

Random Walks in Biology  
 Howard C. Berg Chapters 4 + 5  
 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 with 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-

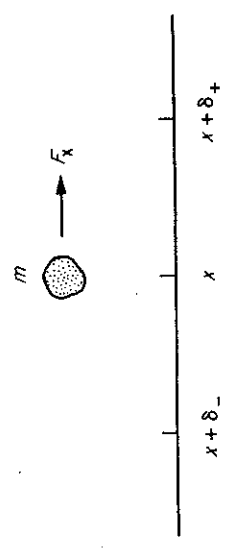


Fig. 4.1. A particle of mass  $m$  subjected to an externally applied force  $F_x$  while undergoing a one-dimensional random walk.

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  $\tau$  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  $\tau$  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  $\tau$  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  $\tau$  is  $a\tau^2/2$ , and the particle drifts to the right with an average velocity

$$v_d = \frac{1}{2} a\tau = \frac{1}{2} \frac{F_x}{m} \tau. \quad (4.1)$$

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

$$v_d = \frac{F_x}{f}. \quad (4.2)$$

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

$$D = \frac{kT}{f}. \quad (4.3)$$

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 Chapter I-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  $v_d C(x)$ . Thus, Fick's first equation, Eq. 2.1,

becomes

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

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

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

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 with the molecules composing 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

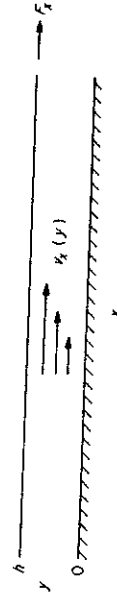


Fig. 4.2. A viscous fluid contained between flat plates at  $y = 0$  and  $y = h$ . The area of each plate is  $A$ . The bottom plate is fixed. The top one is propelled to the right by a force in the  $x$  direction,  $F_x$ . Arrows show the velocity of the fluid relative to the bottom plate at different distances from this plate,  $y$ .

profile is linear:

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

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:

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

This equation provides an operational definition for the coefficient of viscosity,  $\eta$ . The viscous drag is proportional to  $\eta$ , the area of the plate, and the rate of shear of the fluid at the surface of the plate. The units of  $\eta$  can be found from the dimensional equation  $[\eta \text{ cm}^2/\text{sec}^2] = [\eta] [\text{cm}^2] [\text{cm}/\text{sec cm}]$ , which yields  $[\eta] = [\text{g}/\text{cm sec}]$ , which is called a poise (P). The kinematic viscosity,  $\nu$ , is  $\eta$  divided by the specific gravity of the medium,  $\rho$ ; its units are the same as that of the diffusion coefficient,  $\text{cm}^2/\text{sec}$ , which is called a stoke. The viscosity of water at  $20^\circ\text{C}$  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)
$40^\circ\text{C}$	$1.93 \times 10^{-4}$	$6.53 \times 10^{-3}$	2.83
$20^\circ\text{C}$	$1.83 \times 10^{-4}$	$1.00 \times 10^{-2}$	14.1
$0^\circ\text{C}$	$1.71 \times 10^{-4}$	$1.79 \times 10^{-2}$	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,  $\eta$  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  $\Delta P$  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 with 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

$$\eta b w [(\partial v_x / \partial y)_y - (\partial v_x / \partial y)_{y+dy}] = -\eta b w (\partial^2 v_x / \partial y^2) dy.$$

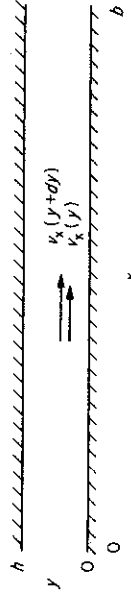


Fig. 4.3. Fluid in a channel between two parallel plates driven from left to right by a pressure gradient  $\Delta P/b$ . Both plates are fixed. The channel is of height  $h$ , length  $b$ , and width  $w \gg h$ .

