3

Basic Biophysical Concepts and Methods

3.1 Chapter Overview

This chapter describes some of the concepts that have been developed to describe and examine the biophysical properties of biological systems at the molecular and cellular level. A comprehensive coverage would include the techniques that have been developed to describe the molecular folding and conformational transitions involved in the functioning of enzymes and nucleic acids; the forces that stabilise the structures of proteins in membranes; cable theory analyses of axons and muscle fibres, for example. Some of the material covered in this chapter will go some way to provide an introduction to such topics, but the main emphasis is to provide an understanding of the main biophysical concepts of relevance to engineers and scientists working in areas such as the development and applications of biosensors, microfluidics and lab-on-chip technologies.

After reading this chapter readers will gain a basic understanding of:

(i) electrostatic forces acting on ions in solution, in membranes and at charged surfaces, and the concept of the Debye screening length;
(ii) the principal modes of transport (diffusion, osmosis, active) of ions and molecules across membranes;
(iii) hydrophobic and hydration forces acting on bioparticles;
(iv) the concepts of osmolarity, tonicity and osmotic pressure of relevance to cells;
(v) electrochemical gradients and ion distributions across membranes;
(vi) osmotic properties of cells;
(vii) the passive and active electrical properties of membranes and cells;
(viii) membrane equilibrium potentials, action potentials and ion channel conduction;
(ix) voltage and patch clamp techniques for investigating membrane ion channels;
(x) biological applications of electrokinetic effects (electrophoresis, electro-osmosis, dielectrophoresis) and electrowetting on dielectric.
3.2 Electrostatic Interactions

3.2.1 Coulomb’s Law

Coulomb’s law, also known as Coulomb’s inverse square law, describes the electrostatic interaction between electrically charged particles, and is named after the French scientist Charles Augustin de Coulomb. The two most significant charged particles are the proton and electron, which carry positive and negative charges of $1.6 \times 10^{-19}$ coulombs, respectively. Charged atoms or molecules in solution are called ions. Positively charged ions will migrate in an electric field to the negative electrode (the cathode) and are called cations, whilst negatively charged ions are called anions and will migrate to the positively charged anode.

Coulomb’s law states that:

*The magnitude of the Electrostatics force of interaction between two point charges is directly proportional to the scalar multiplication of the magnitudes of charges and inversely proportional to the square of the distances between them.*

The idealised point charges are considered to be small in size compared to their separation distance $r$, and the force $F$ (units of newton) acting simultaneously on such point charges $q_1$ and $q_2$ is given by

$$F = K \frac{q_1 q_2}{r^2}. \quad (3.1)$$

A positive value for $F$ implies that the force is repulsive, while a negative force implies it is attractive. In Equation (3.1) the constant $K$ is the coulomb force constant, which in SI units is given by

$$K = \frac{1}{4\pi \varepsilon_0}. \quad (3.2)$$

and is related to the defined electromagnetic properties of free space (vacuum) by the equation derived by James Clerk Maxwell:

$$c^2 = \frac{1}{\mu_0 \varepsilon_0}, \quad (3.3)$$

where $c$ is the speed of electromagnetic radiation (light) in vacuum ($2.99792458 \times 10^8$ m s$^{-1}$) and $\mu_0$ is the magnetic permeability of free space, defined as $4\pi \times 10^{-7}$ H m$^{-1}$. From Equations (3.2) and (3.3) the permittivity of free space $\varepsilon_0$ is derived to be $8.8541872 \times 10^{-12}$ F m$^{-1}$ and the Coulomb force constant as $8.9875517871 \times 10^9$ N m$^2$ C$^{-2}$. In electrostatic units (esu) and gaussian units (statcoulomb) the unit charge is defined in such a way that the Coulomb constant $K$ in Equation (3.1) is a dimensionless quantity equal to 1. The inverse square law described by Equation (3.1) results from the force field due to an isolated point charge being uniform in all directions and attenuating with distance $r$ at the same rate as the expansion of the surface area $(4\pi r^2)$ of a sphere centred on the point charge.
Equation 3.1 represents the scalar form of Coulomb’s law. To obtain both the magnitude and the direction of the force we require the vector-form:

\[ F = \frac{1}{4\pi\varepsilon_0} \frac{q_1 q_2 (r_1 - r_2)}{|r_1 - r_2|^3} = \frac{1}{4\pi\varepsilon_0} \frac{q_1 q_2}{r^2} \hat{r}_{21}. \] (3.4)

The direction of the force \( F \) is given by the unit vector \( \hat{r}_{21} \), lying parallel with the line directed from charge \( q_2 \) to \( q_1 \). If the two charges are alike (either both positive or both negative) the product \( q_1 q_2 \) in Equation (3.4) is positive and charge \( q_1 \) will experience a repulsive force of direction given by \( \hat{r}_{21} \). If the two charges have opposite signs, the product \( q_1 q_2 \) is negative and an attractive force will be felt by charge \( q_1 \) directed along \( -\hat{r}_{21} \). These vector relationships are shown in Figure 3.1.

Coulomb’s law can be applied to describe the forces that hold together the atomic constituents in a molecule, as well as the forces that bind atoms and molecules together to form solids and liquids. It can also be used to describe the force between the positively charged nucleus and each of the negatively charged electrons in an atom. Equations 3.1 and (3.4) assume that the two charges are stationary or moving slowly. This is known as the electrostatic approximation. Rapidly moving charges create magnetic fields that alter the force on the two charges.

