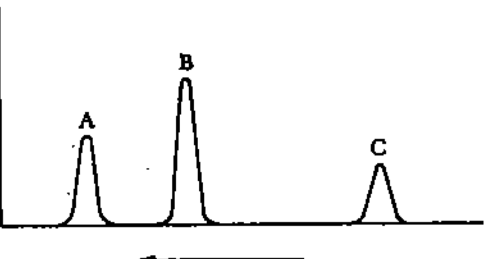
alkaline sucrose gradient, a single peak is seen; after nicking, three peaks appear and the pattern shown below is seen.




Peak A consists of unnicked molecules. The area of B is always 3 × that of C, no matter how large A is. What is the structure of the molecule?

11–41. A sample of circular DNA molecules is X-irradiated. This causes double-strand breaks; that is, the molecule becomes linear. Samples exposed to several doses are centrifuged and two species are observed. The ratio of the amount of slower moving material to the amount of the faster moving material is determined as a function of dose; the data are the following.

| Dose (rads) | Fraction fast | Fraction slow |
|---|---|---|
| 0 | 1.00 | 0 |
| 1000 | 0.61 | 0.39 |
| 2000 | 0.37 | 0.63 |
| 3000 | 0.23 | 0.77 |
| 4000 | 0.14 | 0.86 |

On the average, how many rads are required to convert a circular molecule to a linear molecule?

11–42. A sample of DNA gives one band in CsCl but two in CsCl containing ethidium bromide. The ratio of the areas of the denser to the lighter band is 2:1. The molecular weight of the DNA is $30 \times 10^6$. Suppose that the DNA were treated with an enzyme that produces on the average one single-strand break in each molecule. What would the ratio of the band areas be after such a treatment? (Remember to use the Poisson distribution to determine the fraction of molecules receiving no breaks.)

Set 2 - 1

# 12

# Partial Specific Volume and the Diffusion Coefficient

In the preceding chapter, it was mentioned that measurements of the partial specific volume and the diffusion coefficient are frequently necessary in using hydrodynamic methods to characterize macromolecules. How this is done is the subject of this chapter.

## Measurement of Partial Specific Volume

In the theory of ultracentrifugation, a term that is often encountered is $1 - \bar{v}\rho$, in which $\rho$ is the solution density and $\bar{v}$ is the partial specific volume. The volume increment produced in a solution when unit mass of solute is added is $dv/dm = \bar{v}$. (The partial specific volume is sometimes approximated as the reciprocal of the density of the solute, which is not exactly true but often a good approximation.) Common experience indicates that the volume increment in solution differs from the volume of the solid—for example, a cup of sugar dissolved in a cup of water makes much less than two cups of solution (nearer one cup). It is also important to know that $\bar{v}$ is not an invariant parameter of a particular macromolecule but varies with the solvent composition—that is, the salt concentration, pH, presence of other dissolved substances, and so forth.

An evaluation of $\bar{v}$ is essential in determining both molecular weight and sedimentation coefficients. Furthermore, $\bar{v}$ must be measured with great precision because, due to the range of values for biological macromolecules (0.6–0.75 $cm^3/g$), a 1% error in $\bar{v}$ gives about a 3% error in $M$ or $s_{20,w}$. In fact, the measurement of $\bar{v}$ is frequently the limiting factor in determining molecular weight. The three major methods are the summa-

tion of $\bar{v}$ values of the residues of a macromolecule, those that use density measurement, and parallel sedimentation equilibrium measurements in isotopically labeled solvents.

### Summation of $\bar{v}$ of the Residues of a Macromolecule

If the amino acid composition of a protein is accurately known, $\bar{v}$ can be calculated from the $\bar{v}$ values of the individual amino acids as $n_i m_i \bar{v}_i / \sum n_i m_i$, in which $n_i$ is the number of residues per mole of the $i$th amino acid in the protein, $m_i$ is the residue molecular weight (the molecular weight of the amino acid minus the weight of one mole of water, because one mole of water is removed in the formation of a peptide bond), and $\bar{v}_i$ is the partial specific volume of the $i$th residue. Values of $\bar{v}$ for the residues can be found in various reference tables. If other groups such as lipids, carbohydrates, flavins, and so forth, are present, $\bar{v}$ for the group must be added in. This is an accurate method for proteins but has not been tested for nucleic acids.

## Methods Using Density Measurement

Because $\bar{v} = dv/dm$, $\bar{v}$ can be determined from the variation of the density of a solution with solute concentration. Four methods for accurately measuring density are described first, and then the surprisingly formidable problem of precisely measuring concentration is discussed.

### *Pycnometry*

A pycnometer is simply a container whose volume can be accurately measured and which can be filled with great precision (Figure 12-1). The volume is measured by filling with water and weighing, since the density of water is accurately known. It is then filled with the *solution* and reweighed. Because of the temperature dependence of volume, the temperature of both the water and the solution must be accurately controlled and known. Pycnometry is the most direct way to determine density. However, in order for the weight differences to be large enough to be measured with precision, large volumes of solution of high concentration (approximately 10 ml at 50 mg/ml) are needed; frequently it is very difficult to obtain so much material. Pycnometry usually fails with highly extended molecules such as high-molecular-weight DNA because at 10 mg/ml the solution is a semisolid gel and the pycnometer cannot be filled.

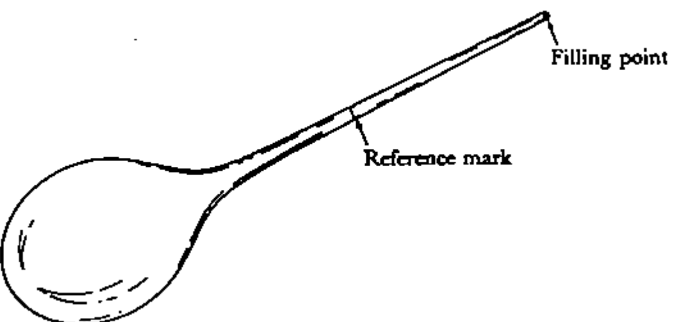



**Figure 12-1**
A pycnometer. The pycnometer is first weighed empty and then filled to the mark and reweighed. The volume up to the mark is accurately known.