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,  $\Delta Ph^2/8\eta b$ . 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

$$\begin{aligned} v_r &= -v_d \cos \theta \left( 1 - \frac{3a}{2r} + \frac{a^3}{2r^3} \right) \\ v_\theta &= v_d \sin \theta \left( 1 - \frac{3a}{4r} - \frac{a^3}{4r^3} \right). \end{aligned} \tag{4.11}$$

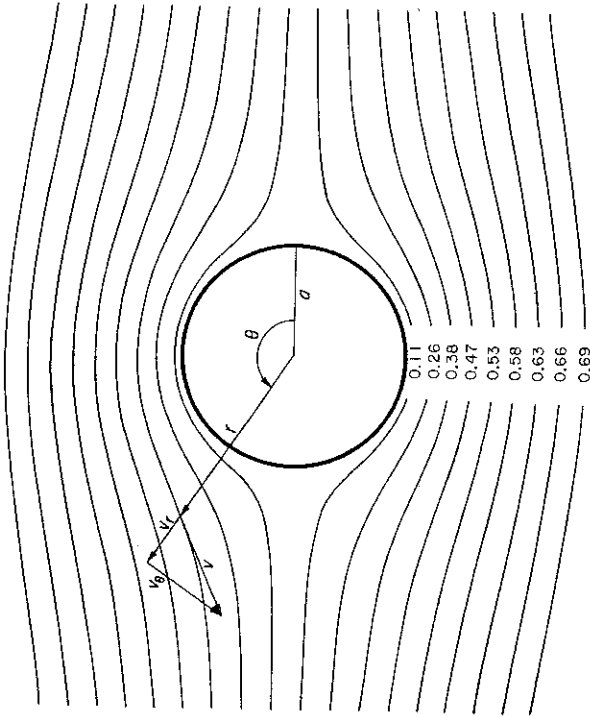


Fig. 4.4. A solid sphere moving at a constant velocity  $v_d$  through an incompressible viscous fluid. The fluid moves around the sphere along the flow lines shown. See Eq. 4.11. The numbers on the flow lines at  $\theta = -90^\circ$  indicate the magnitudes of  $v_\theta$  at these points in units 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.

**Stokes' law:** A calculation of the net force acting on the sphere yields Stokes' 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  $6\pi\eta av_d$ .

Given Eqs. 4.2 and 4.3, this implies that

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

and

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

For a sphere of radius  $a = 10^{-4}$  cm in water at room temperature,  $f_{\text{sphere}} = 1.9 \times 10^{-5}$  g/sec, and  $D_{\text{sphere}} = 2.2 \times 10^{-9}$  cm<sup>2</sup>/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  $\eta'$  in a medium of viscosity  $\eta$ ,

$$f_{\text{droplet}} = 6\pi\eta a \frac{2\eta + 3\eta'}{3\eta + 3\eta'}; \quad (4.14)$$