Two charges are shown in Figure 3.1, but Coulomb’s law can be extended to derive the force acting on a point charge as a result of its electrostatic interaction with any number of other point charges. This is achieved using the law of superposition by the vector addition of the individual electrostatic forces that act alone on that point charge. The force vector \( F \) acting on a small point charge \( q \) by a system of \( N \) discrete charges is thus given by:

\[ F = \frac{q}{4\pi\varepsilon_0} \sum_{i=1}^{N} \frac{q_i (r - r_i)}{|r - r_i|^3} = \frac{q}{4\pi\varepsilon_0} \sum_{i=1}^{N} \frac{q_i}{R_i^2} \hat{R}_i, \] (3.5)

where \( q_i \) and \( r_i \) are the magnitude and position respectively of the \( i^{th} \) charge, \( \hat{R}_i \) is a unit vector pointing in the direction from charge \( q_i \) to charge \( q \), and \( R_i \) is the magnitude of the distance of separation between charges \( q_i \) and \( q \). The resultant force vector \( F \) lies parallel to the electric field vector at the position of the point charge \( q \), with that point charge removed.

\[ F_1 \quad \hat{r}_{21} \quad F_2 \]

Figure 3.1 Two point charges, distance \( r \) apart, experience a force \( F \) given by Coulomb’s law. If the two charges are of the same polarity, charge \( q_1 \) will experience a repulsive force directed along the unit vector \( \hat{r}_{21} \). If the two charges are of opposite polarity the direction of the attractive force acting on charge \( q_1 \) is given by \( -\hat{r}_{21} \).
The work \( W \) required (at constant temperature and pressure) to bring a charge \( q_1 \) from infinity to a distance \( r \) from charge \( q_2 \) is given by:

\[
W = -\int_{\infty}^{r} F \, dr = -\frac{q_1 q_2}{4\pi \varepsilon_0} \int_{\infty}^{r} \frac{1}{r^2} \, dr = \frac{q_1 q_2}{4\pi \varepsilon_0} \frac{1}{r}.
\] (3.6)

Performing work of this amount increases by the same amount the potential energy \( U \) of the system of two charges. The potential energy is therefore given by:

\[
U = \frac{q_1 q_2}{4\pi \varepsilon_0} \frac{1}{r} = q_1 \phi
\] (3.7)

with

\[
\phi = \frac{q_2}{4\pi \varepsilon_0} \frac{1}{r}.
\] (3.8)

The parameter \( \phi \) represents the Coulomb potential arising from the charge \( q_2 \). The difference between potential energy \( U \) (units of Joule) and potential \( \phi \) (units of Volt) should be noted and understood. If the product \( q_1 q_2 \) in Equation (3.7) is positive then work is required to bring the two charges closer together and the potential energy \( U \) is positive. On the other hand, if the product \( q_1 q_2 \) is negative then work is required to keep the two charges apart and the potential energy assumes a negative value.

The magnitude of the electric field \( E \) created by the single point charge \( q_2 \) at a distance \( r \) from that charge is given by the negative gradient of the potential at that point:

\[
E = -\nabla \phi = \frac{1}{4\pi \varepsilon_0} \frac{q_2}{r^2} \hat{r}.
\] (3.9)

Symbol \( \nabla \) is the gradient operator, \( \hat{i}(\partial/\partial x) + \hat{j}(\partial/\partial y) + \hat{k}(\partial/\partial z) \), with \( \hat{i}, \hat{j}, \hat{k} \) unit vectors along axes \( x, y, z \). For a positive point charge (\(+q\)) the electric field is directed radially away from the location of the charge, and for a negative charge (\(-q\)) it is directed in the opposite sense towards the charge. The magnitude of the force acting on charge \( q_1 \) arising from the electric field created by \( q_2 \) is given by:

\[
F = q_2 E.
\] (3.10)

The values obtained for the potential energy \( U \) and potential \( \phi \) using Equations (3.7) and (3.8) assume that a charge, for example an isolated ion, is located in a vacuum. For an ion located in a solution, account must be taken of how easily the molecules surrounding the ion can be electrically polarised. This polarisation will counteract the local electric field \( E \) and reduce the magnitudes of \( U \) and \( \phi \). The polarisability of a material is determined by its dielectric permittivity \( \varepsilon = \varepsilon_0 \varepsilon_r \), where \( \varepsilon_r \) is known as the relative permittivity and is sometimes also referred to as the dielectric constant (\( \varepsilon_r \) varies with temperature and the ac frequency of an applied electric field, and so is not a constant). For ions located in a medium
other than vacuum, the equations for the potential energy, potential and electric field are modified to the forms:

\[
U = \frac{q_1 q_2}{4 \pi \varepsilon_0 \varepsilon_r r} = q_1 \phi \\
\phi = \frac{q_2}{4 \pi \varepsilon_0 \varepsilon_r r} \\
E = \frac{1}{4 \pi \varepsilon_0 \varepsilon_r} \frac{q_2}{r^2} \hat{r}
\]

