch the migration of particles from one to the other. In this case, the initial conditions are
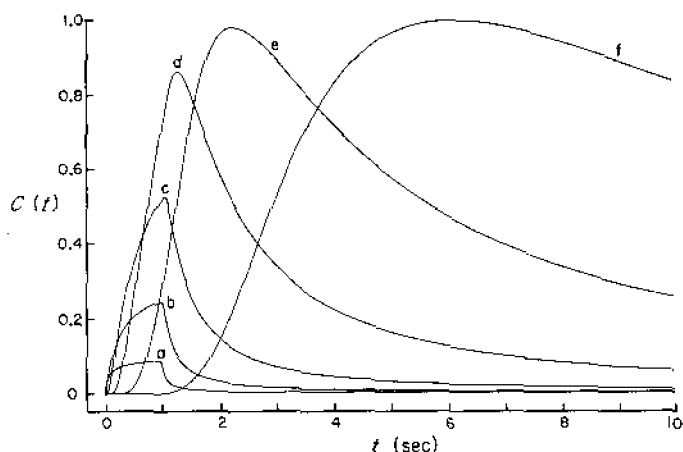
Fig. 2.4. Concentration (in arbitrary units) as a function of time at a distance $r = 10^{-3}$ cm from a point-source in an infinite medium emitting particles at a constant rate from $t = 0$ to $t_0 = 1$ sec, for particles with diffusion coefficient, D(in cm²/sec): (a) 10^{-5}, (b) 3×10^{-6}, (c) 10^{-6}, (d) 3×10^{-7}, (e) 10^{-7}, and (f) 3×10^{-8}. See Eqs. 2.9 and 2.12.

$C = C_0$ for $x > 0$ and $C = 0$ for $x < 0$, and Eq. 2.3 has the solution

$$C(x,t) = \frac{C_0}{2}\left[1 + \text{erf}\frac{x}{(4Dt)^{1/2}}\right]. \qquad (2.13)$$

Since erf $0 = 0$, Eq. 2.13 implies that the concentration of particles at $x = 0$ falls abruptly to $C_0/2$ and remains at that value. This behavior is shown in Fig. 2.5. By taking derivatives of $C(x,t)$ with respect to x or t, we obtain

$$\frac{\partial C}{\partial x} = \frac{C_0}{(4\pi Dt)^{1/2}}\, e^{-x^2/4Dt} \qquad (2.14)$$

and

$$\frac{\partial C}{\partial t} = -\frac{x}{2t}\frac{\partial C}{\partial x}. \qquad (2.15)$$

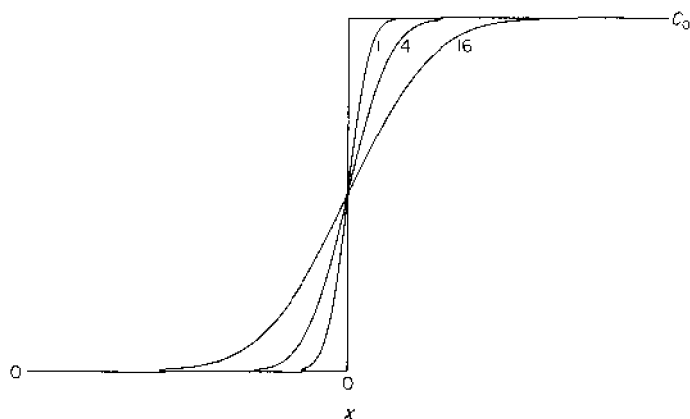Equation 2.14 is similar to Eq. 1.22. Thus, D can be mea-

Fig. 2.5. Concentration as a function of position at times $t = 0, 1, 4$, and 16 for diffusion from a column of liquid initially containing particles at a concentration C_0 (right) into a column of liquid initially devoid of particles (left). The horizontal axis in this figure is drawn on the same scale as that of Fig. 1.3, which shows $\partial C/\partial x$. See Eqs. 2.13–2.15.

sured if one measures C as a function of space and/or time. A numerical solution to a similar problem is given in Appendix B, Fig. B.3.

Steady-state solutions to Fick's equations

If sources and adsorbers are present, the final distribution of particles will not be uniform; instead, the concentration will approach a steady-state value that is higher near sources, lower near adsorbers. In this limit, $\partial C/\partial t = 0$, and Eq. 2.5 reduces to

$$\nabla^2 C = 0. \tag{2.16}$$

For problems with spherical symmetry, Eq. 2.7,

$$\frac{1}{r^2} \frac{d}{dr} \left(r^2 \frac{dC}{dr} \right) = 0. \tag{2.17}$$

We already have seen the steady-state solutions for diffusion in one dimension from a plane at concentration C_2 to a plane at concentration C_1, Fig. 2.2, and for diffusion in three dimensions from a continuous point-source in an infinite medium, Eq. 2.11. Here are some other examples.

Diffusion to a spherical adsorber: Consider a spherical adsorber of radius a in an infinite medium, as shown in Fig. 2.6. Every particle reaching the surface of the sphere is gobbled up, so the concentration at $r = a$ is 0. The concentration at $r = \infty$ is C_0. With these boundary conditions, Eq. 2.17 has the solution

$$C(r) = C_0\left(1 - \frac{a}{r}\right). \qquad (2.18)$$

The flux, Eq. 2.6, is

$$J_r(r) = -DC_0\frac{a}{r^2}. \qquad (2.19)$$

The net migration of molecules is radially inward, as shown by the dashed arrows in Fig. 2.6. The particles are
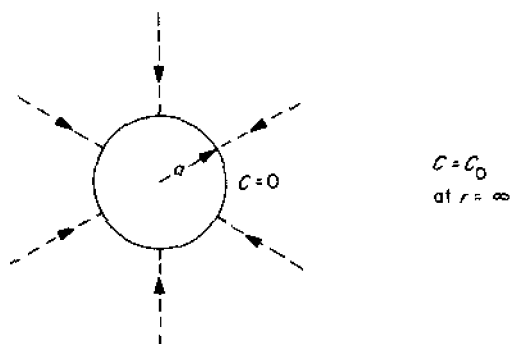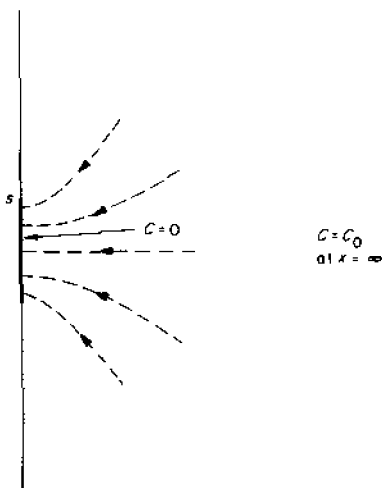

Fig. 2.6. A spherical adsorber of radius a in an infinite medium containing particles at an initial concentration C_0. The dashed arrows are lines of flux.

adsorbed by the sphere at a rate equal to the area, $4\pi a^2$, times the inward flux, $-J_r(a)$:

$$I = 4\pi DaC_0. \tag{2.20}$$

If C_0 is expressed in particles/cm^3, I is in particles/sec. We will refer to this adsorption rate, I, as a diffusion current. Note that this current is proportional not to the area of the sphere but to its radius. As the radius, a, increases, the area increases as a^2, but the concentration gradient, to which the flux is proportional, decreases as $1/a$.

Diffusion to a disk-like adsorber: Next, consider a disk-shaped adsorber of radius s in a semi-infinite medium, as shown in Fig. 2.7. Every particle reaching the surface of the disk is gobbled up, so the concentration at the disk is 0. The concentration at $x = \infty$ is C_0. This problem is cylindrically symmetric rather than spherically sym-
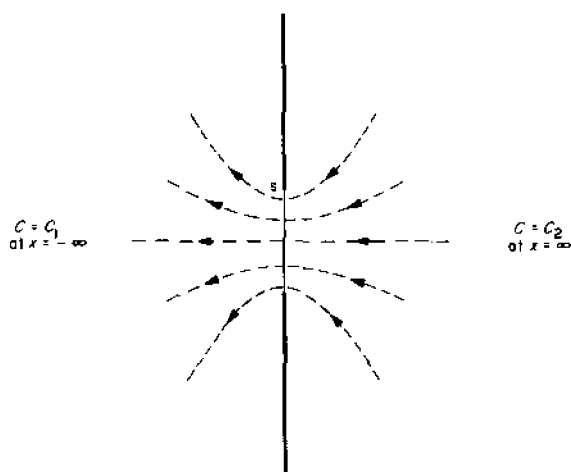

Fig. 2.7. A disk-like adsorber of radius s on one edge of a semi-infinite medium, $x \geq 0$, containing particles at an initial concentration C_0. The dashed arrows are lines of flux.

metric, so the mathematics is not so easy; see, for example, p. 42 of Crank (1975). But the answer turns out to be simple. The diffusion current is

$$I = 4DsC_0. \qquad (2.21)$$

Fig. 2.8. A circular aperture of radius s in a nonadsorbing barrier separating two semi-infinite media, $x < 0$ and $x \geq 0$, containing particles at initial concentrations $C_2 > C_1$. The dashed arrows are lines of flux.

