

Thus far, we have derived Fick's First Law and have shown how it could be used to describe the passive transport of molecules across membranes.

Fick's First Law

$$J_k^x = -D \frac{dC_k(x)}{dx} \quad \text{flux of species } k \text{ along } x\text{-direction}$$

where $D \equiv$ diffusion coefficient

According to Einstein,

$$D = \frac{k_B T}{f} \quad \text{where } f \equiv \text{frictional coefficient}$$
$$= \lambda_k k_B T \quad \lambda_k \equiv \text{mobility of species } k$$

Extend to 3-Dimensions

$$\vec{J}_k = -D \vec{\nabla} C_k \quad \text{if diffusion is isotropic.$$

If the medium is anisotropic, then

$$\begin{pmatrix} J_k^x \\ J_k^y \\ J_k^z \end{pmatrix} = - \begin{pmatrix} D_{xx} & 0 & 0 \\ 0 & D_{yy} & 0 \\ 0 & 0 & D_{zz} \end{pmatrix} \begin{pmatrix} \frac{dC_k}{dx} \\ \frac{dC_k}{dy} \\ \frac{dC_k}{dz} \end{pmatrix}$$

↑↑
Diffusion tensor \underline{D}

\underline{D} is diagonal as shown if the principal axes are chosen; otherwise \underline{D} is non-diagonal:

$$\underline{D} = \begin{pmatrix} D_{x'x'} & D_{x'y'} & D_{x'z'} \\ D_{y'x'} & D_{y'y'} & D_{y'z'} \\ D_{z'x'} & D_{z'y'} & D_{z'z'} \end{pmatrix}$$

x, y, z are related to x', y', z' by a simple rotation of the coordinate system.

Frictional Coefficient

f can be related to the size and shape of a molecule.

Stokes: For a sphere of radius r ,

$$f = 6\pi r\eta$$

where $\eta \equiv$ viscosity of the medium in which the sphere is diffusing

If the macromolecule is spherical and unsolvated, you can determine its radius from the frictional coefficient using the Stoke's relationship.

The measured f for a macromolecule is usually larger than the value predicted by the Stoke's relationship for a molecule of the same volume.

Reasons: Macromolecule is (1) solvated
(2) nonspherical

Effect of Solvation on f

A certain amount of solvent is usually associated with a macromolecule in solution. This solvation increases the effective or hydrodynamic volume of the macromolecule and hence its friction coefficient. The effect can be estimated by measuring the partial specific volume of the macromolecule in solution.

Say you add g_2 grams of the macromolecule to the solution:

$$\text{partial specific volume, } \bar{V}_2 = \left(\frac{\partial V}{\partial g_2} \right)_{T_1 P_1 g_1}$$

For a solvent-impermeable object, such as an insoluble bead, the increase in volume is just equal to the volume of the bead. Such is not the case with a typical macromolecule, because water may solvate and even penetrate the macromolecule.

In general, when a solute (macromolecule) is added to a solution, the volume is increased due to the volume of the added solute, but there may be a compensating decrease arising from solvation if the bound solvent has a smaller volume than the free solvent.

We can describe this mathematically as follows:

$$\bar{V}_2 = v_2 + \delta_1(v_1 - v_1^0)$$

where $\bar{V}_2 \equiv$ partial specific volume of solute (cm^3 / g)

$v_2 \equiv$ specific volume (cm^3 / g) of unsolvated solute

$v_1 \equiv$ specific volume (cm^3 / g) of solvent when it is bound to the solute

$v_1^0 \equiv$ specific volume (cm^3 / g) of "free" solvent
(reciprocal of density of "free" solvent)

$\delta_1 \equiv$ grams of solvent bound to macromolecule per gram of solute

Note that when $\delta_1(v_1 - v_1^0)$ is negative and the decrease is greater than v_2 , $\bar{V}_2 < 0$; that is, the partial specific volume is negative.