Vacuum is completely unresponsive in terms of its electrical polarisability and so will have \(\varepsilon_r = 1\). At 298 K and low ac frequencies, water, ethanol and benzene exhibit \(\varepsilon_r\) values of around 78.5, 24, and 2.3, respectively. The relatively high \(\varepsilon_r\) value for water is related to the \(\text{H}_2\text{O}\) molecule possessing a large dipole moment (see Figure 3.2) enhanced by the formation of hydrogen bonds to neighbouring water molecules, as discussed in Chapter 1 (Sections 1.2.4 and 1.3.1). An isolated water molecule consists of two polar \(\text{O}--\text{H}\) bonds, each having a dipole moment of \(5 \times 10^{-30}\) C m (1.51 debye units). The \(\text{O}--\text{H}\) bond length \((l)\) in a water molecule is 95.8 p.m., so that its dipole moment \((= \delta q)\) implies that the magnitudes of \(\delta^+\) and \(\delta^-\) defined in Figure 3.2 are each \(5 \times 10^{-20}\) C, corresponding to about one-third of a full electronic charge \(q\). The vector sum of these two dipolar bonds gives a total dipole moment for a water molecule of \(6.2 \times 10^{-30}\) C m (1.855 D). As shown in Figure 3.2 the convention is to direct the dipole moment vector from the negative charge to the positive charge. A dipole will experience a torque when subjected to an external electric field, tending to align the dipole vector with the field to give an orientation of lower potential energy. The orientational polarisability of a water molecule in the bulk water phase is increased as a result of the cooperative reorientations required of its neighbouring hydrogen-bonded water molecules. This increases the effective dipole moment of a water molecule in the condensed phase to around \(8.3 \times 10^{-30}\) C m (\(\sim\) 2.5 D) and results in the high relative permittivity exhibited by water. Ethanol possesses a smaller dipole moment (1.69 D) and so exhibits a smaller \(\varepsilon_r\) value. The benzene molecule is symmetrical (see Figure 3.2) and so has a zero dipole moment and

![Figure 3.2](image-url)
does not exhibit orientational polarisability. Its relative permittivity value of 2.3 arises solely from electronic polarisations.

Because we are manipulating the charges under the conditions of constant temperature and pressure, it follows from the discussion in Section 1.2.7 of Chapter 1 that in thermodynamic terms the potential energy $U$ given by Equation (3.11) takes the form of Gibbs free-energy. From the second law of thermodynamics, contributions to the free-energy $U$ of a system (including a system of charges) in thermal equilibrium with its surroundings, at constant temperature and pressure, will come from its heat energy (enthalpy $H$) and entropy $S$ according to the equation (see also Section 1.2.7 of Chapter 1):

$$U = H - TS.$$  

### 3.2.2 Ions in Water

In an ionic crystal such as NaCl we can estimate, from the Van der Waals radii values given in Table 1.5, that the closest separation distance between a Na$^+$ and Cl$^-$ ion is 0.4 nm. From Equation (3.10), and taking the relative permittivity of a sodium chloride crystal as $\varepsilon_r \sim 6$, the Coulomb energy of attraction of the ion pair is about $9.6 \times 10^{-20}$ J (i.e. $23 kT$ at room temperature). When a crystal of NaCl is placed in water the ions at the crystal surface will interface with a medium of relative permittivity $\varepsilon_r \sim 80$, and when fully immersed their Coulomb energy will approach a value of $4 \times 10^{-21}$ J (1.8 $kT$), which is only a little more than the energy of $3 kT/2$ associated with thermal fluctuations and insufficient to result in stabilisation of the ion pair. This is why salt crystals dissolve and dissociate in water.

To understand this dissociation process more clearly we can find the relative contributions of the enthalpy and entropy changes from Equation (3.14). The entropy $S$ term can be approximated as

$$S = -\frac{\partial}{\partial T} \left( \frac{q_1 q_2}{4\pi \varepsilon_0 \varepsilon_r} \right) = \frac{q_1 q_2}{4\pi \varepsilon_0 \varepsilon_r^2} \frac{\partial \varepsilon_r}{\varepsilon_r} = U \frac{1}{\varepsilon_r} \frac{\partial \varepsilon_r}{\varepsilon_r}.$$  

(3.15)

The value of $\varepsilon_r$ for water at 273 K is 87.9 and to a good approximation between 273 and 315 K exhibits a percentage fall of $-0.4\%$ per Kelvin. From Equation (3.15) this gives $S = -4 \times 10^{-3} U$. The value of $TS$ in Equation (3.14) at 298 K is thus $-1.19 U$, in other words larger than the total free energy $U$! This implies that the attraction of sodium and chloride ions is mainly driven by entropy and not enthalpy. A dominating negative value for $TS$ implies that work has been expanded on the system of ions and water so as to create a more ordered system. The ions themselves are only weakly associated and so this increase in order must be associated with how the water molecules interact with the ions. This interaction involves the torques induced on the water molecules by the interactions of their dipole moments with the electric fields around the charged ions. This restricts the orientational rotations of the water molecules and creates a ‘hydration shell’ of orientated water molecules around an ion, as schematically depicted in Figure 3.3. This reduction of orientational mobility and dipole alignments creates the increase of order responsible for the large negative value we have deduced for $ST$ in Equation (3.14). The reduction of orientational mobility of the water molecules in this ‘hydration shell’ will result in a reduction of the local value of $\varepsilon_r$. 
At room temperature the effective \( \varepsilon_r \) value for the water structure closest to the ion could be as low as 10, instead of the value \( \sim 80 \) for normal bulk water. The actual value for \( U \) will therefore be larger than that predicted by Equation (3.11) if the local structuring of water molecules is ignored.