An analogous problem, illustrated in Fig. 2.8, involves diffusion through a circular aperture of radius s in a non-adsorbing barrier separating two semi-infinite media. The concentration at $x = -\infty$ is C_1 and the concentration at $x = \infty$ is C_2. The current through the aperture is

$$I_{2,1} = 2Ds(C_2 - C_1). \qquad (2.22)$$

These currents are proportional not to the area of the disk (or aperture) but to its radius. As the radius, s, increases, the area increases as s^2, but the concentration gradient, to which the flux is proportional, decreases as $1/s$.

Diffusion to an ellipsoidal adsorber: Next, consider a cigar-shaped adsorber, an ellipsoid of revolution with semi-axes $a > b = c$. The concentration at the surface of the ellipsoid is 0, and the concentration at $r = \infty$ is C_0. If the ellipsoid is relatively thin, in particular, if $a^2 \gg b^2$, the diffusion current is

$$I = 4\pi DaC_0/\ln(2a/b), \qquad (2.23)$$

where ln denotes the natural logarithm. This current is smaller than that found for a sphere of radius a given in Eq. 2.20 by the factor $\ln(2a/b)$. This factor is not as large as one might expect. For example, if $b = 10^{-2}a$, $\ln(2a/b) = 5.3$; if $b = 10^{-4}a$, $\ln(2a/b) = 9.9$. Once again, the current is proportional not to the area of the adsorber but roughly to its length.

Appeal to an electrical analogue: The time-independent diffusion equation, Eq. 2.16, is analogous to Laplace's equation for the electrostatic potential in charge-free space. As explained elsewhere (Berg and Purcell, 1977), this implies that the diffusion current to an isolated adsorber of any size and shape can be written as

$$I = 4\pi DcC_0, \qquad (2.24)$$

where c is the electrical capacitance (in cgs units of centimeters) of an isolated conductor of that size and shape. Since the electrical capacitances of a variety of conductors have been worked out, Eq. 2.24 can save some labor. For example, Eq. 2.23 was obtained from an expression for the electrical capacitance of a conducting ellipsoid [by using formula 195.02 of Dwight, 1961, to evaluate the integral 5.02 (4) of Smythe, 1950, and converting from mks to cgs units by multiplying by $1/4\pi\epsilon$]. Smythe used the same integral in another limit to compute the capacitance of a conducting disk, which led us to Eq. 2.21. We

could have derived Eq. 2.20 in a similar fashion, but it was more instructive, given the spherical symmetry, to solve the diffusion equation directly.

Diffusion to N disk-like adsorbers on the surface of a sphere: Given that diffusion currents to spheres, disks, and prolate ellipsoids of similar size are roughly equal, it seems likely that diffusion currents to other adsorbers of similar size should be roughly equal. This turns out to be true, even for nonadsorbing objects sprinkled with small adsorptive patches. Suppose N disk-like adsorbers, each of radius s, are distributed over the surface of an otherwise impenetrable nonadsorbing sphere of radius $a \gg s$, as shown in Fig. 2.9. The concentration at $r = \infty$ is C_0.
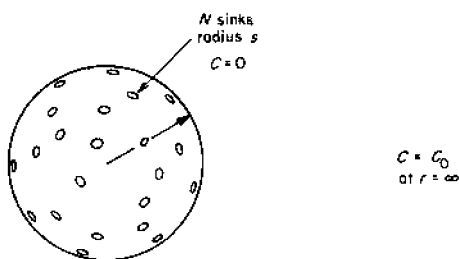
Fig. 2.9. An impenetrable nonadsorbing sphere of radius a covered with N disk-like adsorbers, each of radius s, in an infinite medium containing particles at an initial concentration C_0.

This is a reasonable model for N chemoreceptors or N transport proteins on the surface of a cell. How does the total adsorption rate vary with N? If N is very small, two adsorbers should do twice as well as one, so the rate should increase as $4DNsC_0$, Eq. 2.21. But when N is very large, almost the entire surface of the sphere is adsorbing, and the rate should approach $4\pi DaC_0$, Eq. 2.20. What happens in between? In this regime, the distance between
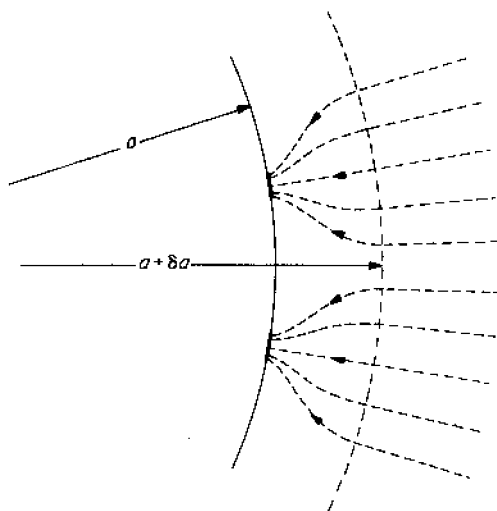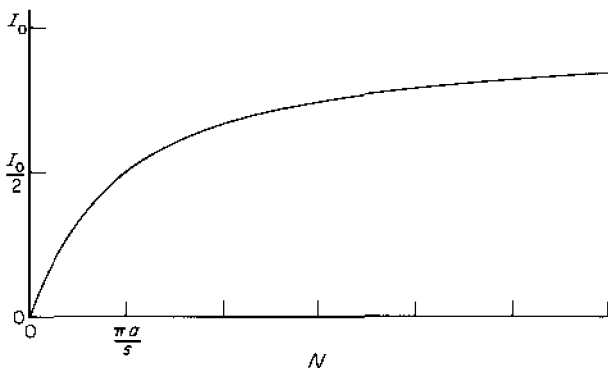
Fig. 2.10. Two disk-like adsorbers of the sphere shown in Fig. 2.9. The dashed arrows are lines of flux. These lines become radial at a distance δa from the surface of the sphere, a distance roughly equal to the distance between adjacent adsorbers.

receptors is large compared to their radius but small compared to the radius of the cell, as shown in Fig. 2.10. The lines of flux are radial for $r > a + \delta a$ (as in Fig. 2.6) but converge on the adsorbers for $a < r < a + \delta a$ (as in Fig. 2.7). Evidently, the concentration at $r = a + \delta a$ is constant at some intermediate value between 0 and C_0.

This problem is formally equivalent to one in electricity in which current flows through a medium of finite resistivity to N conductive patches on an insulating sphere, with the medium a large distance away at potential V_0 and the patches at potential 0. The concentration, C, is an analogue of the potential, V. In the electrical case, we have Ohm's law, which states that the current through a resistor is equal to the potential drop across its terminals divided by its resistance. For steady-state diffusion, we have $I = C/R$, where I is the diffusion current, C is the concentration difference, and R is the diffusion resistance.

Fig. 2.11. An electrical model for the problem of N adsorbers of radius s on the surface of a sphere of radius a, the problem illustrated in Figs. 2.9 and 2.10.

By appealing to this relation, we note that the diffusion resistance for the adsorbing sphere, Eq. 2.20, is $R_a = 1/4\pi Da$, and that the diffusion resistance for the disk-like adsorber, Eq. 2.20, is $R_s = 1/4Ds$. These resistances are shown as discrete elements in Fig. 2.11. The total resistance of this circuit is $R = R_{a+\delta a} + R_s/N = 1/[4\pi D(a + \delta a)] + 1/4DNs$. Since $\delta a \ll a$, $R \approx 1/4\pi Da + 1/4DNs = (1/4\pi Da)(1 + \pi a/Ns) = R_a(1 + \pi a/Ns)$. We conclude that the diffusion resistance for a sphere covered with N disk-like adsorbers is larger than the diffusion resistance for the completely adsorbing sphere by a factor $1 + \pi a/Ns$. The diffusion current is smaller by the same factor:

$$\frac{I}{I_0} = \frac{1}{1 + \pi a/Ns}, \tag{2.25}$$

where $I_0 = 4\pi DaC_0$, Eq. 2.20. I is plotted as a function of

Fig. 2.12. The diffusion current, or rate of adsorption, I, as a function of the number of adsorbers, N, for disk-like adsorbers of radius s on the surface of a sphere of radius a. See Eq. 2.25. I_0 is the diffusion current for the completely adsorbing sphere, Eq. 2.20.

N in Fig. 2.12. If N is small, the rate increases as $4DNsC_0$. If N is large, the rate approaches $4\pi DaC_0$. This is the asymptotic behavior that we predicted.