Example: Addition of MgSO_4 to water at concentrations below 0.07 M.

Rearranging, we have

$$v_2 + \delta_1 v_1 = \bar{V}_2 + \delta_1 v_1^0$$

The left-hand side is merely the specific volume of the solvated solute.

Therefore

$$\begin{aligned} V_s &= \frac{M}{N_A} (v_2 + \delta_1 v_1) \\ &= \frac{M}{N_A} (\bar{V}_2 + \delta_1 v_1^0) \end{aligned}$$

where $V_s \equiv$ volume of solvated macromolecule (solute)

The partial specific volume \bar{V}_2 and the hydration δ_1 have been measured for many proteins and nucleic acids in aqueous solution (buffer).

$$\bar{V}_2$$

For most proteins, $\bar{V}_2 = 0.7$ to $0.75 \text{ cm}^3/\text{g}$. For double-stranded DNA, $\bar{V}_2 = 0.4$ to $0.5 \text{ cm}^3/\text{g}$.

$$\delta_1$$

Values of hydration δ_1 range from 0.2 to 0.6 grams of water per gram of protein for proteins, and 0.5 to 0.7 for DNA.

r, f

If the macromolecule is spherical,

$$\frac{4\pi}{3} r^3 = V_s = \frac{M}{N_A} (\bar{V}_2 + \delta_1 v_1^0)$$

$$\text{or } r = \left[\frac{3M}{4\pi N_A} (\bar{V}_2 + \delta_1 v_1^0) \right]^{1/3}$$

and the frictional coefficient

$$f = 6\pi\eta \left[\frac{3M}{4\pi N_A} (\bar{V}_2 + \delta_1 v_1^0) \right]^{1/3}$$

Because of solvation, f is increased. Typically, solvation increases the frictional coefficient of a protein or nucleic acid by 10–20%.

It is customary in the field to define

$f_0 \equiv$ frictional coefficient of solvated sphere

$f_{\min} \equiv$ frictional coefficient of unsolvated sphere

$$\frac{f_0}{f_{\min}} = \left(\frac{\bar{V}_2 + \delta_1 v_1^0}{\bar{V}_2} \right)^{1/3} \approx 1.1 - 1.2$$

Effect of Shape on Frictional Coefficient

The frictional coefficient of a nonspherical macromolecule is greater than that of a spherical one of the same volume. Let

$f \equiv$ frictional coefficient of macromolecule of interest

$f_0 \equiv$ frictional coefficient of a sphere of same volume

$f_{\min} \equiv$ frictional coefficient of unsolvated sphere

Then

$$\frac{f}{f_{\min}} = \left(\frac{f}{f_0}\right) \left(\frac{f_0}{f_{\min}}\right)$$

where $\left(\frac{f}{f_0}\right) \equiv$ shape factor

$\left(\frac{f_0}{f_{\min}}\right) \equiv$ solvation

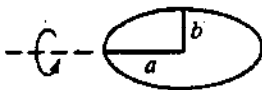
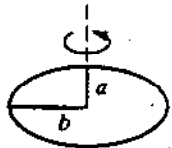
$\left(\frac{f}{f_0}\right)$ has been calculated for various single geometrical forms (see Table 3-1). It can be as large as ~ 4 for a prolate ellipsoid (football shape solute); e.g., a long rod. It can be as large as ~ 3 for an oblate ellipsoid (pancake shape solute); e.g., a flat disk.

Since $\frac{f_0}{f_{\min}} \sim 1.1$ to 1.2 , shape has a much larger effect on the frictional coefficient than solvation.

Note that the above concepts are useful for describing rigid macromolecules, not flexible ones. To illustrate the problem, consider a large DNA molecule, for which length \gg diameter. We know that the DNA is coiled up in solution like a loose ball of thread and there is a lot of solvent associated with the “ball.” Hydrodynamically, the solvent molecules within the domain of the coiled macromolecule can move freely, independent of the motion of the macromolecule, as the macromolecule moves through the solution; or they can be trapped by the macromolecule and move with it. These two limiting possibilities are called “free draining” or “nondraining,” respectively. The ideas expounded here are applicable for the nondraining limit. For DNA, the amount of solvent hydrodynamically associated with the DNA is large, so the nondraining limit applies.