A positively charged ion will orientate a neighbouring water molecule such that the negative component of its dipole is directed towards the ion. This will serve to screen the ion’s positive charge and to reduce its Coulomb potential \( \phi \). Water molecules will orientate in the opposite sense around a negatively charged ion and so also act to screen this charge. This screening is also enhanced by the tendency on average of neighbouring ions to overcome thermal vibrations and to be attracted to a countercharged ion. So, although the interaction between counter ions is sufficiently weak for their salt to dissociate and dissolve in water, the balance between electrostatic forces and thermal agitation is such that on average ions with the same charge will tend to avoid each other and those of opposite charge to spend more time near each other. This weak association of counter ions will increase as their average separation distance decreases (i.e. the salt concentration increases) and this is reflected in the activity coefficient values given in Table 1.3. For example, from Table 1.3 we can deduce that a 10 and 100 mM solution of KCl will exhibit an osmolarity of 180 and 154 mOsm, respectively, rather than the value of 200 mOsm if it were to act as an ideal ionic solution.

### 3.2.3 The Formation of an Ionic Double Layer

The distribution of ions around a charged particle is determined by the balance between electrostatic forces and thermal agitation. A quantitative description of the distribution of ions in thermal equilibrium in an electrostatic field can be obtained by combining the Poisson equation of electrostatics with the Boltzmann distribution. The Poisson equation generalises the electrostatic Coulomb potential \( \phi(r) \) to a volume distribution of charge density \( \rho(r) \):

\[
\nabla^2 \phi(r) = -\frac{\rho(r)}{\varepsilon_0 \varepsilon_r}.
\]

Equation 3.15 reduces to the Coulomb potential described by Equation (3.8) when \( \rho(r) \) is a point charge in a uniform dielectric medium. For an arbitrary collection of ions of number
densities \((m^{-3})\) \(c_i\) and valences \(z_i\) the charge distribution is given by

\[
\rho(r) = q \sum_i z_i c_i(r),
\]

where \(q\) is the charge on an electron. The earliest model, known as the Helmholtz model, describes the distribution of ions at the interface between a charged surface and an electrolyte as a parallel-plate capacitor. One plate of this capacitor contains the charge on the electrified surface and the other plate (known as the Helmholtz plane) contains the ions, of opposite charge polarity to that on the surface, electrostatically attracted to it from the electrolyte. The thickness of this electrical double layer is taken to be the diameter of the ions attracted to the charged surface. If we define \(\sigma\) to be the surface charge density, it is balanced by an equivalent amount of ionic charge of opposite polarity in the solution. This balance can be expressed by the relationship

\[
\sigma = -\int_0^\infty \rho(r) dr.
\]

In the Helmholtz model the counter ionic charge density \(\rho(r)\) takes the form of a layer of charges at the charged surface. An obvious oversimplification of the Helmholtz model is that thermal motions of the ions in the electrolyte are ignored. These thermal motions will cause \(\rho(r)\) to form a diffused distribution rather than a layer.

The concentration \(c_i\) of ions in thermodynamic equilibrium with the electrolyte solution, as a function of their distance \(r\) from a charged surface, is related to the electrostatic potential \(\phi(r)\) using the Boltzmann distribution as follows:

\[
c_i(r) = c_{i\infty} \exp\left(-\frac{-q z_i \phi(r)}{kT}\right).
\]

The parameter \(c_{i\infty}\) is the concentration of ions in the bulk solution, far enough away from the charged object that the value of \(\phi(r)\) is zero. If the electrolyte is an aqueous sodium chloride solution, for example, this corresponds to equal concentrations \([\text{Na}^+]\) and \([\text{Cl}^-]\) of the sodium and chloride ions. As we move from the bulk solution towards a negatively charged surface, for example, we would find that \([\text{Na}^+]\) increases and \([\text{Cl}^-]\) decreases. Substituting Equation (3.18) into Equation (3.16) gives

\[
\rho(r) = q \sum_i z_i c_{i\infty} \exp\left(-\frac{-q z_i \phi(r)}{kT}\right).
\]

Using this result to eliminate \(\rho(r)\) from Equation (3.15) we obtain the Poisson-Boltzmann equation:

\[
\nabla^2 \phi(r) = -\frac{q}{\varepsilon_0 \varepsilon_r} \sum_i z_i c_{i\infty} \exp\left(-\frac{-q z_i \phi(r)}{kT}\right).
\]

This equation describes the electrical potential \(\phi(r)\) at the interface between a charged object and an electrolyte solution, taking into account the screening of this potential by counterions. For example, it can in principle describe the spatial composition of the ionic ‘atmosphere’ around an ion or a charged particle. For such situations, where \(r\) is the only
relevant coordinate, the appropriate form of the vector operator $\nabla^2$ involves spherical coordinates. However, Equation (3.20) cannot be solved analytically using spherical coordinates. Depending on the geometry of the system and the boundary conditions, solving Equation (3.20) may require the use of approximations. One such approximation is to assume that the electrostatic interactions of the ions in the solution are weak ones, so that $qz_i\phi(r)/kT \ll 1$. This allows the linear form ($e^x = 1 + x + x^2/2! + \ldots$) of the exponential function to be used in Equation (3.20) to give:

$$\nabla^2 \phi(r) = -\frac{q}{\varepsilon_o \varepsilon_r} \sum_i z_i c_{i\infty} \left( 1 - \frac{qz_i \phi(r)}{kT} \right).$$