### *Linderstrøm-Lang density gradient column*

A density gradient column of bromobenzene and kerosene, both of which are immiscible with water, is prepared (Figure 12-2). If a small droplet (usually 1 $\mu$l) of an aqueous solution is placed on the surface, it will fall through the column and come to rest at a point at which its density equals that of the column. If the column is calibrated with solutions of known concentration (usually of KCl), the density of the sample can be determined from its position relative to the standards. A large number of standards and sample drops are needed to define the density gradient and to determine the position of the sample with precision. To avoid thermal convection, which would result in the movement of the drops, the temperature of the gradient must be controlled to $\pm 0.01°C$. This method has the great advantage of using tiny amounts of material and is generally reliable although there have been a few instances of error caused by interaction with the solvents in the gradient.

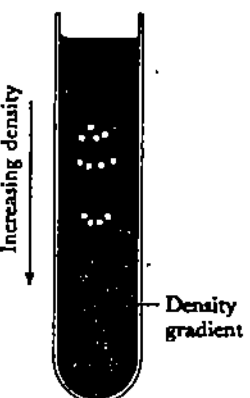



**Figure 12-2**
Linderstrøm-Lang density gradient column. A linear density gradient of two organic liquids (typically kerosene and bromobenzene, which are miscible with one another but immiscible with $H_2O$) is prepared using apparatus of the type shown in Figure 8-5. Droplets of solutions of known density (open circles) are introduced into the column for calibrating the density gradient. The position of the sample droplets (solid circles) is measured with respect to the reference drops.

### *Cahn electrobalance*

Like a pycnometer, this instrument accurately weighs a solution of known volume but uses small (1 ml) volumes at relatively low (~10 mg/ml) concentration. This is possible because its accuracy is ±0.1 μg—roughly, 1000 times as sensitive as standard laboratory balances. However, its cost ($7000) prohibits its widespread use.

### *Mechanical oscillator technique*

This method uses a commercially available mechanical oscillator that can be filled with fluid (Figure 12-3); its resonance frequency is related to the density of the liquid. The advantage of the instrument is high precision with a sample volume of less than 1 ml. The instrument is gaining widespread use.

With each of the methods requiring density measurement, it is necessary to know concentration precisely. This seems trivial because to make a known volume of solution necessitates only weighing a sample and dissolving it. However, the weight must be the *anhydrous weight* and, unfortunately, proteins and nucleic acid invariably contain bound water. With inorganic materials, an anhydrous sample can be obtained by heating to a high temperature, but proteins and nucleic acids are degraded at temperatures higher than 100°C. Hence, a standard method is to dry the protein or nucleic acid sample at 60–80°C in a vacuum until the weight becomes constant and assume that constant weight indicates that all water has been removed. However, to remove all water from

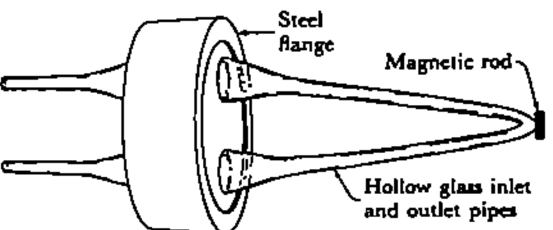



**Figure 12-3**
A mechanical oscillator for density measurement. An oscillating magnet elsewhere in the system causes the magnet rod and therefore the entire V-shaped tube to vibrate. The natural frequency of vibration is determined by the geometry and mass of the tube. The tube is filled with the sample whose density is to be measured, and the natural frequency changes. From the measured frequency, the weight of added liquid is calculated. Because the volume of the V-shaped tube is accurately known, the density can be determined.



proteins requires a temperature so high that degradation occurs. An alternative approach, which has been used only a few times, is to determine the dry weight from the elemental composition. For example, because the chemical formulas of all of the amino acids and nucleotides are known, the weight of amino acids or nucleotides can be calculated from the weight of nitrogen and phosphorus in a sample, both of which can be measured with an error of no more than 1%. Therefore, the solution used for density determination can be analyzed for nitrogen and phosphorus content and dry weight can be calculated from the amino acid or nucleotide composition of the protein or nucleic acid (which must, of course, be determined if not already known).

**Example 12-A**

☐ Calculation of $\bar{v}$ by pycnometry.

The first step in the use of a pycnometer is to determine its volume. This is done by measuring the weight of water that it can hold. A pycnometer weighs 14.3082 g when empty and 24.2651 g when it is filled with water at 20°C. The density of water at 20°C is 0.9982 g/cm so that the volume is (24.2651 − 14.3082)/0.9982 = 9.9749 cm$^3$. The next step is to determine the density of a solution containing a particular ratio of solute and solvent. Thus a solution is prepared from 3.7184 g of solute and 9.9582 g of water. The pycnometer is filled with this solution (that is, with 9.9749 cm$^3$ of solution, as calculated above) and it weighs 25.8237 g at 20°C. The weight of the solution in the pycnometer is 25.8237 − 14.3082 = 11.5155 g and the density is 11.5155/9.9749 = 1.1544 g/cm$^3$. Finally the change in volume per change in mass is calculated. The solution has a total weight of 3.7184 (solute) + 9.9582 (water) = 13.6766 g and a volume of 13.6766/1.1544 = 11.8474 cm$^3$. The volume of water used in preparation of the solution is 9.9582/0.9982 = 9.9762 cm$^3$. The increase in mass (by addition of the solute) is 3.7184 g and the increase in volume is 11.8474 − 9.9762 = 1.8712 cm$^3$. Thus $\bar{v}$ is 1.8712/3.7184 = 0.503 cm$^3$/g.

Note that for high precision the weights must be corrected for the buoyancy of air, when appropriate.