Note that the diffusion current reaches half of its maximum value for $N = \pi a/s$. This number is surprisingly small. Consider a spherical cell of radius $a = 5$ μm equipped with N transport proteins, each with a binding site of radius $s = 10$ Å. This cell can adsorb substrate molecules at half the rate of a cell completely covered by such sites if $N = \pi a/s = 15{,}700$. Only a small fraction of the surface of the cell need be specifically adsorbing, namely $N\pi s^2/4\pi a^2 = 1.6 \times 10^{-4}$. The distance between neighboring sites is about $(4\pi a^2/N)^{1/2} = 0.14$ μm, i.e., about 140 times the site radius. Thus, many hundreds of different transport (or receptor) systems can be accommodated on the surface of the cell, each adsorbing particles of a specific kind with an efficiency approaching that of a cell whose entire surface is dedicated to one such task. For other discussions of this problem, see Berg and Purcell (1977) and DeLisi and Wiegel (1981).

It should be stressed that the electrical analogy used in the derivation of Eq. 2.25 does not extend to time-dependent diffusion; it only applies in the steady-state. The flux is proportional to the concentration gradient, but individual particles are not moving like electrons through a wire; they are moving strictly at random. The same restriction applies to the analogy leading to Eq. 2.24.

Diffusion through N circular apertures in a planar barrier: Consider a system in which two plates a distance b apart are held at concentrations C_1 and C_2, as shown in
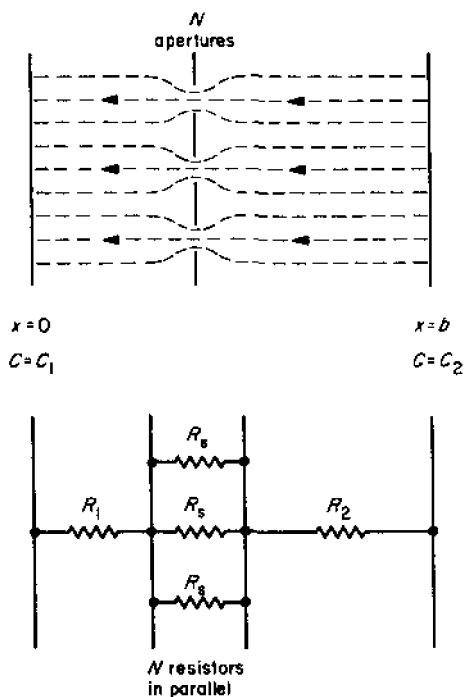
Fig. 2.13. A barrier with $N = nA$ apertures, each of radius s, separating two plates of area A held at concentrations C_1 and C_2 (top), and an electrical model for this system (bottom). See Eq. 2.27.

Fig. 2.2. Let the system have a cross-sectional area A. The diffusion current from one plate to the other is

$$I_{2,1} = DA(C_2 - C_1)/b. \qquad (2.26)$$

Now insert somewhere between the two plates a thin barrier containing N apertures, each of radius $s \ll b$, as shown in Fig. 2.13. What is the diffusion current in the new steady state? The diffusion resistance for one aperture is $R_s = 1/2Ds$, Eq. 2.22. The diffusion resistance of the medium between the plates is $R_1 + R_2 \simeq R_{2,1} = b/DA$, Eq. 2.26. The diffusion resistance of the entire system is $R_1 + R_2 + R_s/N \simeq R_{2,1} + R_s/N = b/DA + 1/2DNs \simeq (b/DA)(1 + 1/2nsb)$, where n is the number of apertures per unit area. It follows that the barrier decreases the diffusion current by a factor

$$\frac{I}{I_{2,1}} = \frac{1}{1 + 1/2nsb}. \qquad (2.27)$$

If this ratio is written as $b/(b + 1/2ns)$, it is evident from the denominator that the effect of the barrier is equivalent to the addition of an extra diffusion path of length $1/2ns$.

The diffusion current reaches half of its maximum value for $n = 1/2sb$. This number is surprisingly small. Consider two cubical cells 10 μm on a side joined on one face. Assume that the membranes comprising this face are of negligible thickness and penetrated by N pores, each of radius 50 Å. How many pores are required for the diffusion current between the cells to be half as large as it would be were the barrier not there? Given $N/A = 1/2sb$, with $A = 10^{-6}$ cm^2, $s = 5 \times 10^{-7}$ cm, and $b = 2 \times 10^{-3}$ cm, we find $N = 500$. Only a small fraction of the surface of the barrier need be penetrated, namely $N\pi s^2/A = 3.9 \times 10^{-4}$. The distance between neighboring pores is about $(A/N)^{1/2} = 0.45$ μm, i.e., about 90 times the pore radius.

A similar problem involves the diffusion of gases through the stomata of leaves. In this case, the concentration is clamped at 0 inside the leaf, just to the left of the barrier; so $R_1 = 0$, and the diffusion resistance for each aperture is the same as that for the disk-like adsorber, $R_s = 1/4Ds$. Thus, the stomata add an extra diffusion path of length $1/4ns$, and the diffusion current reaches half of its maximum value for $n = 1/4sb$. This analysis is valid only for the boundary layer near the surface of the leaf, i.e., in still air. For a discussion of this problem, see Chapter 3 of Meidner and Mansfield (1968).

Chapter 3

Diffusion to Capture

In Chapter 2 we compared the steady-state rates of uptake of particles by completely adsorbing objects of various shapes, such as spheres, disks, and ellipsoids. We found that these rates are proportional to the linear dimension of the object rather than to its area, and that the shape is not of crucial importance. For example, the diffusion currents to a sphere of radius a, a two-sided disk of radius a, and an ellipsoid of revolution of length $2a$ and radius $a/10$ fall in the ratios 1 to 0.64 to 0.33. We also found that a reflecting object of a given size and shape sparsely covered with adsorbent patches is nearly as good at sequestering particles as a completely adsorbing object of the same size and shape. From a microscopic point of view, both sets of results reflect the fact that a diffusing particle that finds itself in a given region of space is destined, by that very circumstance, to wander around that region for a time, probing it rather thoroughly before wandering away for good. A particle that finds itself in a spherical space of radius a has a fair chance of blundering into a disk or an ellipsoid inscribed in that space. A particle that bumps into a reflecting object has a fair chance of hitting an adsorbent patch nearby on its surface. This property of the random walk is apparent in the two-dimensional simulation shown in Fig. 1.4. That particle wandered about in some regions at great length but ignored others completely. It is very difficult to get a feel for this mindless ramble from a casual study of the diffusion equation (Eq. 2.5). In this chapter, we try to make these ideas more quantitative by working out some probabilities of capture and mean times to capture.

Probability of capture

Suppose a particle is released near a spherical adsorber of radius a at a point $r = b > a$? What is the probability that the particle will be adsorbed at $r = a$ rather than wander away for good? Naively, one might think that as b increases, this probability would decrease as $1/b^2$, as would be expected if the probability of capture depended on the solid angle subtended by the adsorber at the point of release. In fact, the probability decreases only as $1/b$.

To see this, consider a spherical shell source of radius b between a spherical adsorber of radius a and a spherical shell adsorber of radius c, as shown in Fig. 3.1. The concentration rises from 0 at $r = a$ to a maximum value C_m at $r = b$ and then falls again to 0 at $r = c$. With these
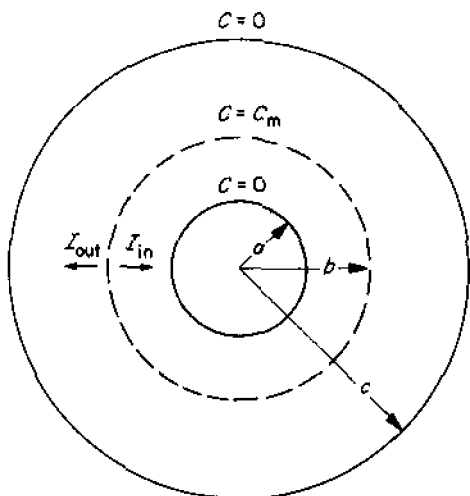
Fig. 3.1. A spherical shell source, radius b, between a spherical adsorber of radius a and a spherical shell adsorber of radius c. Particles released at $r = b$ move inward and are adsorbed at $r = a$ at rate I_{in} or move outward and are adsorbed at $r = c$ at rate I_{out}. Their steady-state concentration rises from 0 at $r = a$ to C_m at $r = b$ and then falls again to 0 at $r = c$. See Eqs. 3.1–3.5.

boundary conditions, Eq. 2.17 has the solution

$$C(r) = \begin{cases} \dfrac{C_m}{1 - a/b}\left(1 - \dfrac{a}{r}\right) & a \le r \le b \\[2ex] \dfrac{C_m}{c/b - 1}\left(\dfrac{c}{r} - 1\right) & b \le r \le c. \end{cases} \quad (3.1)$$

The radial flux, Eq. 2.6, is

$$J_r(r) = \begin{cases} -\dfrac{DC_m}{1 - a/b}\dfrac{a}{r^2} & a \le r \le b \\[2ex] \dfrac{DC_m}{c/b - 1}\dfrac{c}{r^2} & b \le r \le c. \end{cases} \quad (3.2)$$

Thus, the diffusion current from the spherical shell source to the inner adsorber is

$$I_{in} = 4\pi DC_m \frac{a}{1 - a/b}, \quad (3.3)$$

and the diffusion current from the spherical shell source to the outer adsorber is

$$I_{out} = 4\pi DC_m \frac{c}{c/b - 1}. \quad (3.4)$$

The ratio

$$\frac{I_{in}}{I_{in} + I_{out}} = \frac{a(c - b)}{b(c - a)} \quad (3.5)$$

is the probability that a particle released at $r = b$ will be adsorbed at $r = a$. In the limit $c \to \infty$, this probability is just a/b. This is the probability of capture for the sphere of radius a immersed in an infinite medium. As b increases, this probability decreases as $1/b$, as predicted.