(3.21)

A further simplification can be made by noting that for a sufficiently large distance $r$ the electrical potential $\phi(r)$ is zero, and $d\phi(r)/dr$ is also zero. This corresponds to electrical neutrality of the solution, so that

$$q \sum_i z_i c_{i\infty} = 0.$$

Adopting this boundary condition as $r$ tends to infinity, Equation (3.21) can then be written as:

$$\nabla^2 \phi(r) = \frac{q^2}{\varepsilon_o \varepsilon_r kT} \sum_i z_i^2 c_{i\infty} \phi(r).$$

(3.22)

Equation 3.22 is referred to as the linear form of the Poisson-Boltzmann equation, and can be written as

$$\nabla^2 \phi(r) = \kappa^2 \phi(r).$$

(3.23)

### 3.2.3.1 The Debye Screening Length

If the variable $\phi(r)$ in Equation (3.23) is transformed to a variable with units of $1/\kappa$, this equation will contain no parameters. This means that $1/\kappa$ must represent a fundamental unit when considering electrostatic interactions in ionic solutions. From Equations (3.22) and (3.23)

$$\kappa^2 = \frac{q^2}{\varepsilon_o \varepsilon_r kT} \sum_i z_i^2 c_{i\infty}$$

From which we can determine that $1/\kappa$ has units of length, and is an important parameter known as the Debye length. We can interpret its significance by stating that for distances $r$ shorter than the Debye length the electrostatic interactions will be strong, but for much larger distances the interactions will be weak because of ionic screening. The factor $1/\kappa$ can thus be taken to be the ionic screening distance. Its value is given by

$$1/\kappa = \sqrt{\frac{\varepsilon_o \varepsilon_r kT}{2q^2IN_A10^3}}.$$

(3.24)
In this equation \( N_A \) is the Avogadro constant \((6.022 \times 10^{23} \text{ mol}^{-1}\)); we have converted the ionic density \( c \) \((\text{m}^{-3})\) to the ionic strength \( I \) \((\text{mol} \text{ l}^{-1})\) of the solution by assuming a simple monovalent salt \((z_i = 1)\) such as NaCl, so that

\[
\sum z_i^2 c_{i\infty} = 2IN_A10^3.
\]

For an aqueous 10 mM solution of NaCl at 298 K we can calculate \( 1/k \) to be 3.07 nm (assuming \( \varepsilon_r = 80 \)). From Equation (3.24) we note that the Debye length is inversely proportional to the square root of the solution’s ionic strength, so that for a 1 M solution it decreases to 0.31 nm. We would expect the ionic screening to increase as the number of ions per unit volume increases. The ionic strength also increases as \( z_i^2 \), so that solutions containing multivalent salts (e.g. CaCl\(_2\)) will be more effective at screening electrostatic interactions.

3.2.3.2 The Gouy-Chapman Equation

If we wish to consider the case of a charged membrane surface, or to approximate the curved surface of a particle as a planar surface, the only important dimension is the distance normal to the surface, which we will take to be the x-direction. In one dimension Equation (3.15) is written as

\[
\frac{d^2 \phi(x)}{dx^2} = -\frac{\rho(x)}{\varepsilon_o \varepsilon_r},
\]

so that Equation (3.20) takes the form:

\[
\frac{d^2 \phi(x)}{dx^2} = -\frac{q}{\varepsilon_o \varepsilon_r} \sum_i z_i c_{i\infty} \exp \left( \frac{-qz_i \phi(x)}{kT} \right).
\]

This form of the Poisson-Boltzmann equation can be solved analytically without converting it to the linear form. For a monovalent salt solution such as NaCl (i.e. \( z = \pm 1 \)) of number density \( c \), and noting that as the distance \( x \) tends to infinity \( d\phi/dx \) tends to zero, integration of Equation (3.26) gives:

\[
\frac{d\phi(x)}{dx} = \sqrt{\frac{2kTc}{\varepsilon_o \varepsilon_r} \cdot (\exp(-q\phi(x)/2kT) - \exp(q\phi(x)/2kT))}.
\]

Using the relationship between the surface charge density \( \sigma \) and the counter charge density \( \rho(r) \) given by Equation (3.17), then from Equation (3.25):

\[
\sigma = \varepsilon_o \varepsilon_r \int_0^\infty \frac{d^2 \phi(x)}{dx^2} \, dx = -\varepsilon_o \varepsilon_r \frac{d\phi(0)}{dx}.
\]
This equation relates the electric field (i.e. the gradient of the electric potential) at the surface to the surface charge density, where we can define \( \phi(0) \) to be the surface potential. Substituting Equation (3.27) into Equation (3.28) we obtain the important Gouy-Chapman equation:

\[
\sigma = -\sqrt{2\varepsilon_0 \varepsilon_r kTc} \cdot \left[ \exp(-q\phi(0)/2kT) - \exp(q\phi(0)/2kT) \right]. \tag{3.29}
\]