## Parallel Sedimentation Equilibrium Measurement in $H_2O$ and $D_2O$ Solutions

At equilibrium, the distribution of materials in a centrifuge cell is described by

$$M_H(1 - \bar{v}\rho_H) = \frac{2RT}{\omega^2}\left(\frac{d\ln c}{dr^2}\right)_{H_2O} \tag{1}$$

in which $M_H$ is the molecular weight in $H_2O$, $\rho$ is the density of the aqueous solution, $R$ is the gas constant, $T$ is the absolute temperature, $\omega$ is the angular velocity in radians per second, $c$ is the concentration, and $r$

is the distance from the axis of rotation in centimeters. A similar equation can be written for a $D_2O$ solution. Dividing one equation by the other yields

$$\frac{M_H(1 - \bar{v}_H\rho_H)}{M_D(1 - \bar{v}_D\rho_D)} = \left(\frac{d\ln c}{dr^2}\right)_H \bigg/ \left(\frac{d\ln c}{dr^2}\right)_D \quad (2)$$

in which the subscript D refers to $D_2O$. Howard Schachman and his colleagues have shown that these two equations can be solved to yield $\bar{v}$ because $\bar{v}$ is virtually the same in $H_2O$ as in $D_2O$. The values of $M_H$ and $M_D$ are not the same because the amide hydrogens exchange with deuterium. However, the ratio $M_H/M_D$ is easily calculated from the chemical formula. Thus, simultaneous equilibrium centrifugation analyses, one in $H_2O$ and one in $D_2O$, which allow the cancellation of the $2RT/\omega^2$ terms in equation (1) to obtain equation (2), yield the value of $\bar{v}$, since the $d\ln c/dr^2$ terms are measurable quantities. This method has the great advantages that only a tiny amount of material is needed and $\bar{v}$ is measured at the same time that $M$ is being measured. It is the only method available if material is limiting; yet, in principle, it is not as accurate as the methods using the measurement of density because the difference in the two differentials in equation (2) is small. However, because dry weight determination is generally inaccurate, this method probably gives better precision in practice than the others. A considerable increase in accuracy can be achieved by the use of $D_2^{18}O$, which has recently become available.

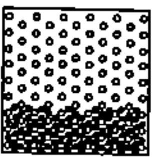
## Measurement of the Diffusion Coefficient

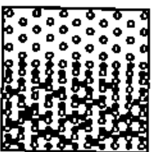
As discussed in Chapter 11, molecular weight, $M$, can be determined quite accurately from a measurement of the sedimentation coefficient, $s$, and the frictional coefficient, $f$. The direct determination of $f$ is very difficult; fortunately, this can be bypassed by measuring the diffusion coefficient, $D$.

Diffusion is the net flow of molecules from a region of high concentration to one of low concentration *if there is no driving force*—that is, the result of random movement (Figure 12-4). The diffusion coefficient, $D$, of a molecule can be simply defined by Fick's first law of diffusion, which states that the number of molecules, $dn$, passing through an area, $A$, in time $dt$ is related to the concentration gradient, $dc/dx$ by this equation:

$$\frac{dn}{dt} = -DA\left(\frac{dc}{dx}\right) \quad (3)$$

The minus sign is introduced so that $D$ is positive. From the fact that molecules will move more slowly if the frictional coefficient, $f$, is large,





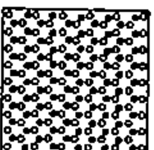



**Figure 12-4**
The mechanics of diffusion. The solid circles are originally located at the bottom of the box. They diffuse upward until they are distributed uniformly throughout the system.

it can be shown that

$$D = \frac{kT}{f} \quad (4)$$

in which $k$ is the Boltzmann constant and $T$ is the absolute temperature. Hence, the Svedberg equation (Chapter 11, equation 1) can be written with $D$ instead of $f$ as

$$M = \frac{RTs}{D(1 - \bar{v}\rho)} \quad (5)$$

in which $R$ is the gas constant, $T$ is the absolute temperature, $\bar{v}$ is the partial specific volume of the macromolecule, and $\rho$ is the solution density, and $M$ can be calculated if $s$ and $D$ are known.

The diffusion coefficient for macromolecules is usually measured by creating a boundary between a buffer and a solution of macromolecules of known concentration and observing the spreading of the boundary with time. The theory behind the measurement is simple, but in practice the measurement is filled with potential error. For example, $D$ for a macromolecule is so small, from $10^{-8}$ to $10^{-5}$ cm²/sec, that it normally takes a day for measurable spreading to occur. During this time, the system must be free of all mechanical disturbances, and thermal convection must be avoided by accurate temperature control. Furthermore, if a molecule is charged (e.g., a net positive charge) the extra $OH^-$ ions in solution, which diffuse more rapidly than the macromolecule, create an electric potential gradient that drives the charged molecules to the region of low concentration. Hence, it is necessary to conduct diffusion experiments at or near the isoelectric point and in the presence of sufficiently high ionic strength to neutralize or eliminate the effect of the developed electric field. In addition, because in theory the molecules must move independently of one another (i.e., they must not collide), it is necessary to extrapolate to infinite dilution.

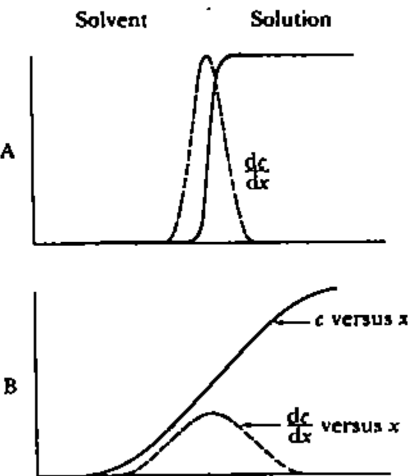



**Figure 12-5**
Measurement of diffusion: (A) initial concentration distribution in which the solvent and the solution are in contact; the curve $c$ versus $x$ shows the concentration of the solution across the cell and $dc/dx$ versus $x$ is the concentration gradient or the schlieren pattern; (B) at a later time.