An individual particle has no way of knowing that an adsorber is present unless it blunders into it. Thus, Eq. 3.5 gives the probability that a particle released at $r = b$ reaches $r = a$ before diffusing as far as $r = c$, even when the adsorbers are not there. Suppose a particle is released at $r = b$ near the surface of a reflecting sphere of radius a. How many times, on the average, does it reach the surface of the sphere and return to $r = b$ before wandering away for good? The probability that the particle visits the sphere at least once before wandering away for good is $p = a/b$. The probability that it visits the sphere once, returns to $r = b$, and then wanders away for good is $p(1 - p)$. The probability that the particle makes this round trip twice and then wanders away for good is $p^2(1 - p)$. The probability that it does so n times is $p^n(1 - p)$. Therefore, the mean number of round trips is

$$\langle n \rangle = \sum_{n=0}^{\infty} np^n(1 - p). \tag{3.6}$$

By factoring out $p(1 - p)$ and noting that $1 + 2p + 3p^2 + \cdots = (1 - p)^{-2}$, we find

$$\langle n \rangle = p/(1 - p) = a/(b - a). \tag{3.7}$$

It follows that a particle close to the surface of the sphere makes a large number of trips from b to a and back again before wandering away for good; the number increases without limit as b approaches a. In the process, the particle probes the sphere at a large number of points, some far enough apart to allow it to find adsorbent patches, even when these patches cover only a small fraction of the surface. For a derivation of Eq. 2.25 from this point of view, see pp. 196–198 of Berg and Purcell (1977).

What is the probability that a particle released at $r = b$ near the surface of an adsorbing sphere of radius a wan-

ders as far as $r = c$ or farther before being captured? The probability that a particle is adsorbed at $r = a$ after diffusing to an arbitrary radius is a/b. The probability that a particle is adsorbed at $r = a$ without diffusing as far as $r = c$ is given by Eq. 3.5. The probability that a particle is adsorbed at $r = a$ after diffusing as far as $r = c$ or farther is the difference of these two probabilities, namely, $a/b - a(c - b)/b(c - a) = a(b - a)/b(c - a)$. The fraction of particles that do so is this number divided by a/b, i.e., $(b - a)/(c - a)$. Thus, on the average, if 100 particles are released at $r = 2a$, $(a/2a) \times 100 = 50$ will be adsorbed at $r = a$, and 50 will wander away for good. Of the 50 that are adsorbed at $r = a$, $[(2a - a)/(3a - a)] \times 50 = 25$ will have wandered as far as $r = 3a$ or farther before being captured. One will have wandered as far as $r = 51a$ or farther before being captured.

We noted earlier that a particle executing a random walk has no inkling of its past. These calculations emphasize the fact that it also has no inkling of its future. The behavior of an individual particle is not affected by an adsorber unless, by chance, it happens to bump into it.

The one-dimensional case is shown in Fig. 3.2. The diffusion currents are $I_{left} = DAC_m/a$, and $I_{right} = DAC_m/(b - a)$. The probability that a particle released at $x = a$ will be adsorbed at $x = 0$ is

$$\frac{I_{left}}{I_{left} + I_{right}} = \frac{b - a}{b}. \tag{3.8}$$

In the limit $b \to \infty$, this probability is 1. The average number of trips that a particle makes from $x = a$ to a reflecting barrier at $x = 0$ before reaching $x = b$ is $(b - a)/a$. The probability that a particle released at $x = a$ is adsorbed at $x = 0$ after diffusing as far as $x = b$ or farther is

$1 - (1 - a/b) = a/b$. Thus, on the average, if 100 particles are released at $r = a$, 50 will have wandered as far as $x = 2a$ or farther before being captured. One will have wandered as far as $x = 100a$ or farther before being captured. None of the particles will wander away for good.

Mean time to capture

When a particle is released at position $x = a$, as indicated in Fig. 3.2, how long does it take to blunder into an adsorber at $x = 0$ or $x = b$? If this experiment is repeated many times, what is the mean time to capture, $W(a)$? To find out, we return to the formalism of the random walk, release a particle at position x at time $t = 0$, and allow it to step to the right or to the left a distance δ every τ sec. At time τ, the particle will be at position $x + \delta$ with probability $1/2$, or at position $x - \delta$ with probability $1/2$. The mean times to capture from these positions are $W(x + \delta)$
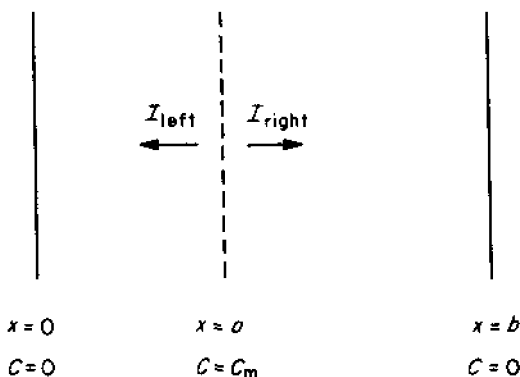
Fig. 3.2. A planar source at $x = a$ between two planar adsorbers at $x = 0$ and $x = b$. The cross-sectional area of the system is A. Particles released at $x = a$ move to the left and are adsorbed at $x = 0$ at rate I_{left} or move to the right and are adsorbed at $x = b$ at rate I_{right}. Their steady-state concentration rises from 0 at $x = 0$ to C_m at $x = a$ and then falls again to 0 at $x = b$. See Eq. 3.8.

and $W(x - \delta)$, respectively. Thus, the expectation value of $W(x)$ is

$$W(x) = \tau + \frac{1}{2}\,[W(x + \delta) + W(x - \delta)]. \quad (3.9)$$

By adding and subtracting $W(x)/2$ and multiplying through by $2/\delta$, we obtain

$$\frac{1}{\delta}\,[W(x + \delta) - W(x)] - \frac{1}{\delta}\,[W(x) - W(x - \delta)]$$

$$+ \frac{2\tau}{\delta} = 0.$$

In the limit of very small δ, by the definition of a derivative, we have

$$\left.\frac{dW}{dx}\right|_x - \left.\frac{dW}{dx}\right|_{x-\delta} + \frac{2\tau}{\delta} = 0.$$

Dividing once more by δ, appealing again to the definition of a derivative, and noting that $2\tau/\delta^2 = 1/D$, we obtain

$$\frac{d^2W}{dx^2} + \frac{1}{D} = 0. \quad (3.10)$$

This differential equation can be solved for W given suitable boundary conditions. At an adsorbing boundary, the mean time to capture is 0, so $W = 0$. At a reflecting boundary, the mean time to capture does not vary with x, so $dW/dx = 0$.

If there are adsorbing boundaries at $x = 0$ and $x = b$, as shown in Fig. 3.2, then $W(0) = W(b) = 0$, and Eq. 3.10 has the solution

$$W(x) = \frac{1}{2D}\,(bx - x^2). \quad (3.11)$$

The mean time to capture a particle released halfway in between, at $x = b/2$, is $b^2/8D$. The mean time to capture a particle released at random anywhere in between $x = 0$ and $x = b$ is given by the average

$$\frac{1}{b} \int_0^b W(x)\, dx, \qquad (3.12)$$

which, on substitution of Eq. 3.11, gives $b^2/12D$.

If there is an adsorbing boundary at $x = 0$ and a reflecting boundary at $x = b$, then $W(0) = 0$, $dW/dx = 0$ at $x = b$, and

$$W(x) = \frac{1}{2D}(2bx - x^2). \qquad (3.13)$$

The mean time to capture a particle released at $x = b/2$ is now $3b^2/8D$, three times longer than before. Sometimes a particle released at $x = b/2$ wanders from $x = b/2$ to $x = b$ and back again before capture at $x = 0$. Eq. 3.8 tells us that the probability for this is $1/2$. Events of this kind raise the mean. The mean time to capture a particle released at random is now $b^2/3D$, four times longer than before.