If we adopt the linear approximation for the exponential terms and insert the Debye screening length \( 1/\kappa \) introduced for Equation (3.24), the Gouy-Chapman equation reduces to the simple form:

\[
\sigma = \varepsilon_0 \varepsilon_r k \phi(0). \tag{3.30}
\]

Equation 3.30 describes a proportionality between the surface charge \( \sigma \) and the surface potential \( \phi(0) \) analogous to the relationship \( q = VC \) between charge and voltage for a capacitance \( C \). From Equation (3.30) we can consider the term \( \varepsilon_0 \varepsilon_r k \) to be an effective capacitance per unit area, with the Debye length \( 1/\kappa \) representing the distance between the two charge-carrying plates. This further supports the concept of the charge distribution of ions at the interface between a charged surface and an electrolyte taking the form of an electrical double layer.

Integration of Equation (3.27) and adopting the assumption that \( qz_i \frac{\partial \phi(r)}{kT} \ll 1 \), together with the linear form of the exponential, we obtain the relationship:

\[
\phi(x) = \phi(0) \exp(-x\kappa) \tag{3.31}
\]

Equation 3.31 indicates that the electrostatic potential falls exponentially with distance into the electrolyte, reaching a value of \( 0.37\phi(0) \) at a distance equal to the Debye screening length \( 1/\kappa \).

3.2.3.3 Stern’s Modification of the Gouy-Chapman Equation

The assumptions used in deriving Equation (3.29) do not hold for high surface charge densities and high potential gradients (fields). The predicted concentrations of ions attracted to the charged surface can be unrealistically high, sometimes above the saturation level for a salt. Multivalent salt ions can also be attracted so strongly to the surface as to bind to it. Otto Stern introduced two modifications to the Gouy-Chapman theory, described in detail in the book by Aveyard and Haydon ([1], p. 231). The first modification simply takes account of the fact that an ion cannot get closer to the charged surface than its own radius, and the second modification is to allow for specific binding of ions to the charged surface in what is called the Stern layer. Within a distance of the Debye length other ions form a diffuse layer, and the electrostatic potential falls exponentially as described by Equation (3.31), with the potential \( \phi(0) \) being replaced with the value \( \phi(a) \) at the interface of the Stern and diffuse layers. These two ion distributions and the corresponding profile of the electrostatic potential are shown in Figure 3.4.

Bedzyk et al. [2] have determined the ion distribution in an electrolyte solution in contact with a charged polymerised phospholipid membrane using x-ray standing waves, and found it to qualitatively agree with the Gouy-Chapman-Stern model.
The charge densities $\sigma_{St}$ and $\sigma_{Dl}$ in the Stern layer and the diffuse layer add up to the total charge $\sigma$ on the surface, and together these charged layers act as two capacitors in series. The total capacitance $C$ is given by:

$$C = \frac{C_{St}C_{Dl}}{C_{St} + C_{Dl}}. \quad (3.32)$$

The contribution to the total capacitance of the Stern layer tends to be unaffected by changes in the ionic strength of the solution. At low ionic strengths the Debye screening length $1/\kappa$ is relatively large so that the effective capacitance $\varepsilon_0\varepsilon_r\kappa$ of the diffuse layer is low and the total capacitance $C$ given by Equation (3.32) tends to be dominated by the diffuse layer. At high ionic strengths the Debye length is small, $C_{Dl}$ is large and now the total capacitance tends to be dominated by $C_{St}$.

### 3.2.3.4 Activity Coefficient of Ions in Solution

We discussed in Chapter 1 (Table 1.3) and Section 3.2.2 of this chapter that the activity coefficient of ions in solution decreases as the ionic concentration increases. We are now in a position to quantify this effect. The distribution of ions around a central ion in solution will have spherical symmetry, and this can be obtained by writing the linearised form of the Poisson-Boltmann Equation (3.23) in spherical coordinates:

$$\frac{1}{r} \frac{d^2(r\phi(r))}{dr^2} = \kappa^2 \phi(r). \quad (3.33)$$

Integration of this differential equation, after multiplying through by $r$, gives the result

$$r\phi(r) = A \exp(-r\kappa) + B \exp(r\kappa), \quad (3.34)$$

in which $A$ and $B$ are constants of integration. To satisfy the boundary condition that as $r$ tends to infinity, the potential $\phi(r)$ tends to zero, constant $B$ must equal zero. Thus, from Equation (3.34)
\[ \phi(r) = \frac{A \exp(-rk)}{r} \]  

(3.35)

and the electric field \( E(r) \) is given by

\[ E(r) = \frac{d\phi(r)}{dr} = \frac{A \exp(-rk)}{r} \left( \kappa + \frac{1}{r} \right). \]  

(3.36)

We can now use the boundary condition that at the surface of an ion of radius \( a \) there is no ionic screening of the potential, so that the electric field at the surface of the ion is given by Equation (3.13). For \( r = a \), and setting \( E(r) \) in Equation (3.36) to the value given by Equation (3.13), we obtain the following value for \( A \):

\[ A = \frac{q}{4\pi\epsilon_0\epsilon_r} \left[ \frac{\exp(ak)}{1 + ak} \right]. \]

Substituting this result into Equation (3.35) gives

\[ \phi(r) = \frac{q}{4\pi\epsilon_0\epsilon_r} \left[ \frac{\exp(-k(r-a))}{1 + ak} \right]. \]  

(3.37)

This result gives the electrostatic potential energy around an ion in solution. Substituting this into Poisson’s Equation (3.15) in the form of Equation (3.33) the corresponding counter-ion charge density around a single ion is given as

\[ \rho(r) = \frac{qk^2}{r} \left[ \frac{\exp(-k(r-a))}{1 + ak} \right]. \]  

(3.38)

The charge contained in a cylindrical shell of thickness \( dr \) at a distance \( r \) from the ion centre is \( 4\pi r^2 \rho(r)dr \). Plots of this function for two different ionic strengths of NaCl are given in Figure 3.5.