Each of the methods that make use of boundary spreading starts with a concentration distribution such as that shown in Figure 12-5A, and the change in time is measured as shown in Figure 12-5B. The measurement is facilitated if the concentration gradient is observed by schlieren optics (Chapter 11). If $A$ and $H$ are the area and height of the schlieren curve,

$$4\pi Dt = \left(\frac{A}{H}\right)^2 \tag{6}$$

and a plot of $(A/H)^2$ versus $t$ gives a straight line of slope $4\pi D$ (Figure 12-6).

The initial boundary can be prepared in several ways. In a standard diffusion cell, the solvent is layered onto the solution of macromolecules or a very thin zone is formed between two solutions having two different densities.

These procedures are, however, frought with experimental difficulties. This is because diffusion occurs very slowly. (The time required for a molecule to move a particular distance $d$ is proportional to $d^2$.) For example, it takes several hours for a typical protein to produce a boundary 1 mm wide and about a week to reach 1 cm. A typical diffusion experiment requires several days during which time many external agents may disturb the spreading boundary. Small temperature changes cause convective flow within the solution; to reduce this, the temperature variation must be kept to less than 0.01°C. Mechanical vibrations are a major problem although they may be minimized by using special "vibration-free" mounts for the apparatus. Both problems are reduced somewhat by using a stabilizing density gradient. The usual criteria for



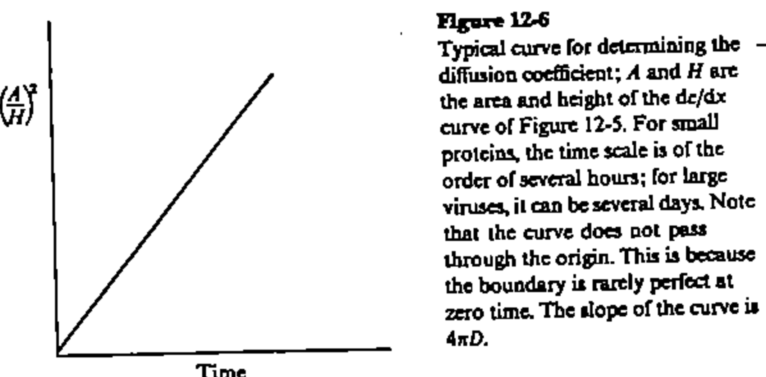



**Figure 12-6**
Typical curve for determining the diffusion coefficient; $A$ and $H$ are the area and height of the $dc/dx$ curve of Figure 12-5. For small proteins, the time scale is of the order of several hours; for large viruses, it can be several days. Note that the curve does not pass through the origin. This is because the boundary is rarely perfect at zero time. The slope of the curve is $4\pi D$.

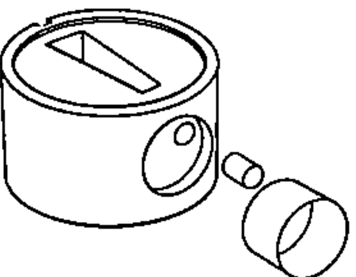


**Figure 12-7**
One type of synthetic boundary cell (valve type) for the Beckman ultracentrifuge: an aluminum centerpiece with a flat-bottomed cavity in place of the usual filling hole (see aluminum centerpiece in Figure 11-3). There is a small, round hole, which has a groove on its perimeter (not shown), on the bottom of the cavity in which a rubber plug is placed. A large cup containing a hole in which the plug fits snugly is placed in the cavity. This cup contains the solvent. The sector of the centerpiece contains the solution. At rest, the plug prevents entry of the solvent, but, during centrifugation, the plug is compressed and solvent leaks from the cup along the groove into the sector. [Courtesy of Beckman Instruments.]

a "disturbance-free" measurement is linearity of the curve shown in Figure 12-6.

The initial boundary can also be formed in a special analytical centrifuge cell—a synthetic boundary cell as shown in Figure 12-7, in which, at a critical speed, solvent passes through a fine capillary into the solution. The centrifuge is then operated at a speed sufficiently low that sedimentation does not occur appreciably. This method is useful if samples of only

small size are available. In a variation of the centrifugal method, the cell is filled with the solution only and centrifuged: a boundary forms as a result of the sedimentation and the spreading of the boundary as sedimentation proceeds is measured, but a different equation is needed to analyze the data.

A remarkable method—optical mixing spectroscopy—involving the scattering of a laser light beam, has been developed. Because the molecules in solution undergo translational motion (i.e., diffusion), there is a Doppler shift in the scattered light so that the scattered light has a slightly different frequency. This frequency shift is measured by mixing the scattered and unscattered light (i.e., beating the scattered light against the incident light) and measuring the beat frequency (i.e., the frequency difference). This frequency can be simply related to $D$. This method gives a 1% error, uses small samples (a few hundred $\mu g$), is very rapid, and is not affected by convection or electrical effects. Its only disadvantage is that it often fails with very highly extended molecules. The technique has not yet become popular.

To date, there has been no satisfactory measurement of $D$ for large nonspherical molecules such as DNA, because $D$ is so small for such molecules.