These results are shown graphically in Fig. 3.3. The average height of each curve is given by the area that it subtends divided by its width, as specified by Eq. 3.12. In the case of two adsorbing boundaries, the largest contributions to the average occur when the particle is released near $x = b/2$; in the case of one adsorbing and one reflecting boundary, the largest contributions occur when the particle is released near $x = b$.

An example of a one-dimensional process of this kind of enormous practical significance is the diffusion of a molecule of repressor along a strand of DNA, in quest of its binding site at the promoter. If the repressor, after
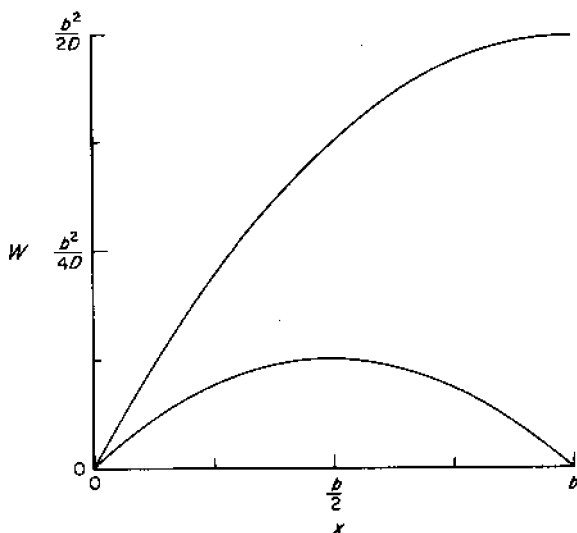
Fig. 3.3. Plots of the mean time to capture for particles released at position x with adsorbing boundaries at $x = 0$ and $x = b$ (lower curve, Eq. 3.11) or with an adsorbing boundary at $x = 0$ and a reflecting boundary at $x = b$ (upper curve, Eq. 3.13). If x is chosen at random, the mean time to capture is $b^2/12D$ or $b^2/3D$, respectively; see Eq. 3.12.

having arrived at a random point on a segment of DNA of length b terminated at one end by the promoter, is adsorbed strongly enough that it cannot desorb but loosely enough that it can move along the DNA with a finite diffusion coefficient D', then its mean time to capture is $b^2/3D'$. Given the surprisingly high rate at which particles can be adsorbed by a thread-like object, Eq. 2.23, it is reasonable to suppose that the repressor actually finds its binding site by such a two-stage mechanism. But, as discussed for an analogous problem in two dimensions (pp. 198–200 of Berg and Purcell, 1977), the importance of this mechanism depends on the strength of the nonspecific binding. Unless it is strong, the repressor can do as well or better by staying in three dimensions.

The equation for the mean time to capture in two or three dimensions is

$$\nabla^2 W + \frac{1}{D} = 0, \tag{3.14}$$

where ∇^2 is the two- or three-dimensional Laplacian. This equation also can be solved by appeal to an electrical analogue; it is Poisson's equation for the potential (W) in a region of uniform charge density $(1/4\pi D)$. For some sample solutions, see Appendix B of Berg and Purcell (1977).

Note, finally, that the logic used in the derivation of Eq. 3.10 can be applied as well to the problem posed at the beginning of this chapter, the probability of capture of a particle at an adsorbing boundary. The recursion relation for the probability of capture, $P(x)$, of a particle released at point x, corresponding to the recursion relation for the mean time to capture, Eq. 3.9, is

$$P(x) = \frac{1}{2} \left[P(x + \delta) + P(x - \delta) \right]. \tag{3.15}$$

This leads to the differential equation

$$\frac{d^2P}{dx^2} = 0. \tag{3.16}$$

The boundary conditions for capture by an adsorber at $x = 0$ rather than at one at $x = b$ are $P(0) = 1$ and $P(b) = 0$. With these boundary conditions, Eq. 3.16 has the solution

$$P(x) = \frac{b - x}{b}, \tag{3.17}$$

which, for $x = a$, is the result obtained earlier from computation of diffusion currents, Eq. 3.8.

In two or three dimensions we have

$$\nabla^2 P = 0, \tag{3.18}$$

where, as before, ∇^2 is the two- or three-dimensional Laplacian. For a particle released near a spherical adsorber of radius a at a point $r = b > a$ the boundary conditions are $P(a) = 1$ and $P(\infty) = 0$. With these boundary conditions, Eq. 3.18 has the solution

$$P(r) = \frac{a}{r}, \tag{3.19}$$

which, for $r = b$, is the result obtained earlier from computation of diffusion currents, a/b. Equation 3.5 follows from the solution of Eq. 3.18 for a spherical adsorber of radius a inside a spherical shell adsorber of radius c with boundary conditions $P(a) = 1$ and $P(c) = 0$.
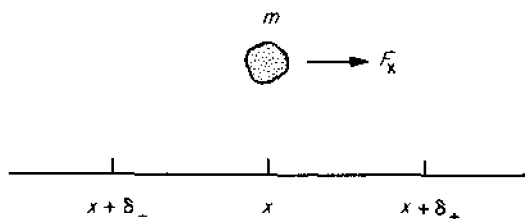
Chapter 4

Diffusion with Drift

The theory of diffusion developed in Chapters 1–3 would be more useful if we had a means of estimating the values of diffusion coefficients from first principles, given the sizes and shapes of the particles of interest. It turns out that this can be done if we compute the velocity at which a particle drifts through the medium when exposed to an externally applied force, such as that due to a gravitational, centrifugal, or electrical field. In practice, the velocity at which the particle moves in response to such a field is infinitesimal when compared to the instantaneous root-mean-square velocity given by Eq. 1.2. This means that the particles diffuse much as they would in the absence of the field, but with a small persistent directional bias, as indicated in Fig. 1.1c.

Random walk with drift

Consider a particle of mass m at position x subjected to an externally applied force, F_x, acting in the $+x$ direction, as shown in Fig. 4.1. In accordance with Newton's second law, the force causes the particle to accelerate uni-

Fig. 4.1. A particle of mass m subjected to an externally applied force F_x while undergoing a one-dimensional random walk.

formly to the right with acceleration $a = F_x/m$. The random walk proceeds as before, according to the rules set down in Chapter 1, with a particle stepping to the right or the left once every τ seconds with an initial velocity $+v_x$ or $-v_x$. A particle starting at position x with an initial velocity $+v_x$ moves in time τ a distance $\delta_+ = v_x\tau + a\tau^2/2$, while a particle starting at position x with an initial velocity $-v_x$ moves in time τ a distance $\delta_- = -v_x\tau + a\tau^2/2$. Since steps to the right and left are equally probable, the average displacement in time τ is $a\tau^2/2$, and the particle drifts to the right with an average velocity

$$v_d = \frac{1}{2}\,a\tau = \frac{1}{2}\frac{F_x}{m}\,\tau. \tag{4.1}$$

It is customary to relate the drift velocity to the applied force by a parameter, f, called the frictional drag coefficient:

$$v_d = \frac{F_x}{f}. \tag{4.2}$$

In our model, $f = 2m/\tau$. Multiplying both the numerator and the denominator of this expression by $(\delta/\tau)^2$ and noting that $v_x = \delta/\tau$ and $D = \delta^2/2\tau$, we find $f = mv_x^2/D$. But by Eq. 1.1, $mv_x^2 = kT$; therefore, $f = kT/D$, or

$$D = \frac{kT}{f}. \tag{4.3}$$

This result, known as the Einstein-Smoluchowski relation, turns out to be very general. It does not depend on any assumptions made about the structure of the particle or the details of its motion, a point to which we will return in Chapter 5. Given Eqs. 4.2 and 4.3, we have a procedure for estimating D. First, apply a force F_x, measure v_d, and use Eq. 4.2 to compute f; then, use Eq. 4.3 to compute D.

A reader who knows more physics might be perturbed by our derivation of Eq. 4.3. Real particles do not step in

synchrony at a fixed interval, move solely in one dimension, or start each step at a fixed velocity. Step intervals, directions, velocities, and lengths continuously vary as the particle exchanges energy with the molecules of the fluid in which it is suspended. In a more rigorous treatment, one worries about the distributions of these quantities and defines a mean collision time—or for a large particle diffusing in a medium of small particles, a directional correlation time—a mean velocity, and a mean free path. The functional dependence of D and f on these parameters is the same as in our model, but some of the numerical coefficients differ. The final result is the same. The essential point is that a particle is accelerated by the externally applied force; it forgets about this acceleration when it exchanges energy with the molecules of the fluid in which it is suspended, and then it is accelerated once again. As a result, the particle drifts through the medium with a velocity proportional to the externally applied force. The constant of proportionality is D/kT. For further discussion of these points, see Chapters 1-43 of Feynman, Leighton, and Sands (1963).