From Figure 3.5 the maximum number density of counterions around an ion occurs at a distance corresponding to the Debye length \( 1/k \). Beyond this distance the central ion is effectively screened from further electrostatic interactions. Equation 3.37 can thus be separated into two terms – the Coulombic term given by Equation (3.12) and the term that describes the ionic screening by the counterion distribution shown in Figure 3.5:

![Figure 3.5](image)

**Figure 3.5** Plots of the counterion charge density around a single ion in water, as a function of radial distance \( r \), for two NaCl solutions. The 10 mM solution at 298 K has a Debye length of 3.07 nm, which reduces down to 0.31 nm for the 1 M solution.
\[ \phi(r) = \frac{q}{4\pi\varepsilon_0 r} + \frac{q}{4\pi\varepsilon_0 r} \left[ \frac{\exp(-\kappa(r-a))}{1 + ak} - 1 \right]. \]

Following the logic described for Equation (3.6) we can calculate the work \( W \) done on the ionic cloud that screens the central ion (the right-hand term of the above equation) on incrementally charging the central ion (the left-hand term) by integrating the right-hand term to give:

\[
W = -\frac{1}{4\pi\varepsilon_0 \alpha} \left( \frac{1}{1 + ak} - 1 \right) \int_0^1 q dq = -\frac{q^2}{8\pi\varepsilon_0 \varepsilon_r} \left[ \frac{1}{\alpha + 1/k} \right].
\] (3.39)

To correct for ionic screening, this work \( W \) is added to the Gibbs free energy \( G \):

\[ G = G^0 + RT \log c. \]

of an ideal solution of ionic concentration \( c \). The activity coefficient \( \gamma \) for a single ion is defined as:

\[ kT \log\gamma = W. \]

At relatively low ionic concentration the Debye screening length \( 1/\kappa \) is much larger than the ion radius \( a \), so that from Equation (3.39) we can approximate the activity coefficient to be

\[ \log\gamma = -\frac{q^2 \kappa}{8\pi\varepsilon_0 \varepsilon_r}. \] (3.40)

Equation 3.40 is known as the Debye-Hückel limiting law for the activity coefficient of an ionic solution. From Equation (3.24) we see that \( \kappa \) is proportional to the square root of the ionic strength of the solution. Equation 3.40 thus predicts the expected result that the activity coefficient approaches zero as the ionic strength approaches zero. All electrolyte solutions are ideal at infinite dilution of their ions.

### 3.2.4 Ion–Dipole and Dipole–Dipole Interactions

The interaction between an ion of charge \( Q \) and a neighbouring water molecule is shown in Figure 3.6. The dipole moment of the water molecule is represented as two equal and opposite charges, \( +\delta q \) and \( -\delta q \), separated by a distance \( d \) of the order 0.1 nm.

\[ \text{Figure 3.6} \quad \text{The interaction between a charge} \ Q \ \text{and a water dipole. The dipole is represented as two equal and opposite charges separated by a distance} \ d. \]
The electrostatic interaction between charge $Q$ and the dipole will be the sum of the Coulomb interaction between $Q$ and $+\delta q$ and that between $Q$ and $-\delta q$:

$$U = \frac{\delta q \cdot Q}{4 \pi \varepsilon_0 \varepsilon_r r^+} - \frac{\delta q \cdot Q}{4 \pi \varepsilon_0 \varepsilon_r r^-},$$

where the distances $r^+$ and $r^-$ are as defined in Figure 3.6. For the case where $d (~0.1 \text{ nm})$ is small compared to $r$, this equation can be approximated through the following steps:

$$U \approx -\frac{1}{4 \pi \varepsilon_0 \varepsilon_r} \frac{\delta q \cdot Q d \cos \theta}{r^2 - (d^2/4) \cos^2 \theta} \approx -\frac{m \cdot Q \cos \theta}{4 \pi \varepsilon_0 \varepsilon_r r^2}.$$