In Chapter 11, the utility of the value of $D$ in determining $M$ was explained. Another important use is to give information about the shape of a macromolecule. How this is done is shown by the following argument. In any arrangement in which diffusion is occurring, molecules are moving from a region of high concentration to one of low concentration. The driving force is determined by the concentration difference; however, as soon as the movement begins, the driving force is counteracted by the frictional drag of the particles moving through the solvent. Stokes' Law expresses the frictional force on a sphere of radius $a$ moving through a fluid of viscosity $\eta$ at constant velocity, and a simple calculation yields the value of $D$ for a sphere—namely,

$$D = kT/6\pi a\eta \tag{7}$$

The units of this equation are poise for $\eta$ (=0.01 for water at 20°C), cm for $a$, and a value of $1.38 \times 10^{-16}$ erg deg$^{-1}$ for $k$; $D$, therefore, is in cm$^2$/sec. The mass $m$ of a sphere is $\frac{4}{3}\pi a^3\rho$, in which $\rho$ is the density. Thus,

$$D_{\text{sphere}} = \frac{kT}{6\pi\eta}\left(\frac{4\pi\rho}{3m}\right)^{\frac{1}{3}} \tag{8}$$

The density can be approximated as the reciprocal of the partial specific volume $\bar{v}$ so that

$$D_{\text{sphere}} = \frac{kT}{6\pi\eta}\left(\frac{4\pi}{3m\bar{v}}\right)^{\frac{1}{3}} \tag{9}$$



If the mass (in grams) of a molecule is known, from an independent measurement, it is possible to calculate the value of $D$ that the molecule would have *if the molecule were spherical*. The difference between that value and the experimentally observed value is a measure of the departure of the shape of the molecule from that of a sphere.

It is common to rewrite these expressions in terms of the frictional coefficient $f$, using equation (4) and the notation $f_0$ for the frictional coefficient of a sphere. Thus,

$$f_0 = 6\pi\eta\left(\frac{3m\bar{v}}{4\pi}\right)^{\frac{1}{3}} \tag{10}$$

If $f$ is a measured value, the frictional ratio $f/f_0$ (also called the Perrin factor) is a measure of the departure of the molecule from spherical shape.

Several quantitative theories have been developed for relating the value of $f/f_0$ to molecular dimensions. In each theory, the value of $f$ is calculated for simple regular shapes, just as has been done for the sphere. Since molecules never have a really simple shape, the information provided by these theories is primarily suggestive. The two shapes examined in greatest detail are the oblate and prolate ellipsoid. An oblate ellipsoid is a disc-shaped ellipsoid generated by rotating an ellipse about its short axis. A prolate ellipsoid is rodlike (actually more like a cigar or an American football) and is generated by rotating an ellipse about its long axis. The ratio of the long axis of either ellipsoid to its short axis is called the *axial ratio*. A commonly used relation between the axial ratio and $f/f_0$ is shown in Table 12-1. Note that it is not really necessary to distinguish the oblate and prolate ellipsoids until the axial ratio is greater than ten.

The following examples show how to use equation (10) and Table 12-1.

**Example 12-B**

☐ Estimation of the shapes of two proteins from a diffusion measurment.

Protein P has a molecular weight of 40,000, $D = 9.3 \times 10^{-7}$ cm$^2$/sec, and $\bar{v}$ is 0.75 g/cm$^3$ (a typical value for a protein). The value of $m$ is $40{,}000/6 \times 10^{23} = 6.67 \times 10^{-20}$ g. Assuming that $D$ is measured in water at 20°C so that $\eta = 0.01$ poise (usually $\eta$ would be measured for the solvent used in the diffusion measurement), then $f_0 = 4.31 \times 10^{-8}$. From the value of $D$, $f = kT/D = (1.38 \times 10^{-16})(293)/(9.3 \times 10^{-7}) = 4.35 \times 10^{-8}$ g cm$^{-1}$ sec$^{-1}$. Thus $f/f_0 = 1.01$ and the shape of the molecule is not very different from a sphere. Actually one means by this that the molecule could fit neatly in a sphere.

Myosin, a muscle protein, has a molecular weight of 493,000, $\bar{v} = 0.728$ cm$^3$/g, and $D = 1.1 \times 10^{-7}$ cm$^2$/sec. A calculation similar to that just performed yields $f/f_0 = 3.65$. Referring to Table 12-1 the axial ratio of myosin is very large. Other measurements indicate that it is more like a prolate ellipsoid than an oblate one so that its

**Table 12-1**
Values of $f/f_0$ for Prolate and Oblate Ellipsoids Having Various Axial Ratios.

| Axial ratio | $f/f_0$ prolate | $f/f_0$ oblate |
|---|---|---|
| 1 | 1.00 | 1.00 |
| 2 | 1.04 | 1.04 |
| 3 | 1.11 | 1.11 |
| 4 | 1.18 | 1.17 |
| 5 | 1.25 | 1.22 |
| 6 | 1.31 | 1.28 |
| 8 | 1.43 | 1.37 |
| 10 | 1.54 | 1.46 |
| 15 | 1.78 | 1.64 |
| 20 | 2.00 | 1.78 |
| 30 | 2.36 | 2.02 |
| 40 | 2.67 | 2.21 |
| 50 | 2.95 | 2.38 |
| 60 | 3.20 | 2.52 |
| 80 | 3.66 | 2.77 |
| 100 | 4.07 | 2.93 |

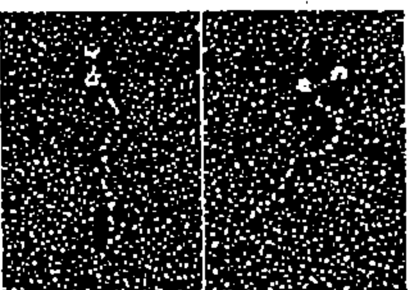


**Figure 12-8**
An electron micrograph of two myosin molecules prepared by the replica method. Note the two-headed region of the protein attached to the long tail. [From S. Lowey, H. S. Slayter, A. G. Weeds, and H. Baker, *J. Mol. Biol.* 42(1969):1.]

axial ratio is about 80. Figure 12-8 shows an electron micrograph of myosin taken many years after the diffusion measurement was done; the predicted high axial ratio is clearly correct although the diffusion measurement could not have indicated the dual beads at the end of the polypeptide strand.