Note, finally, that we could have obtained the same drift velocity, $v_d = (D/kT)F_x$, from a biased random walk, with the step rate, velocity and distance constant, given a probability of stepping in the $+x$ direction $p = 1/2 + F_x\delta/4kT$ and a probability of stepping in the $-x$ direction $q = 1/2 - F_x\delta/4kT$. To see this, use Eqs. 1.17 and 1.18 with $n = t/\tau$, $D = \delta^2/2\tau$. Since the bias is small, $p/q = 1 + F_x\delta/kT$.

Fick's equations for diffusion with drift

If all the particles in a distribution drift in the $+x$ direction at velocity v_d, then the flux at point x must increase by an amount $v_dC(x)$. Thus, Fick's first equation, Eq. 2.1,

becomes

$$J_x = -D \frac{\partial C}{\partial x} + v_d C. \qquad (4.4)$$

The derivation of Fick's second equation, Eq. 2.3, proceeds as before, giving

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v_d \frac{\partial C}{\partial x}. \qquad (4.5)$$

We will use this equation in the general proof of the Einstein-Smoluchowski relation given in Chapter 5.

Viscous drag

If a particle is large compared to the molecules comprising the medium in which it is suspended, it is possible to use the equations of motion of viscous fluids and calculate the frictional drag coefficient. These equations, called the Navier-Stokes equations, become relatively simple when applied to small things moving slowly, i.e., under conditions in which viscous forces are important but inertial forces are not. Viscous forces arise whenever a fluid is sheared, i.e., whenever the velocities of adjacent regions of fluid differ. Shear is generated, for example, when two parallel plates are moved relative to one another, as shown in Fig. 4.2. In this case, the velocity
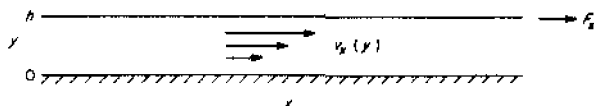
Fig. 4.2. A viscous fluid contained between flat plates at $y = 0$ and $y = h$. The area of each plate is A. The bottom plate is fixed. The top one is propelled to the right by a force in the x direction, F_x. Arrows show the velocity of the fluid relative to the bottom plate at different distances from this plate, y.

profile is linear:

$$v_x(y) = \frac{v_x(h)}{h} y, \qquad (4.6)$$

and the shear, $\partial v_x/\partial y$, does not depend on the position, y. Note that there is no slip at the boundaries; the fluid at the surface of each plate moves at the velocity of that plate. The force, F_x, is balanced by the viscous drag due to the shear:

$$F_x = \eta A \left.\frac{\partial v_x}{\partial y}\right|_{y=h}. \qquad (4.7)$$

This equation provides an operational definition for the coefficient of viscosity, η. The viscous drag is proportional to η, the area of the plate, and the rate of shear of the fluid at the surface of the plate. The units of η can be found from the dimensional equation $[\text{g cm/sec}^2] = [\eta][\text{cm}^2][\text{cm/sec cm}]$, which yields $[\eta] = [\text{g/cm sec}]$, which is called a poise (P). The kinematic viscosity, ν, is η divided by the specific gravity of the medium, ρ; its units are the same as that of the diffusion coefficient, cm^2/sec, which is called a stoke. The viscosity of water at 20°C is 0.01 P, or 1 centipoise (cP). The viscosities of air, water, and glycerol are compared in the table below.

Viscosities of various fluids at different temperatures, in g/cm sec (poise)

	Air	Water	Glycerol (dry)
40°C	1.93×10^{-4}	6.53×10^{-3}	2.83
20°C	1.83×10^{-4}	1.00×10^{-2}	14.1
0°C	1.71×10^{-4}	1.79×10^{-2}	120.

The viscosity of air increases slowly with temperature; it is nearly independent of pressure (data not shown). The

viscosities of the liquids decrease rapidly with temperature. For most liquids encountered in the laboratory, η is a constant at a given temperature; it does not depend on the rate of shear. However, this is not the case for solutions containing molecules with long unbranched chains, such as methylcellulose; see, for example, Berg and Turner (1979). Indeed, some media are so complex that the viscous drag is not even in the direction of flow. Fortunately, we do not encounter these problems with dilute aqueous solutions of globular particles or with substances of low molecular weight.

Flow through a thin rectangular channel: Here is a sample calculation. Consider a channel of length b bounded by flat plates at $y = 0$ and $y = h$, as shown in Fig. 4.3. If a pressure difference ΔP is applied between the left and right ends of this channel, what is the flow profile $v_x(y)$? We can solve this problem using Eq. 4.7, assuming that the width of the channel, w, is so large compared to its height, h, that edge effects can be neglected. Consider a thin layer of fluid extending from y to $y + dy$. The net force due to the pressure difference at the ends of this layer tending to drive the fluid through the channel is $\Delta P w dy$. The net drag due to viscous shear at the bottom and top edges of this layer tending to resist such flow is

$$\eta b w[(\partial v_x/\partial y)_y - (\partial v_x/\partial y)_{y+dy}] = -\eta bw(\partial^2 v_x/\partial y^2)dy.$$
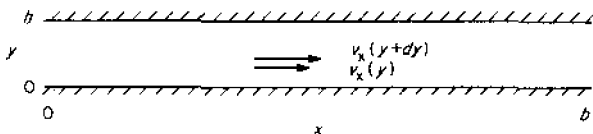
Fig. 4.3. Fluid in a channel between two parallel plates driven from left to right by a pressure gradient $\Delta P/b$. Both plates are fixed. The channel is of height h, length b, and width $w \gg h$.

Thus,

$$\frac{\partial^2 v_x}{\partial y^2} = -\frac{\Delta P}{\eta b}. \tag{4.8}$$

Integrating this equation twice and applying the boundary conditions $v_x(0) = v_x(h) = 0$, we obtain

$$v_x(y) = \frac{\Delta P}{2\eta b}(hy - y^2) = \frac{4v_m}{h^2}(hy - y^2), \tag{4.9}$$

where v_m is the maximum velocity, $\Delta P h^2/8\eta b$. The flow profile is parabolic; the maximum velocity occurs in the middle of the channel at $y = h/2$. The volume of fluid passing through the channel per unit time is

$$w\int_0^h v_x(y)dy = \frac{2}{3}whv_m. \tag{4.10}$$

The average velocity of the fluid is $2v_m/3$.

In its most general form, Eq. 4.8 states that the Laplacian of the velocity is equal to the gradient of the pressure divided by the coefficient of viscosity. This is the basic equation of slow viscous flow; see, for example, Chapter 2 of Landau and Lifshitz (1959).

Flow around a sphere: Viscous flows around small particles are highly regular. Figure 4.4 shows the flow lines around a sphere, radius a, moving to the right through a viscous fluid at constant velocity v_d. The radial and azimuthal components of the velocity of the fluid relative to the sphere are

$$v_r = -v_d \cos\theta\left(1 - \frac{3a}{2r} + \frac{a^3}{2r^3}\right)$$

$$v_\theta = v_d \sin\theta\left(1 - \frac{3a}{4r} - \frac{a^3}{4r^3}\right). \tag{4.11}$$
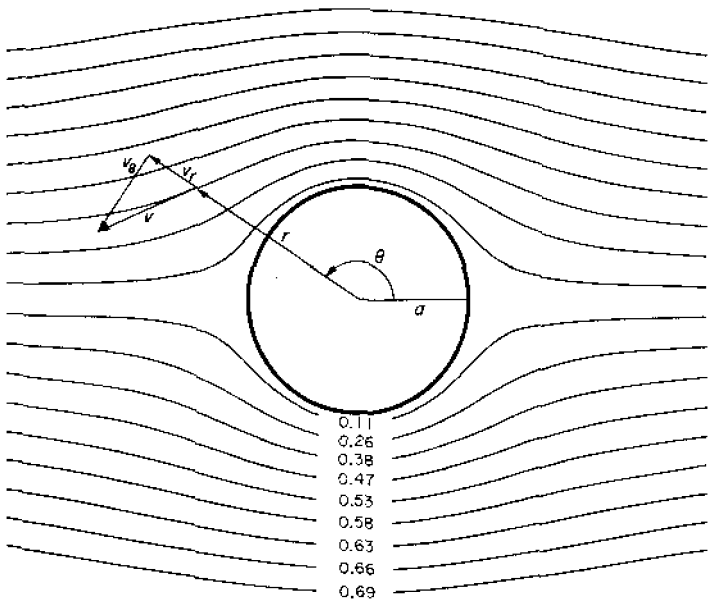
Fig. 4.4. A solid sphere moving at a constant velocity v_d through an incompressible viscous fluid. The fluid moves around the sphere along the flow lines shown. See Eq. 4.11. The numbers on the flow lines at $\theta = -90°$ indicate the magnitudes of v_θ at these points in units of v_d.