For flexible coils, the frictional coefficient $f \propto M^{1/2}$ or $D \propto M^{-1/2}$.

Table 6.2 Ratio of frictional coefficient of ellipsoid of revolution to frictional coefficient of sphere of same volume, f/f_0 , as a function of axial ratio

		f/f_0	
		(a) <i>Prolate ellipsoids</i> Ellipsoid of revolution generated by an ellipse of major axis a and minor axis b revolving about its major axis (football)	(b) <i>Oblate ellipsoids</i> Ellipsoid of revolution generated by an ellipse of major axis b and minor axis a revolving about its minor axis (pancake)
Major axis	Minor Axis		
1		1.000	1.000
2		1.044	1.042
3		1.113	1.105
4		1.182	1.166
5		1.250	1.223
6		1.314	1.277
8		1.433	1.373
10		1.543	1.458
12		1.644	1.534
14		1.739	1.603
16		1.829	1.667
18		1.914	1.727
20		1.995	1.783
25		2.184	1.909
30		2.357	2.020
35		2.518	2.120
40		2.668	2.212
45		2.811	2.293
50		2.947	2.375
60		3.201	2.519
70		3.437	2.648
80		3.658	2.765
90		3.867	2.873
100		4.066	2.974

Electrophoresis

Electrophoresis is the separation of macromolecular species according to their electrophoretic mobility. Here the thermodynamic driving force is $z|e|\vec{E}$

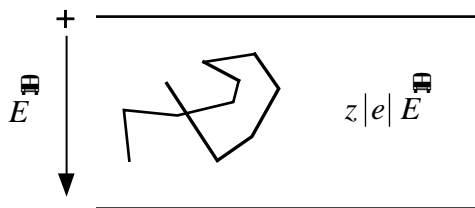


Figure 3-1

and the limiting velocity of migration of the charged macromolecule in a nonconducting solvent is given by

$$f\vec{v} = z|e|\vec{E} \quad \text{or}$$

$$\vec{v} = \frac{z|e|\vec{E}}{f}$$

where $|e| = 1.6 \times 10^{-19}$ coulomb, E is in volt / cm

or the electrophoretic mobility (u), i.e., velocity per unit field is

$$u = \frac{\vec{v}}{E} = \frac{z|e|}{f}$$

In principle, macromolecules can be separated according to their electrophoretic mobilities. For example, HbA (normal) and sickle cell HbS can be readily separated this way. HbS differs from HbA by the substitution of valine for glutamic acid in each of the two β -chains. As a consequence, the two proteins differ by two charges per molecule. The f 's are otherwise identical.

In general, the interpretation of electrophoretic mobility is complicated because

- (1) the solvent is not nonconducting;
- (2) small ions are always present as counterions, buffers and so on;
- (3) the charged macromolecule is surrounded by an atmosphere of small ions.

Nevertheless, electrophoresis is a powerful analytical tool for the separation of charged macromolecules (according to their electrophoretic mobilities).

Isoelectric Focusing

Isoelectric focusing is the separation of charged macromolecules according to their isoelectric point. At the isoelectric point, the net charge on a macromolecule = 0

$$\sum_k z_k |e| = 0, \quad \text{for all residues}$$

and its electrophoretic mobility $u = 0$. So if a pH gradient is set up between the two electrodes in an electrophoresis experiment, the macromolecule will migrate to its isoelectric point and come to rest there. When a mixture of proteins is introduced, the proteins will be separated according to their varying isoelectric pH's or isoelectric points.