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

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

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

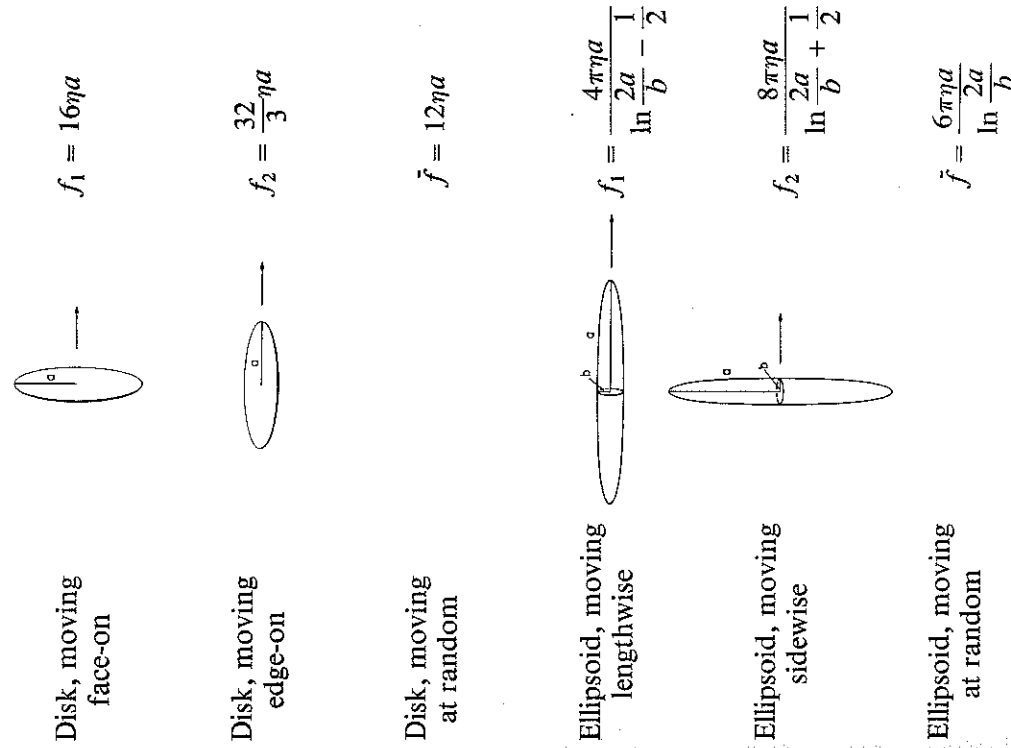


Fig. 4.5. Comparison of the viscous drag coefficients of a solid circular disk of radius  $a$  and a prolate ellipsoid of revolution of semi-major axis  $a$  and semi-minor axes  $b$ . The expressions for the ellipsoid are valid in the limit  $a^2 \gg b^2$ . For general expressions for oblate and prolate 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  $1/f = (1/f_1 + 1/f_2 + 1/f_3)/3$ , 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.

Stokes' 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  $\rho$  and viscosity  $\eta$  and subjected to a gravitational field of acceleration  $g$ , as shown in Fig. 4.6. The net downward force on the particle is

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

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

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

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-

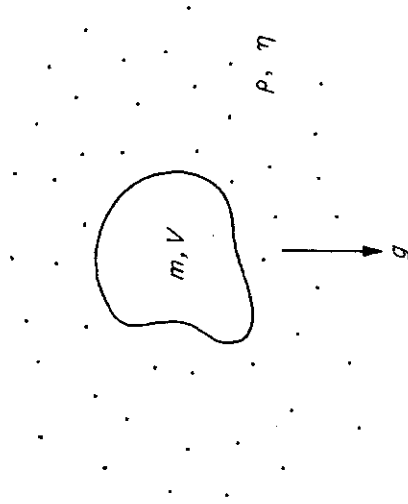


Fig. 4.6. A particle suspended in a fluid and subjected to a gravitational field. The particle has mass  $m$  and volume  $V$ . The fluid has specific gravity  $\rho$ , viscosity  $\eta$ . The downward acceleration is  $g$ .

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

$$v_d = \frac{F_{\text{down}}}{f} = \frac{m'gD}{kT}. \quad (4.18)$$

This is the Svedberg equation. It usually is written

$$\frac{v_d}{g} = S = \frac{m'D}{kT}, \quad (4.19)$$

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  $1 \text{ cm/sec}^2$  at the rate  $70 \times 10^{-13} \text{ cm/sec}$ . The acceleration due to gravity,  $g$ , is about  $980 \text{ cm/sec}^2$ , so in an ultracentrifuge at

100,000  $\times g$ , the 70 S particle sediments at the rate  $70 \times 10^{-5}$  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  $\omega$  is the angular velocity of the rotor in radians/sec ( $2\pi$  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

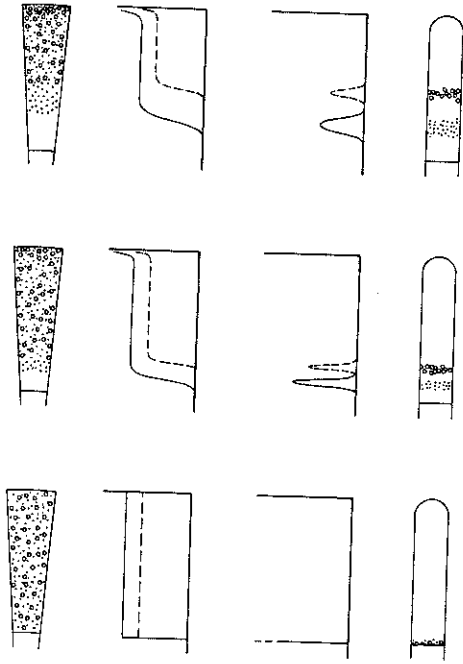


Fig. 4.7. Sedimentation rate experiments involving a mixture of two kinds of particles having sedimentation rates that differ by a factor of about two, shown at times 0 (left),  $t_0$  (middle), and  $2t_0$  (right). In the upper experiment, the mixture initially fills a sector cell in an analytical 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  $\rho_s$  is

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

The sedimentation rate of the sphere is

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

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<sup>3</sup> sediments in water in a field  $1 \times g$  at the rate  $v'_{d,\text{sphere}} = 4.4 \times 10^{-5}$  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. Either one 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 with the pores in the gel can diffuse through it, almost as if the gel were not there. A particle that is large compared with 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.