**Table 12-2**
Several Results of Diffusion Measurements.

| Molecule | $M$ | $D$ (cm²/sec) | $\bar{v}$ (cm³/g) | $f/f_0$ | Maximum possible axial ratio |
|---|---|---|---|---|---|
| Pancreatic ribonuclease | 13,683 | $1.19 \times 10^{-6}$ | 0.728 | 1.14 | 3.4 |
| Egg-white lysozyme | 14,100 | $1.04 \times 10^{-6}$ | 0.688 | 1.32 | 6.1 |
| Bovine serum albumin | 66,500 | $6.1 \times 10^{-7}$ | 0.734 | 1.31 | 6.0 |
| Human fibrinogen | 330,000 | $1.98 \times 10^{-7}$ | 0.706 | 2.35 | 31.0 |
| DNA | $2.5 \times 10^7$ | $<10^{-8}$ | 0.55 | $\gg 12$ | known to be $6 \times 10^4$ |
| Tobacco mosaic virus | $4 \times 10^7$ | $4.4 \times 10^{-8}$ | 0.73 | 2.19 | 24.0 |

Values of $D$ and the axial ratios for several molecules are listed in Table 12-2.

Diffusion measurements can also give information about the arrangement of subunits in a protein because different arrangements would have distinct shapes. This is shown in the following example.

**Example 12-C**

☐ The arrangement of subunits in hemoglobin.

Hemoglobin consists of four subunits, each having a molecular weight of 16,125; the value of $D$ for a subunit is $1.13 \times 10^{-6}$ cm²/sec. Repeating the calculation in Example 12-B yields a value of $f/f_0 = 1.1$ so that the axial ratio of a subunit is about 3 (Table 12-1). The value of $D$ for the tetramer is $6.9 \times 10^{-7}$ cm²/sec so that $f/f_0 = 1.16$. Thus, the axial ratio of hemoglobin is about 3.5. This means that the subunits cannot be arranged linearly but must form a compact cluster; if there were a linear array, the axial ratio of hemoglobin would be about 12 and $f/f_0$ would have to be about 1.6.

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## *PROBLEMS*

12–1. The partial specific volumes of amino acids have different values if the amino acids are in LiCl and KCl. Would you expect this to be true of proteins? Explain and estimate the magnitude of the effect.

12–2. Would $\bar{v}$ of RNA differ if the RNA were in NaCl rather than $MgCl_2$?

12–3. A pycnometer is being used to measure $\bar{v}$ of a solute. The pycnometer weighs 14.2056 g if empty and 24.1305 g if filled with $H_2O$ at 20°C. A solution is prepared by dissolving 3.5921 g of the solute in 9.9413 g of $H_2O$. The pycnometer is filled with this solution at 20°C and weighs 25.5307 g. What is $\bar{v}$ for the solute? (The density of $H_2O$ at 20°C is 0.9982 g/cm$^3$.)

12–4. A macromolecule is known to bind the $Hg^{2+}$ ion. How is $\bar{v}$ for the molecule to which the $Hg^{2+}$ ion is bound related to $\bar{v}$ of the molecule lacking the metal ion?

12–5. If in determining $D$ from measurements of $A$ and $H$, $(A/H)^2$ plotted against $t$ produces a curve rather than a straight line, what conclusion might you draw? Suppose that the curve has two distinct components, each asymptotic to a straight line. What conclusion might you draw?

12–6. A macromolecule with $\bar{v} = 0.74$ cm$^3$/g is sedimented in $H_2O$ at 20°C; $s_{20,w}$ is 14.2 S; $D_{20} = 5.82 \times 10^{-6}$ cm$^2$/sec. What is the molecular weight?

12–7. In the absence of convection, the width of a sedimentation boundary, observed in an analytical ultracentrifuge, is determined almost entirely by diffusion. Indeed, the rate of boundary spreading can be used to determine $D$. Would you expect the measurement of $D$ to be more accurate at high or at low centrifugal speed? Explain. Would it ever be reasonable to perform such a measurement with a mixture of two components? When?

12–8. Will $D$ increase or decrease as axial ratio increases? Which has the greater $D$, a rigid rod or a flexible rod, both having the same length and cross section and made of the same material?

12–9. Answer the following:

a. What is the diffusion coefficient in water at 20°C of a virus particle whose molecular weight is $5 \times 10^7$ and which is spherical? The particle is assumed to be 50% protein (density = 1.3 g/cm$^3$) and 50% DNA (density = 1.7 g/cm$^3$). The viscosity of water is 0.01 poise.

b. Many phages have long protein tails used for attachment to bacteria. If this phage had a tail, would $D$ be greater or smaller than the value for a spherical, tailless phage?

12–10. A macromolecule whose molecular weight is 22,600,000 and density is 1.79 g/cm$^3$ has a value of $D$ of $2.1 \times 10^{-8}$ cm$^2$/sec in water at 20°C. What may you conclude about the shape of the molecule?

12–11. A protein has a molecular weight of 366,000 and a diffusion coefficient of $2.58 \times 10^{-7}$ cm$^2$/sec in water at 20°C. After heating to 75°C and restoring the temperature to 20°C, the molecular weight is found to be 61,000 and the diffusion coefficient is $1.07 \times 10^{-6}$ cm$^2$/sec. What can be said about the structure of the molecule?

12–12. A protein is sedimented at 25°C in pure water (density = 0.998 g/cm$^3$). Its sedimentation coefficient is $8.6 \times 10^{-13}$ sec and the diffusion coefficient is $6 \times 10^{-7}$ cm$^2$/sec. What is the molecular weight? $\bar{v} =$ 0.74 cm$^3$/g.

12–13. The sedimentation coefficient and the diffusion coefficient for a particular protein are found to be $18.3 \times 10^{-13}$ sec$^{-1}$ and $4.62 \times 10^{-7}$ cm$^2$/sec at 20°C. The partial specific volume of the protein is 0.73 cm$^3$/g. What is the molecular weight of the protein? At 20°C the density of water is 0.998 g/cm$^3$.

11–28. No. The bands would be too wide.

11–29. The density difference between [$^{14}$N]DNA and [$^{15}$N]DNA = 0.015 g/ml. Therefore, the density gradient is 0.015/1.32 g cm$^{-3}$ mm.$^{-1}$ Because *E. coli* is 50% G+C, the difference in density of each from that of *E. coli* DNA is 0.098 × 0.20 = 0.0196 g/cm$^3$. This corresponds to a distance of (0.0196/0.015) × 1.32 = 1.72 mm from *E. coli* DNA.