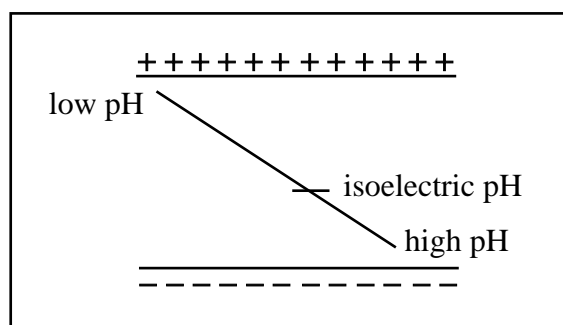


Figure 3-2

Gel Electrophoresis

Gel electrophoresis is electrophoresis through a gel, a 3-D polymer network dispersed in a solvent. A variety of gels have been used.

- (a) Agarose gel (polysaccharide obtained by agar in aqueous medium).
- (b) Acrylamide gel (a copolymer of acrylamide ($\text{CH}_2 = \text{CH} - \text{CO} - \text{NH}_2$) and N, N'-methylenebisacrylamide ($\text{CH}_2 = \text{CH} - \text{CO} - \text{NH} - \text{CH}_2 - \text{NH} - \text{CO} - \text{CH} = \text{CH}_2$) in aqueous medium); the degree of cross-linking depends on the ratio of bis-compound to acrylamide.

In gel electrophoresis:

- (a) The actual path traveled by a macromolecule through the porous gel is considerably longer than the gel tube.
- (b) The gel imposes additional frictional resistance to the macromolecules as they move through pores of comparable dimensions.
- (c) The macromolecules may interact with the gel network, e.g. through electrostatic interactions if the gel network contains charged groups.
- (d) The parts of the gel with pores smaller than a particular macromolecular species are impassable.
- (e) It may be exploited to separate macromolecules of different charges!
- (f) It may be exploited to separate macromolecules of varying sizes and shapes.

Molecular Weight Determination of Proteins by Gel Electrophoresis

Recall $u = \frac{z|e|}{f}$

The determination of the molecular weight of macromolecules by gel electrophoresis is complicated by the fact that u depends on both the charge and f . The charges on a protein vary from protein to protein. Also, f depends on shape. Before gel electrophoresis can be useful for molecular weight determination, you must make the charge and f contribution to $u \propto$ number of residues.

To accomplish this, denaturing gels were introduced.

- (a) Treat proteins with the anionic detergent, sodium dodecylsulfate (SDS) and 2-mercapto-ethanol; the latter disrupts sulfur-sulfur linkages in proteins; the combination denatures protein.
- (b) At SDS concentrations $> 10^{-3}$ M, a nearly constant amount of SDS is bound per unit weight of protein (~ 0.5 detergent molecule/amino acid residue). So

$$\text{charge of protein - SDS complex} = \text{charge of SDS } (-1) \times \# \text{ of bound SDS} \\ + \text{protein charge}$$

$$\cong \text{charge of bound SDS}$$

$$\propto M$$

$$f \text{ of SDS complex} \propto M^{1/3}$$

Thus, under these conditions, the mobilities of the SDS-treated proteins are determined by their molecular weights. Empirically,

$$\log M = a - bx$$

where $M \equiv$ molecular weight

$x \equiv$ distance of migration in the gel

$a, b \equiv$ constants for a given gel at a given electric field

Fig. 6.18 Electrophoretic mobilities of proteins as a function of the acrylamide concentration in the gel. The acrylamide concentrations illustrated are 15% (\blacktriangle), 10% (\bullet), 7.5% (\square), and 5% (\circ). The weight ratio of acrylamide to methylenebisacrylamide is 37:1. The numbers 1 to 11 refer to the following proteins: β -galactosidase, phosphorylase α , serum albumin, catalase, fumarase, aldolase, glyceraldehyde-phosphate dehydrogenase, carbonic anhydrase, trypsin, myoglobin, and lysozyme. [From K. Weber, J. R. Pringle, and M. Osborn, *Methods Enzymol.* 26, 3 (1972).]