## Chapter 5

### Diffusion at Equilibrium

What happens if the sedimentation-rate experiment shown at the top of Fig. 4.7 is continued for a very long time? Eventually, the particles will pile up near the bottom of the cell and form a distribution that does not change. The particles still will diffuse up and down, but the average number at any given point will remain constant. It is often argued that equilibrium will be established when the upward flux of particles due to diffusion balances the downward flux due to sedimentation, the equilibrium concentration,  $C$ , being the steady-state solution of Eq. 4.5, with  $v_g$  given by Eq. 4.18. This is true but misleading. One ought to be able to figure out what the equilibrium distribution is without having to appeal to fluxes, quantities that involve the movement of particles with time. The equilibrium distribution is, by definition, time invariant. In any event, if we were to work out the equilibrium distribution from Eq. 4.5, the argument would be circular, because we derived Eq. 4.18 from Eq. 4.3 following an appeal to Eq. 1.1, which refers to particles at equilibrium. Evidently, the answer we seek and Eq. 1.1 are intimately related.

For the sake of simplicity, consider the concentration of particles at height  $x$  above a horizontal barrier in a uniform field,  $g$ , as shown in Fig. 5.1. At equilibrium, there will be fewer particles at large values of  $x$  than at small values of  $x$ , just as there are fewer molecules of air at high altitudes than at the surface of the earth. The work required to lift a particle a distance  $x$  is  $m'gx$ , the downward force of Eq. 4.16 times the distance  $x$ . Thus,

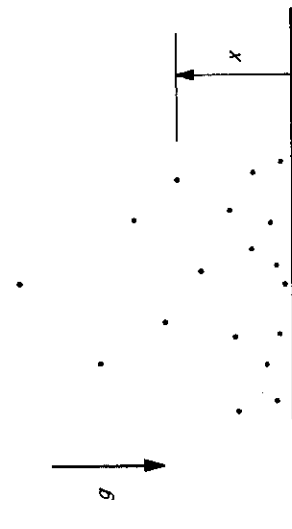


Fig. 5.1. Equilibrium distribution of particles of effective mass  $m'$  above a horizontal barrier in a field of uniform acceleration  $g$ . The height above the barrier is  $x$ . See Eq. 5.4.

particles at large values of  $x$  have larger gravitational energies. Given that the particles are able to move around and interact with the molecules of the fluid in which they are suspended, what is the probability that a particle will be found with gravitational energy  $m'gx$ ? Or to state the problem more generally, given that the particles can exist in states  $1, 2, \dots, i, \dots$ , having different energies  $E_1, E_2, \dots, E_i, \dots$ , what is the probability that a given particle will be found in state  $i$ ? The answer is

$$P_i = \frac{1}{Q} e^{-E_i/kT}, \quad (5.1)$$

where

$$Q = \sum_i e^{-E_i/kT} \quad (5.2)$$

is a normalization constant called the partition function, the summation extending over all possible states  $i$ . If the energy levels are continuous rather than discrete, the sum becomes an integral. Eq. 5.1 is the Boltzmann equation,

the basic equation of classical equilibrium statistical mechanics. If there are  $N$  particles in all,  $N_i = NP_i$  particles will be found in state  $i$ . If we are interested in the ratio of the probability of finding a particle in state  $i$  to the probability of finding a particle in state  $j$ , or in the ratio of the number of particles in state  $i$  to the number of particles in state  $j$ , then Eq. 5.1 implies that

$$\frac{N_i}{N_j} = \frac{P_i}{P_j} = e^{-(E_i - E_j)/kT}, \quad (5.3)$$

and we need not concern ourselves with  $Q$ . For the particles of Fig. 5.1, Eq. 5.3 takes the form

$$\frac{C(x)}{C(0)} = \frac{N(x)}{N(0)} = e^{-m'gx/kT}. \quad (5.4)$$

The concentration of particles decreases exponentially with height, by a factor  $e$  in a distance  $kT/m'g$ . We will refer to this distance as the scale height. For molecules of air over the earth, the scale height is about 8 km.