11–30. $Mg^{2+}$ must be bound to the DNA in CsCl and MgDNA is less dense than CsDNA.

11–31. When the molecule is 10% replicated, it contains 90 units of density equal 1.75 g/cm$^3$ and 20 of density = $\frac{1}{2}$(1.75 + 1.7) = 1.725 g/cm$^3$. Therefore the density of the partially replicated molecule is (1/110)[(90)(1.75) + (20)(1.725)] = 1.745 g/cm$^3$.

11–32. The density, $\rho$, of $^{14}$N- and $^{15}$N-labeled DNA is 1.700 g/cm$^3$ and 1.714 g/cm$^3$, respectively. The density of the linked DNA is

$$\frac{M_1 + M_2}{(M_1/\rho_1) + (M_2/\rho_2)}$$

where the subscripts 1 and 2 refer to the normal and deleted DNA, respectively. Using 30.24 × $10^6$ instead of 30 × $10^6$ for the value of $M_1$ for [$^{15}$N]DNA, the value of $M_2$ of the molecule with the deletion is 22.5 × $10^6$.

11–33. ($\frac{1}{3}$)(1.700) + ($\frac{2}{3}$)(1.300) = 1.433 g/cm$^3$.

11–34. Since the speed and temperature are the same when both DNA and transfer RNA are studied, the relation between $M$ and band width $d$ can be written $M = k\rho/d^2$, in which $k$ is a constant including all of the necessary factors and $\rho$ is the density. Thus, for two different molecules 1 and 2, $d_2 = d_1\sqrt{\rho_2 M_1/\rho_1 M_2}$. The densities of DNA and RNA are 1.7 and 1.8 g/cm$^3$, respectively, so that the band width of the tRNA is 42.3 mm, which is larger than the length of an analytical centrifuge cell.

11–35. All fragments do not have exactly the same G+C content so that there is density heterogeneity, which spreads the band thus yielding an artificially low value of the molecular weight.

11–36. a. They are identical.
b. With saturating amounts of ethidium bromide, the nicked circle, like a linear molecule, binds more ethidium bromide than a covalent circle. Therefore the density of a nicked circle is less than that of a covalent circle.

11–37. The sample contains fragments. These produce a broader band that is superimposed on the narrower main band, which makes the observed band broader than a band without fragments.

11–38. In CsCl, one obtains the molecular weight of CsDNA. In NaCl it is for NaDNA. Since the nucleotide molecular weight is 330 and $M_{NaCl}$ = 23 and $M_{CsCl}$ = 137, if the $M_{NaDNA}$ = 25 million, then $M_{CsDNA}$ is [(137 + 330)/(23 + 330)] × 25 = 33 million.

11–39. Use equation (13) with $a$ = 6.425 cm, $c_a$ = 1.012 mg/cm$^3$, $r$ = 6.703 cm, and $C_r$ = 6.905 mg/cm$^3$ and remember to express angular velocity in radians per second [20,000 rpm = (2000)(2$\pi$)($\frac{1}{60}$) = 2094 radians per second]. The value of $R$ in the appropriate units is 8.314 × $10^7$ erg deg$^{-1}$ mol$^{-1}$ and $T$ = 293 K. Thus, $M$ = 20,600.

11–40. The molecule consists of two linked covalent circles; each has the same molecular weight. One nick yields a covalent circle linked to a nicked circle; the density of this structure is an average of 1.55 and 1.60 or 1.575 g/cm$^3$. In alkali, the nicked strand falls off (this is C) leaving behind a denatured double-stranded covalent circle linked to a single circle (B). This structure contains three units of molecular weight compared to one in C; this accounts for the 3:1 ratio of areas of B and C.

11–41. The fast and slow material consist of circles and linears respectively. A survival of 1/$e$ = 0.37 is obtained after irradiation with 2000 rads. This is the dose that produces, on the average, one break per circle.

11–42. Two-thirds of the molecules in the DNA sample are covalent circles; one-third are open circles or linear molecules. If there were an average of one break per molecule, 1/$e$ = 0.37 of the covalent circles would not receive a break. Therefore, 0.37 × ($\frac{2}{3}$) = 0.246 of the molecules would remain covalent circles and 0.754 would not. The ratio of the area of the denser band to that of the lighter band would be 0.246/0.754 = 0.326.

## Chapter 12

12–1. The effect would be quite small for proteins because the cause of the effect for amino acids is that $Li^+$ and $K^+$ can bind to the free carboxyl groups and alter the density of the molecules. Proteins have few free carboxyl groups.

12–2. Yes, because of the relative mass increases due to the binding of $Na^+$ and $Mg^+$ to the phosphates.

12–3. The pycnometer holds 9.9249 g of $H_2O$ and therefore has a volume of 9.9249/0.9982 = 9.9428 cm$^3$. The weight of the solution in the pycnometer is 11.3251 g and the density is 11.3251/9.9428 = 1.1390 g/cm$^3$. The solution consisting of 3.5921 g of solute and 9.9413 g of $H_2O$ has a total weight of 13.5334 g and a volume of 13.5334/1.1390 = 11.8818 cm$^3$. The volume of water used to prepare the solution is 9.9413/0.9982 = 9.9592 cm$^3$. Hence, if the increase in mass is 3.5921 g, the change in volume is 11.8818 − 9.9592 = 1.9226 cm$^3$. Therefore, $\bar{v}$ = 1.9226/3.5921 = 0.535 cm$^3$/g.

12–4. Binding of the $Hg^{2+}$ ion increases the density and thus decreases $\bar{v}$.

12–5. Curvature usually means that the sample is not homogeneous but contains many components. However, thermal convection can cause curvature. If the curve has two components, the sample contains two types of molecules.