Note that both are 0 at the surface of the sphere and that both increase as r increases. The fluid at the surface of the sphere moves with the sphere; the fluid a long distance away does not. The motion of the sphere causes the fluid to shear. Even at the outermost flow line shown in the figure, the fluid is moving 30% as fast as the sphere. The sphere carries fluid with it. Evidently, it must move several diameters before it can shed its local environment.

Stoke's law: A calculation of the net force acting on the sphere yields Stoke's law, which states that the force necessary to drag a sphere of radius a at a velocity v_d through an incompressible, viscous liquid is $6\pi\eta a v_d$.

Given Eqs. 4.2 and 4.3, this implies that

$$f_{sphere} = 6\pi\eta a, \qquad (4.12)$$

and

$$D_{sphere} = \frac{kT}{6\pi\eta a}. \qquad (4.13)$$

For a sphere of radius $a = 10^{-4}$ cm in water at room temperature, $f_{sphere} = 1.9 \times 10^{-5}$ g/sec, and $D_{sphere} = 2.2 \times 10^{-9}$ cm^2/sec.

The net force acting on a liquid droplet is slightly smaller than that for a solid sphere of the same size, because the liquid can flow backward along the sides of the droplet (in the $+\theta$ direction, Fig. 4.4) and return along its axis. This reduces the shear in the external medium. For a sphere of viscosity η' in a medium of viscosity η,
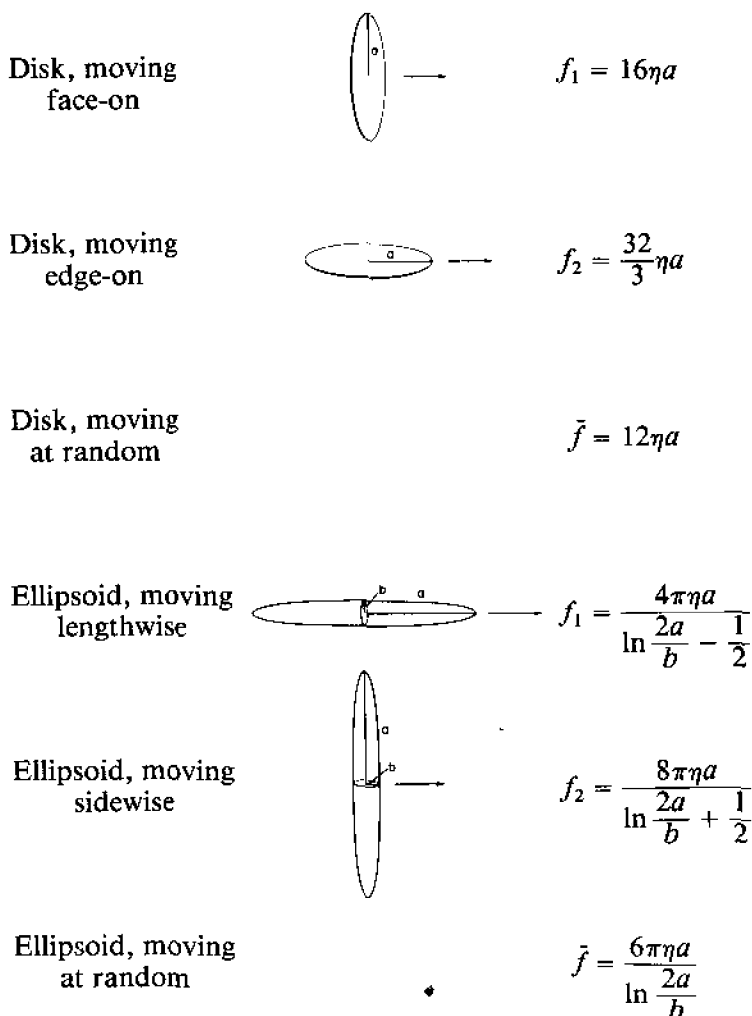
$$f_{droplet} = 6\pi\eta a\, \frac{2\eta + 3\eta'}{3\eta + 3\eta'}; \qquad (4.14)$$

see §337 of Lamb (1932). In the limit $\eta' \to \infty$, this reduces to Eq. 4.12. In the limit $\eta' \to 0$, we get the frictional drag coefficient for a bubble, e.g., of air:

$$f_{bubble} = 4\pi\eta a. \qquad (4.15)$$

Note that a spherical cell behaves as a solid rather than as a liquid, because the plasma membrane is not free to flow through the middle of the cell. The same thing is true for a droplet or bubble in a medium containing surface-active agents that form a monolayer at the interface.

The frictional drag coefficients of a disk and an ellipsoid of revolution are compared in Fig. 4.5. When a particle diffuses, it continuously changes its orientation. The frictional drag coefficient, $\bar{f}$, that characterizes the average drift velocity (and average diffusion coefficient) of

Disk, moving face-on		$f_1 = 16\eta a$
Disk, moving edge-on		$f_2 = \dfrac{32}{3}\eta a$
Disk, moving at random		$\bar{f} = 12\eta a$
Ellipsoid, moving lengthwise		$f_1 = \dfrac{4\pi\eta a}{\ln\dfrac{2a}{b} - \dfrac{1}{2}}$
Ellipsoid, moving sidewise		$f_2 = \dfrac{8\pi\eta a}{\ln\dfrac{2a}{b} + \dfrac{1}{2}}$
Ellipsoid, moving at random		$\bar{f} = \dfrac{6\pi\eta a}{\ln\dfrac{2a}{b}}$

Fig. 4.5. Comparison of the viscous drag coefficients of a solid circular disk of radius a and a prolate ellipsoid of revolution of semi-major axis a and semi-minor axes b. The expressions for the ellipsoid are valid in the limit $a^2 \gg b^2$. For general expressions for oblate and prolate ellipsoids, see p. 499 of Perrin (1934). The average coefficient $\bar{f}$ was obtained from f_1 and f_2 as described in the text; see also pp. 10–11 of Perrin (1936). The frictional drag coefficient for a solid sphere of radius a is $6\pi\eta a$, Eq. 4.12.

such a particle is given by $1/\bar{f} = (1/f_1 + 1/f_2 + 1/f_3)/3$, where f_1, f_2, and f_3 are the drag coefficients for motion along the principal axes. For the particles shown in Fig. 4.5, $f_2 = f_3$. Note that even though a particle is highly asymmetric, f_1 and f_2 differ by less than a factor of 2.

Stoke's law gives a good ballpark estimate for the viscous drag on globular things, even for particles as small as a molecule of the protein lysozyme. As a first approximation, do not worry about the shape of the particle, just think in terms of a sphere of roughly the same linear size. However, situations do arise in which asymmetries in viscous drag matter. One that we will encounter in Chapter 6 is flagellar propulsion. A great deal of effort has gone into figuring out the hydrodynamic properties of objects of complex shape; see, for example, García de la Torre and Bloomfield (1981).

Sedimentation rate

We are now in a position to write down the equations governing the sedimentation of particles in a gravitational or centrifugal field. A particle of mass m and volume V is suspended in a fluid of specific gravity ρ and viscosity η and subjected to a gravitational field of acceleration g, as shown in Fig. 4.6. The net downward force on the particle is

$$F_{down} = m'g, \qquad (4.16)$$

where m' is the effective mass of the particle, the mass of the particle less the mass of the fluid it displaces:

$$m' = m - V\rho. \qquad (4.17)$$

In a vacuum, the downward force on the particle would be mg. In a fluid, it is reduced by an amount $V\rho g$, the force due to buoyancy. If $V\rho$ is smaller than m (m' posi-
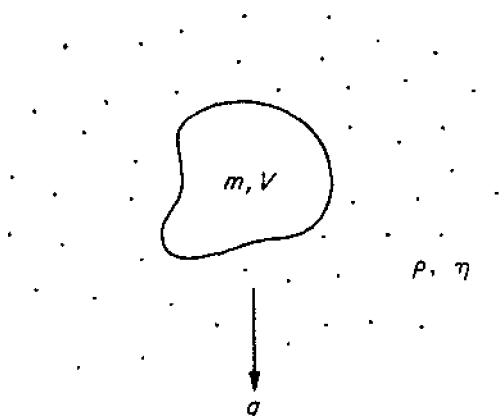
Fig. 4.6. A particle suspended in a fluid and subjected to a gravitational field. The particle has mass m and volume V. The fluid has specific gravity ρ, viscosity η. The downward acceleration is g.

tive), the particle sinks; if it is larger than m (m' negative), the particle floats; if it is equal to m (m' zero), the particle is neutrally buoyant. Equation 4.17 often is expressed as $m' = m(1 - \bar{v}\rho)$, where $\bar{v}$ is the specific volume of the particle, V/m.