A particle in a Boltzmann distribution still diffuses. It moves up and down, spending most of its time near  $x = 0$ , but sometimes wandering several scale heights away. Equation 5.4 tells us the probability that the particle will be found at any given height  $x$ . If there are a large number of similar particles, then Eq. 5.4 tells us the average number that will be found at any given height  $x$ . The mean height is just the scale height,  $x_s$ . Figure 5.2 shows the vertical displacement of a particle diffusing in a Boltzmann distribution. The plot extends over a period of time equal to 10 times the period required for the particle to diffuse a root-mean-square distance equal to its scale height.

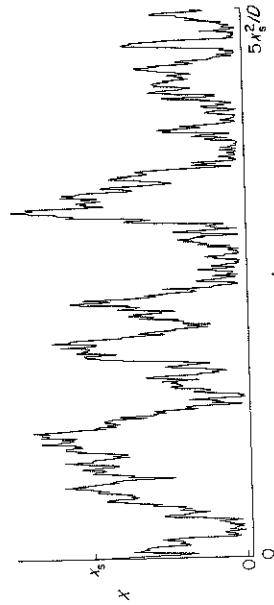


Fig. 5.2. The height of a particle in a Boltzmann distribution of scale height  $x_s$  shown for a period of time  $10x_s^2/2D$ . The motion was simulated on a computer by dividing a segment of the  $x$  axis into 200 equal units, assigning the particle a scale height of 40 units, and allowing it to step up or down one step at a time 16,000 times. Sedimentation was accounted for by making the probability of stepping up smaller than the probability of stepping down by a factor  $e^{-1/40}$ ; at  $x = 0$  the particle only was allowed to diffuse up. This ensures that the particle will be found at height  $x$  with probability  $e^{-x/x_s}$ . For the period of time shown, the particle spent most of its time at a height smaller than the scale height, on the average at about  $0.6x_s$ .

### Derivation of the Boltzmann equation

Consider an ensemble of  $N$  particles with total energy  $E$ . Assume that  $N_j$  particles are in a state with energy  $E_j$ ,  $N_j$  particles are in a state with energy  $E_j$ , etc., subject to the constraints  $\sum_j N_j = N$  and  $\sum_j N_j E_j = E$ . If  $N$  is large, there are many different ways of parceling out this energy, i.e., of assigning particles to states. In classical statistics, the particles are distinguishable; for a given set of occupation numbers  $[N_j] = N_1, N_2, \dots, N_j, \dots$ , there are

$$W = \frac{N!}{N_1! N_2! \dots N_j! \dots} \quad (5.5)$$

different ways of assigning particles to states. To see this, note that if all the particles were in different states, a different assignment would arise every time we exchanged any two particles, i.e., for every one of the  $N!$  possible

permutations in the order of the particles. However, if there are  $N_1$  particles in state 1,  $N_2$  particles in state 2, etc., this number must be reduced by a factor  $N_1! N_2! \dots$ , because the exchange of two particles in the same state does not change the assignment, and  $N_1! N_2! \dots$  is the total number of assignments that differ by such exchanges.

We now take a leap of faith and postulate that every possible way of parceling out the energy is, a priori, equally probable. If a given set of occupation numbers  $[N_j]$  turns out to give a number of assignments that is enormous compared with that possible with any other set of occupation numbers, then the set of occupation numbers  $[N_j]$  will be the one observed in practice. Although we will not prove it, this turns out to be the case. The problem is reduced to one of finding  $[N_j]$  such that  $W$  is a maximum, subject to the constraints  $\sum_j N_j = N$  and  $\sum_j N_j E_j = E$ , where  $N$  and  $E$  are constants. The result is

$$\frac{N_j}{N} = \frac{e^{\beta E_j}}{\sum_j e^{\beta E_j}}, \quad (5.6)$$

where  $\beta$  is a constant.

One can show that  $\beta$  is solely a function of temperature by considering two ensembles of particles  $N$  and  $N'$  separated by a barrier that blocks the passage of matter but allows an exchange of energy. The number of different ways,  $W + W'$ , of assigning particles to states, subject to the constraint that the total energy  $E + E'$  is constant, is maximum when both sets of occupation numbers satisfy Eq. 5.6, with the same value of  $\beta$ . It is a matter of common experience (and thermodynamic postulate) that different systems in thermal contact come to equilibrium at the same temperature; therefore,  $\beta$  is a measure of temperature. By using Eq. 5.6 to compute the expectation

value of a suitable macroscopic parameter, such as the pressure of an ideal gas, it is possible to show that  $\beta = -1/kT$ . Thus Eq. 5.6 leads to Eq. 5.1.