12–6.
$$M = \frac{s^0_{20,w} RT}{D^\circ(1 - \bar{v}\rho)}$$
$$= \frac{14.2 \times 10^{-13}}{5.82 \times 10^{-6}} \cdot \frac{(8.3100 \times 10^7)293}{1 - 0.74(0.9982)}$$
$$= 2.275 \times 10^5.$$

12–7. Lower speed is preferable because this would allow a longer time for diffusional spreading to occur. A mixture of two components could be measured as long as the two boundaries are totally resolved.

12–8. Decrease due to increased friction. The flexible rod has greater $D$ because it encounters less friction.

12–9. a. $D = kT/f$ and for a sphere, $f = 6\pi r\eta$, in which $r$ is the radius. The mass $m$ of the sphere $= \frac{4}{3}\pi r^3 \rho$, in which $\rho$ is the density, which is 1.5 g for the virus. The mass must be expressed in grams so that $m = (5 \times 10^7)/(6 \times 10^{23}) = 8.3 \times 10^{-17}$ g. Substituting all values, $D = 9.1 \times 10^{-8}$ cm²/sec.

b. Smaller, because the asymmetry of the particle will increase friction.

12–10. Repeating the calculation of problem 12-9a, $D$ would be $1.26 \times 10^{-7}$ cm²/sec if the molecule were spherical. The value of $f/f_0$ is 5.7 so that the molecule is very long and thin.

12–11. The molecule has six subunits. Repeating the calculation of problem 12–9, the values of $D$ for the protein and one subunit, if each were spherical, would be $4.45 \times 10^{-7}$ cm²/sec and $8.1 \times 10^{-7}$ cm²/sec, respectively. The values of $f/f_0$ for the subunit and the protein are 1.30 and 1.03 respectively. Thus, the axial ratio of the subunit is 6 and that of the protein is 2 and the six subunits could not be arranged end-to-end but could be in a hexagonal array.

12–12. $2.28 \times 10^{-19}$ or a molecular weight of $1.37 \times 10^4$.

12–13. $6 \times 10^{-19}$ or a molecular weight of $3.6 \times 10^4$.

## Chapter 13

13–1. At the higher NaCl concentration, intrastrand hydrogen bonds form and the single-stranded DNA is more compact; hence $[\eta]$ is lower. In formaldehyde, the amino groups of the bases are titrated and intrastrand hydrogen bonds cannot form. There would be no effect in 0.01 M NaCl because there are no hydrogen bonds in the absence of formaldehyde. In 0.5 M NaCl, the single-stranded DNA would be less compact if formaldehyde were present so that $[\eta]$ would be greater than 3 ml/g. However, $[\eta]$ would be less than 20 ml/g because, in 0.5 M NaCl, the negatively charged phosphates would be neutralized and a random coil could be assumed.

13–2. The bacteria have become permeable to proteins and the intracellular proteins have come out of the cells.

13–3. (a) The sphere with larger radius; (b) the solid sphere; (c) the rigid rod; (d) the lollipop.

13–4. Both will be the same; see equation (2).

13–5. (a) The circle because in a shear field it will be deformed to a structure of half length and double thickness; (b) the denatured DNA because it will be very compact owing to intramolecular hydrogen bonds; (c) the DNA in 1 M NaCl because it will be more compact; (d) they will be nearly the same, but the twisted circle will be slightly more resistant because it will be shorter.

13–6. If $[\eta]$ increases, it is probably compact. If $[\eta]$ decreases, it is probably an extended fiber. With a decrease of $[\eta]$ uncertainty arises because the



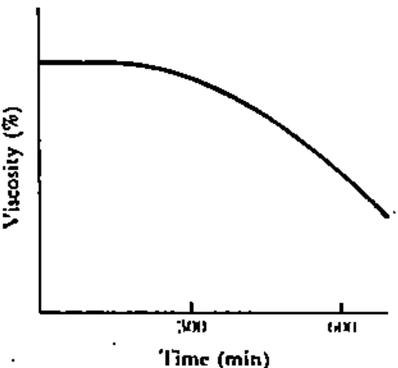
protein might consist of subunits that dissociate in 6 M guanidine chloride.

13–7. The ions might disrupt the quasi-crystalline water lattice (i.e., decrease the interaction between water molecules).

13–8. The polynucleotide has become more extended. If the ionic strength is low, the pH change might break intrastrand base pairing with the result that charge repulsion between the phosphates would produce an extended single-stranded molecule. At high ionic strength, a likely possibility is that the polynucleotide would undergo a transition from being single-stranded to being double-stranded.

13–9. The DNA sample probably contains basic proteins. At low ionic strength, the proteins bind to the DNA and cause intramolecular aggregation, which decreases viscosity. If the DNA is diluted before decreasing the ionic strength, the concentration of DNA and protein can be sufficiently low that aggregation does not occur.

13–10.




13–11. Both $\eta/r$ and $[\eta]$ would be higher after joining.

13–12. The viscosity should decrease as the optical density increases.

## Chapter 14

14–1. The frequency is $2989 \times c = 2989(3 \times 10^{10}) = 8.97 \times 10^{13}$ s⁻¹. The wavelength = 3345.6 nm. It is in the infrared part of the spectrum.

14–2. Tyrosine absorbs ultraviolet light; isoleucine does not.

14–3. a. 1/100 = 0.01 M.

b. Use equation (3). The concentration is 0.02 M.

c. The molecular weight is 78 so that the concentration of benzene is 10.26 M. Thus, 0.001 cm would have an absorbance of 1.

14–4. 32 μg/ml = 0.032 g/l = 0.032/423 = 0.000076 M. Therefore, $\varepsilon$ = 0.27/0.000076 = 3552 M⁻¹ cm⁻¹.

14–5. By mutual dilution of both A and B, the optical densities of the mixture should be $OD_{260} = 0.301$ and $OD_{450} = 0.27$. There appears to be an interaction between A and B. It has been assumed that Beer's law is obeyed during the dilution.