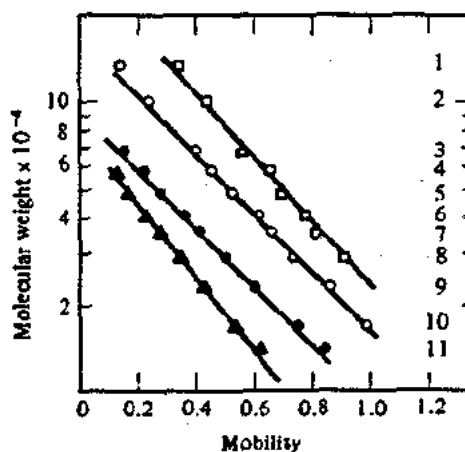


Figure 3-3

This method doesn't always work. Deviations for the relation occur when a protein binds an abnormal amount of SDS (such as glycoproteins) or when the protein carries a number of charges (such as histones or highly acidic/basic ones).

Gel Electrophoresis of Double-Stranded DNA

At neutral pH's, large DNA's behave like random coils with charge densities that are uniform along the length of the molecules. Therefore, expect gel electrophoresis to work well. Very good resolution is obtained for $MW < 10^7$. For the smaller fragments, gel electrophoresis provides a versatile and accurate method of molecular weight measurements.

The method is also capable of distinguishing among DNA's of different topological forms. Circular DNA's, supercoiled DNA's have different electrophoretic mobilities from those of linear DNA molecules of the same molecular weight.

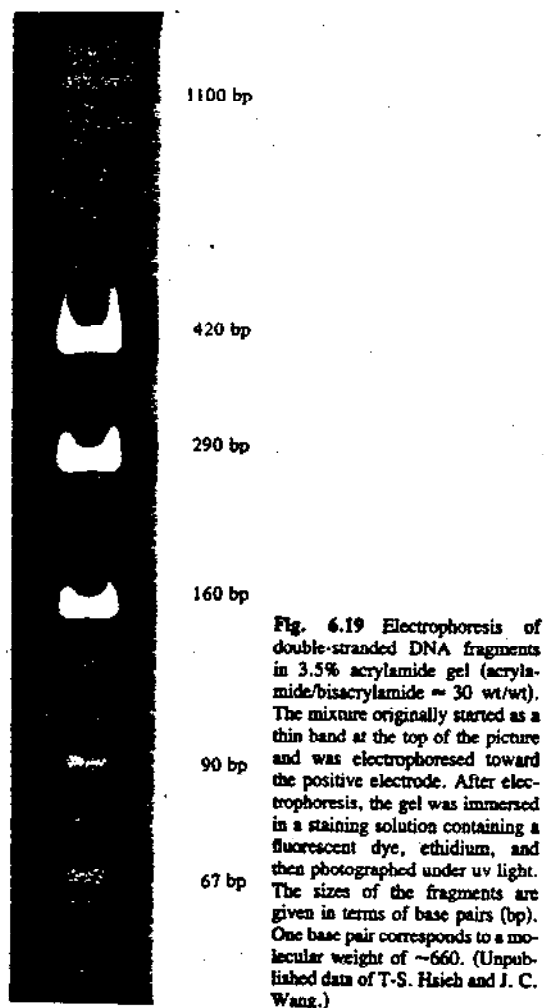


Figure 3-4

Gel Electrophoresis of Single-Stranded Nucleic Acids

Single-stranded nucleic acids usually contain regions of complementary base sequences. As a consequence, intramolecule base pairing can occur. Such pairings affect frictional coefficients, so electrophoretic mobility of single-stranded nucleic acids is not a simple function of molecular weight.

To obviate this difficulty, electrophoresis is carried out in a gel containing a high concentration of urea, which disrupts base pairing. The mobility of the nucleic acid is determined by the number of phosphate groups in the strand.