This is a remarkable result. It follows from the assumption that every possible microscopic description of a system of  $N$  particles of energy  $E$  is equally probable. If  $N$  is large, essentially all these descriptions involve the set of occupation numbers specified by the Boltzmann equation; the most probable set is overwhelmingly most probable. If a particle has different degrees of freedom—i.e., if it can translate along the  $x$ ,  $y$  and  $z$  axes, rotate about these axes, vibrate internally, etc.—then the Boltzmann equation applies to each of these degrees of freedom separately. The total available energy is distributed among the different degrees of freedom in such a way that each satisfies the Boltzmann equation with the same value of the temperature  $T$ .

In quantum statistics, the particles are indistinguishable, and assignments such as those made in Eq. 5.5 that differ by the exchange of two particles are not counted separately. The occupation number of a state of a particle of half-integral spin, such as an electron, can be either 0 or 1: this leads to Fermi-Dirac statistics. The occupation number of a state of a particle of integral spin, such as a photon, can be anything from 0 to  $N$ : this leads to Bose-Einstein statistics. In other respects, the derivations are similar to that of Boltzmann statistics.

### The importance of $kT$

At thermal equilibrium, the probability of finding a particle in a state with an energy large compared with  $kT$  is quite small. For example, the probability of finding a particle in a state of energy  $10kT$  or more is only  $4.5 \times 10^{-5}$ . However, the probability of finding a particle in a state

with an energy of the same order of magnitude as  $kT$  is quite large. The probability of finding a particle in a state of energy  $kT$  or more is 0.37. Thus,  $kT$  can be thought of as the energy of thermal fluctuation. When a particle interacts with its surroundings at temperature  $T$ , it frequently gains and loses amounts of energy of this order of magnitude. This is evident, for example, in the changes of height of a particle that has sedimented to equilibrium, as illustrated in Figs. 5.1 and 5.2.

### Mean-square velocity

A particle of mass  $m$  moving along the  $x$  axis at velocity  $v_x$  has a kinetic energy  $mv_x^2/2$ . Equation 5.1 implies that

$$\begin{aligned} \langle v_x^2 \rangle &= \int_0^\infty v_x^2 P(v_x) dv_x \\ &= \frac{\int_0^\infty v_x^2 e^{-mv_x^2/2kT} dv_x}{\int_0^\infty e^{-mv_x^2/2kT} dv_x}. \end{aligned} \quad (5.7)$$

On consulting a table of definite integrals, we find

$$\langle v_x^2 \rangle = \frac{(\pi/16)^{1/2} (2kT/m)^{3/2}}{(\pi/4)^{1/2} (2kT/m)^{1/2}} = \frac{kT}{m}, \quad (5.8)$$

the value asserted in Eq. 1.1.

### Einstein-Smoluchowski relation

We used Eq. 1.1 in deriving the Einstein-Smoluchowski relation, Eq. 4.3. A more general proof is now possible via Eq. 4.4, which, given Eq. 4.2, takes the form

$$J_x = -D \frac{\partial C}{\partial x} + \frac{F_x}{f} C. \quad (5.9)$$

The forces of interest,  $F_x$ , are conservative: the work that they do in moving a particle from position 1 to position 2 is independent of the path taken by the particle; it only depends on the end points. Such a force can be expressed as a gradient of a potential energy,  $F_x = -\partial E/\partial x$ , so that

$$J_x = -D \frac{\partial C}{\partial x} - \frac{C}{f} \frac{\partial E}{\partial x}. \quad (5.10)$$

At equilibrium,  $J_x = 0$ , and Eq. 5.10 implies that

$$\frac{dC}{C} = -\frac{1}{Df} dE. \quad (5.11)$$

Integrating this equation, we find

$$\frac{C(x)}{C(0)} = e^{-[E(x)-E(0)]/Df}, \quad (5.12)$$

which on comparison with Eq. 5.3 requires that  $D = kT/f$ , the desired result.