From Eqs. 4.2 and 4.3 we have

$$v_d = \frac{F_{down}}{f} = \frac{m'gD}{kT}. \tag{4.18}$$

This is the Svedberg equation. It usually is written

$$\frac{v_d}{g} = S = \frac{m'D}{kT}, \tag{4.19}$$

where S is the sedimentation rate per unit acceleration. The units of S are in sec; 1 Svedberg = 10^{-13} sec. A 70 S particle, such as a ribosome from the bacterium *Escherichia coli*, sediments in a field of 1 cm/sec² at the rate 70×10^{-13} cm/sec. The acceleration due to gravity, g, is about 980 cm/sec², so in an ultracentrifuge at

$100,000 \times g$, the 70 S particle sediments at the rate 70×10^{-5} cm/sec. This velocity is only 10^{-5} as large as the instantaneous root-mean-square velocity of such a particle, which is about 100 cm/sec. The centrifugal field adds a small but persistent bias to the much more riotous motion due to thermal energy.

Note that the sedimentation rate depends both on the effective mass, m', and on the diffusion coefficient, D (or the frictional drag coefficient, f). If two particles have the same effective mass, the one that is more compact sinks more rapidly. To cite an extreme example, a man wearing a parachute reaches a much higher terminal velocity when his chute fails to open than when it functions properly, even though his effective mass is the same in either case.

In a centrifuge, we deal not with the gravitational acceleration g, but with a centrifugal acceleration $r\omega^2$, where r is the distance from the axis of rotation and ω is the angular velocity of the rotor in radians/sec (2π times the rotation rate in revolutions per sec). The sedimentation rate increases with distance from the axis of rotation, because r increases, but for now we ignore this complication. In general, there are two ways of doing an experiment. Consider a solution containing two kinds of particles whose sedimentation rates differ by a factor of about two. Either we start with a centrifuge tube (or sector cell) filled with the mixture, as shown at the top of Fig. 4.7, or we layer a small sample at the top of a tube containing something else, usually a density gradient of sucrose, as shown at the bottom of Fig. 4.7. In the former case, the particles with the smaller sedimentation rate lag behind near the top of the tube. In the latter case, the two species separate out into different bands. The sucrose gradient is absolutely essential; without it, the bands would have larger specific gravities than the fluid beneath them, and they would sink in bulk by convective flow. At
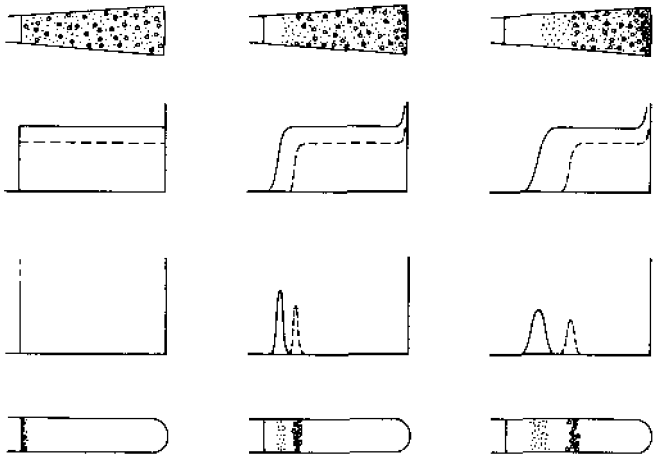
Fig. 4.7. Sedimentation rate experiments involving a mixture of two kinds of particles having sedimentation rates that differ by a factor of about two, shown at times 0 (left), t_0 (middle), and $2t_0$ (right). In the upper experiment, the mixture initially fills a sector cell in an analytical centrifuge. In the lower experiment, it is layered at the top of a sucrose gradient in a swinging bucket of a preparative centrifuge. The same centrifugal field is applied in either case; the particles sediment to the right. The upper and lower graphs show the concentrations of particles observed in the two experiments. The lower graph also depicts $\partial C/\partial r$, where C is the concentration in the upper experiment. In the analytical centrifuge, this function can be displayed directly with schlieren optics. Note in the upper experiment that the particles pile up at the bottom of the cell.

higher sucrose densities, the particles sediment more slowly, but we ignore this buoyant effect. Diffusion broadens the moving boundaries shown in the upper graph of Fig. 4.7, in accordance with Eq. 2.13 (see Fig. 2.5), and it broadens the moving bands shown in the lower graph, in accordance with Eq. 1.22 (see Fig. 1.3). The relative displacement of the boundaries (or bands) increases linearly with time, while the spreading increases only as the square-root of the time; therefore, the separation improves with the square-root of the time. One can

always improve the separation by working at higher fields, because this increases the sedimentation rates but does not change the diffusion coefficients. From the spreading, one can compute D; from the sedimentation rate, given D, one can compute the effective mass, m'. If the specific volume of the particle is known, one can use Eq. 4.7 and compute the mass, m.

From the spreading shown in Fig. 4.7, it is evident that more rapidly sedimenting particles have smaller diffusion coefficients. This is generally true for globular particles made of a similar material. The effective mass of a sphere of radius a and specific gravity ρ_s is

$$m'_{sphere} = m - V\rho = V(\rho_s - \rho) = 4\pi a^3(\rho_s - \rho)/3.$$
(4.20)

The sedimentation rate of the sphere is

$$v_{d,sphere} = \frac{F_{down}}{f} = \frac{m'g}{6\pi\eta a} = 2a^2(\rho_s - \rho)g/9\eta,$$
(4.21)

a quantity that increases as a^2. As we already have seen in Eq. 4.13, the diffusion coefficient of the sphere is inversely proportional to a. Therefore, a sphere that sediments twice as fast has a diffusion coefficient that is smaller by a factor of $2^{-1/2}$. A sphere of radius $a = 10^{-4}$ cm and specific gravity $\rho_s = 1.2$ g/cm^3 sediments in water in a field $1 \times g$ at the rate $v_{d,sphere} = 4.4 \times 10^{-5}$ cm/sec.

Electrophoresis

If a particle carries an electric charge, then one can exert a force on it with an electric field. An ion carrying charge q (esu) in an electric field of intensity E (statvolts/cm) experiences a force in the direction of the field Eq (dynes). Unfortunately, q is not easy to define. Parti-

cles of biological interest contain a variety of ionizable groups whose charges depend strongly on pH. These charges are shielded by counter-ions attracted from the medium in which the particles are suspended. The effectiveness of the shielding depends on the ionic strength. So you do not hear much about particles that have specified electrophoretic drift rates per unit field (as you do, for example, about 30, 50, or 70 S ribosomes). Nevertheless, electrophoretic methods of separating and characterizing biological materials are extremely useful. In practice, they are remarkably simple.

As in the case of sedimentation, Fig. 4.7, there are essentially two ways of doing an experiment. One either creates a sharp interface between a column of liquid containing a mixture of the particles to be studied and a column of liquid devoid of such particles and then passes an electrical current from one to the other, generating patterns analogous to those shown at the top of Fig. 4.7, or one layers a mixture of particles at the top of a medium designed to suppress convective stirring and passes an electrical current through that, generating patterns analogous to those shown at the bottom of Fig. 4.7. The physics is much the same: the relative displacement of the boundaries (or bands) increases linearly with time, while the spreading increases as the square-root of the time; so the separation improves as the square-root of the time. But in the case of electrophoresis, it is not always possible to improve the separation by increasing the field, because the electric current generates heat. The heat is generated at the same rate at all points across a transverse section of the electrophoretic column, but it is dissipated only at the edges, so the center of the column becomes relatively hotter. In most of the media used to suppress convection (see below), this increases the electrophoretic mobility, and the bands become curved.

As noted earlier, convective stirring is suppressed in the ultracentrifuge by the use of density gradients, e.g., of sucrose or CsCl. In an electrophoresis experiment, it is more convenient to use a gel, e.g., polyacrylamide or agarose. At the end of the experiment the bands can be precipitated into the gel and/or stained, e.g., with colored or fluorescent dyes, or the gel can be dried down and exposed to X-ray film to reveal components that are radioactive. Gels are not used in the ultracentrifuge, because they collapse in large centrifugal fields.

Gels not only suppress convective stirring, they act as molecular sieves. The rate of migration of a particle through the gel is strongly dependent on size. A particle that is small compared to the pores in the gel can diffuse through it, almost as if the gel were not there. A particle that is large compared to the pores in the gel simply is immobilized. Particles of intermediate size get through with varying degrees of difficulty. Particles that would move through a dilute aqueous medium at roughly the same rate move through the gel at rates that decrease exponentially with size; as a result, an estimate of size (or mass) can be made from a measurement of the logarithm of the displacement. Pieces of DNA and RNA are routinely sorted in this way, as are proteins dissolved in ionic detergents, such as sodium dodecyl sulfate. It is easy to distinguish gels of this kind, because the faster-moving bands always are broader; the molecules that drift more rapidly are smaller and have larger diffusion coefficients.