Sequencing Gel Electrophoresis

Maxam and Gilbert¹ and Sanger et al.² used this method of sequencing nucleic acids. For example:

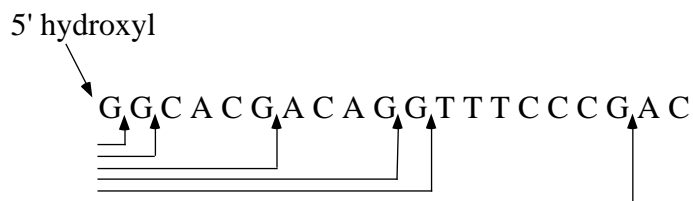


Figure 3-5

The procedure consists of the following:

- (a) Label the strand at the 5' hydroxyl group of the first nucleotide with radioactive ^{32}P .
- (b) Cleave after guanines (G).
- (c) Separate oligonucleotides according to their μ 's on a denaturing gel (7 M urea).
- (d) Repeat steps a through c, but cleave after adenines (A).
- (e) Repeat steps a through c, but cleave after cytosines (C).
- (f) Repeat steps a through c, but cleave after thymines (T).

This method has the resolution of 1 base!

¹ A. M. Maxam and W. Gilbert, *Proc. Nat. Acad. Sci. USA* **74**, 560-564 (1977).

² Sanger et al., *Proc. Nat. Acad. Sci. USA* **74**, 5463-5467 (1977).

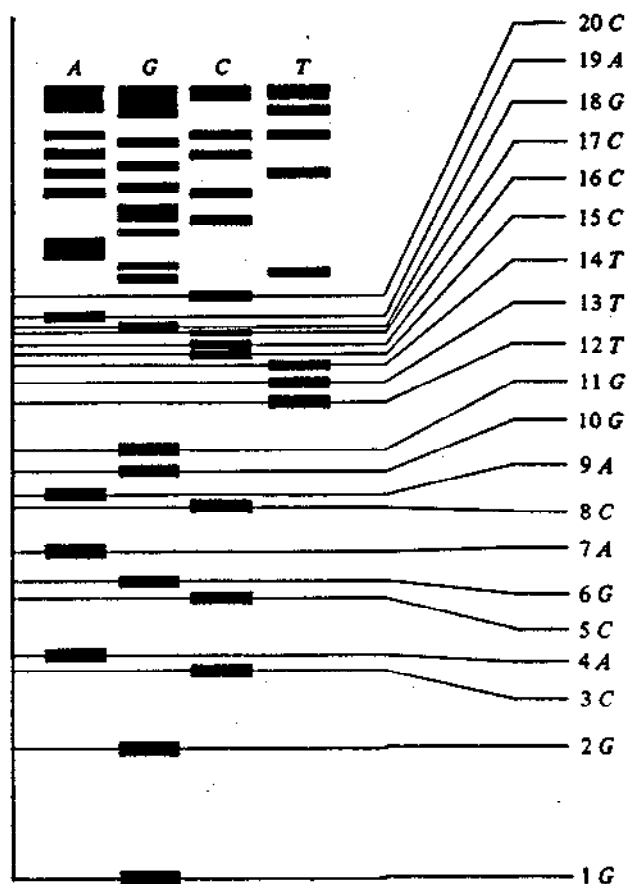


Fig. 6.20 Schematic illustration of a sequencing gel electrophoresis pattern. The sequence of the first 20 bases of a single strand of DNA is shown. The strand is labeled at the 5' hydroxyl group of the first nucleotide with radioactive ^{32}P . The strand is broken at adenines (A), guanines (G), cytosines (C), and thymines (T) and placed in four lanes at the top of a denaturing gel of 20% acrylamide, 0.67% methylene bisacrylamide, 7 M urea. After electrophoresis, autoradiography for 8 h produced the pattern shown. The sequence is now simply read off. To continue the sequence determination, longer electrophoresis times must be used. [A.M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. USA* 74, 560-564 (1977).]

Figure 3-6