### Equilibrium sedimentation

Given the Boltzmann equation, we know at once the equilibrium distribution of particles in a gravitational field, Eq. 5.4. Things are slightly more complicated in the centrifuge, because the downward acceleration,  $r\omega^2$ , varies with  $r$ . The work required to lift the particle from radius  $r_0$ , the bottom of the cell, to radius  $r$ , is

$$\begin{aligned} E(r) - E(r_0) &= -\int_{r_0}^r m'r\omega^2 dr \\ &= m'\omega^2(r_0^2 - r^2)/2, \end{aligned} \quad (5.13)$$

so that

$$\frac{C(r)}{C(r_0)} = e^{-m'\omega^2(r_0^2 - r^2)/2kT}. \quad (5.14)$$

### Density-gradient sedimentation

If particles are suspended in a dense salt, e.g., in a solution of CsCl or CsSO<sub>4</sub>, and the experiment is run long enough at high enough fields, then the salt will sediment to equilibrium, and the density of the fluid (water + salt) will be larger near the bottom of the cell than near the top. In practice, the difference is not large, because the scale height of the distribution of salt is larger than the height of the column of liquid. Under these conditions, the particles will form a band centered at their own buoyant density,  $m/V$ . Particles above this point will sediment downward; particles below this point will sediment upward. How broad is the band? What is its shape? We return to the integral in Eq. 5.13 and note that  $m'$  is now a function of  $r$ , since the density of the fluid,  $\rho$ , is a function of  $r$ . Suppose the band is narrow and centered at  $r = r_b$ . The density of the fluid near  $r_b$  can be expressed in a Taylor expansion as

$$\begin{aligned} \rho(r) &= \rho(r_b) + \left. \frac{\partial \rho}{\partial r} \right|_{r=r_b} (r - r_b) \\ &+ \text{terms of order } (r - r_b)^2 \text{ and higher.} \end{aligned} \quad (5.15)$$

By substituting this approximate value of  $\rho(r)$  into Eq. 5.13, noting that  $1 - \bar{v}\rho(r_0) = 0$ , evaluating the integral, and using it in the Boltzmann equation, we find

$$\frac{C(r)}{C(r_b)} = e^{-m'\omega^2 \bar{v}\rho(r_b)(r-r_b)^2/2kT}, \quad (5.16)$$

where  $\rho(r_b) = \partial\rho/\partial r|_{r=r_b}$ . This is a Gaussian distribution (compare Eq. 1.22) with standard deviation

$$\sigma_r = [kT/m'r_b\omega^2 \bar{v}\rho(r_b)]^{1/2}. \quad (5.17)$$

Thus, the band is narrow for particles of large mass (large  $m$ ), in high fields (large  $r_b\omega^2$ ), and in steep gradients (large

$\rho(r_b)$ ). If the material under study is inhomogeneous, the band will be broader, with particles of lower mass predominating at larger values of  $(r - r_b)$ . The method is particularly useful for isolating large molecules of a given kind that come in different sizes or tend to fragment, such as DNA, since particles with the same buoyant density band in the same region, regardless of their size.

### Isoelectric focusing

An analogous situation arises in electrophoresis when the experiment is run in a pH gradient. At equilibrium, a protein will form a band centered at the pH at which it is electrically neutral, i.e., at its isoelectric point. A particle at a more acid pH is positively charged and moves toward the cathode; a particle at a more basic pH is negatively charged and moves toward the anode. Thus, the pH gradient must be acidic near the anode and basic near the cathode. The band is narrow for particles with large numbers of titratable groups, in high fields, and in steep gradients. The gradient is established by the migration of an array of polyelectrolytes that are electrically neutral at different values of pH. These ampholytes play a role in isoelectric focusing analogous to that of the heavy salts in density-gradient sedimentation.

## Chapter 6

### Movement of Self-propelled Objects

The drift imposed on the diffusion of particles discussed in Chapter 4 was due to forces externally applied: to gravitational, centrifugal, or electrical fields. There are many microscopic objects in this world that are self-propelled. Among the most remarkable of these are flagellated bacteria: spherical, rod-shaped, or helical cells as small as  $10^{-4}$  cm in diameter that swim steadily at speeds of order  $2 \times 10^{-3}$  cm/sec. While it may be difficult to imagine oneself as an inert particle falling in a centrifugal field, it is less so to contemplate the plight of a tiny living thing. What is it like to be so small?

#### Life at low Reynolds number

In the discussion leading to Stokes' law, we noted that when small things move through fluids slowly, viscous forces are important but inertial forces are not. A hydrodynamicist would say that we are dealing with fluids at low Reynolds number. The Reynolds number is a dimensionless parameter in the equations of motion of a fluid that indicates the relative size of terms that describe inertial forces (forces required to accelerate masses) and viscous forces (forces due to viscous shear). The Reynolds number is

$$R = \frac{vL\rho}{\eta}, \quad (6.1)$$