The potential energy of the interaction between the field created by a charge $Q$ and an induced dipole moment is then given by inserting the above expression for the induced moment into Equation (3.41), using Equation (3.9) as the field, and assuming that the induced moment is aligned with the field:

$$U = -\frac{\alpha Q^2}{(4 \pi \varepsilon_0 \varepsilon_r)^2 r^4}.$$

In Chapter 1 the weak attractive force between neighbouring atoms, known as the Van der Waals force, was described in terms of an induced dipole–dipole interaction. In Figure 1.2 this force is shown to vary as $1/r^6$. This represents a very short-range force, and we are now able to approach an understanding of why this is so. Molecules that carry no charge or lack a permanent dipole moment can have an induced moment given to them as a result of an interaction with an electric field. The magnitude of this induced moment ($m_i$) is determined by magnitude of the field and the polarisability $\alpha$ of the molecule according to the relationship:

$$m_i = \alpha E.$$

The potential energy of the interaction between the field created by a charge $Q$ and an induced dipole moment is then given by inserting the above expression for the induced moment into Equation (3.41), using Equation (3.9) as the field, and assuming that the induced moment is aligned with the field:
From Equations (3.11) and (3.41) the potential \( \phi_m \) and field \( E_m (\nabla \phi_m) \) of a dipole of moment \( m \) is given by:

\[
\phi_m = -\frac{m}{4\pi\varepsilon_0\varepsilon_r r^2}, \quad E_m = \frac{3m}{4\pi\varepsilon_0\varepsilon_r r^3}.
\] (3.44)

Using Equation (3.44) for the dipole fields, and following the procedure described to obtain Equation (3.41), the sum of the four Coulomb terms in the interaction between a dipole and an induced dipole leads to the following form for the potential energy:

\[ U \propto \frac{am^2}{r^6}. \]

This provides an insight into the attractive dipole-dipole force represented by the \( 1/r^6 \) term in the Lennard-Jones 6–12 potential described in Chapter 1 and shown in Figure 1.2. The steric repulsion term \( (1/r^{12}) \) simply describes the fact that atoms and their electron shells cannot occupy the same space, and so describes a steep repulsion at a short range that balances out the longer range attraction force. Assigning to the steric repulsion term a \( 1/r^{12} \) dependence is not based on any real physical theory, but is mathematically convenient and describes quite well the experimental data on intermolecular spacing in crystals [3].

### 3.2.5 Ions in a Membrane or Protein

A modified form of Equation (3.11) can be used to calculate the self free-energy (also known as the electrostatic self-energy or Born energy) of a single charge in a medium. The potential energy change when a small increment of charge \( dq \) is brought to the surface of a sphere that already carries a charge \( q \) can be described by:

\[ \partial U = \frac{q\partial q}{4\pi\varepsilon_0\varepsilon_r r}. \] (3.45)

The total work required, and hence the total free-energy, to carry out this charging process from an initial zero charge to a final charge \( Q \), is given by:

\[ U = \frac{1}{4\pi\varepsilon_0\varepsilon_r r} \int_0^Q q\partial q = \frac{Q^2}{8\pi\varepsilon_0\varepsilon_r r^5}. \] (3.46)

As an approximation we can assume the value for the radius \( r \) to be used in Equation (3.46) to be the ionic radius, namely 0.095 nm and 0.133 nm for the sodium and potassium ion, respectively. If the medium surrounding the ion is water we should be careful in choosing the appropriate value for \( \varepsilon_r \). The value of 78.5 at 298 K results from unhindered rotational freedom of a water dipole and the cooperative motions of neighbouring hydrogen bonded water molecule. The concept of permittivity is thus a macroscopic one, and so assigning an effective \( \varepsilon_r \) for the structured water immediately surrounding an ion is not straightforward. The relative permittivity of 5 obtained for water at infrared frequencies, where dipolar orientations contribute nothing to the polarisability, can be taken as the lower value for the first
layer of water molecules around an ion. Normal tetrahedral hydrogen-bond associations will be restricted for the second layer of water, and by the fourth layer the normal bulk dielectric property of water can reasonably be assumed to have been attained. The 1/r dependency given by Equation (3.43) indicates that the coulomb potential is a relatively long range one. Taking a value for \( \varepsilon_r \) of 30 ~ 40 in Equation (3.46) for the case of an ion in water is probably appropriate.

We will now employ Equation (3.46) to estimate the free energy transfer required to take a sodium ion from an aqueous environment into the hydrocarbon interior of a cell membrane. The relative permittivity of the membrane interior can be taken as \( \varepsilon_r \sim 2.35 \) based on values of 2.31 for stearic acid (CH\(_3\)(CH\(_2\))\(_{16}\)COOH) and 2.42 for palmitic acid (CH\(_3\)(CH\(_2\))\(_{14}\)COOH) given in the CRC Handbook of Chemistry & Physics [4]. From Equation (3.21), with \( r = 0.095 \) nm and assigning an effective value for \( \varepsilon_r \) of 35, the potential energy of a Na\(^+\) ion in water is \( 3.4 \times 10^{-20} \) J, and when located in a medium of the same permittivity as a hydrocarbon membrane (\( \varepsilon_r = 2.35 \)) is \( 2.45 \times 10^{-19} \) J. The difference between these potential energies is \( 2.1 \times 10^{-19} \) J, which at 298 K corresponds to an energy difference of 51.3 \( kT \). The factor \( kT \) corresponds to the mean thermodynamic energy available to an ion when it is in equilibrium with its environment at temperature \( T \). A value of 51.3 \( kT \) (127 kJ/mole) therefore represents a considerable energy barrier to be surmounted, and is depicted schematically in Figure 3.7. Equation 3.43 does not take account of the image force that will be present at the interface between two materials of different permittivity, but provided that the membrane thickness is of the order of 4 nm or more (which it is for a cell membrane) this correction can be ignored [5].

A thermally activated process will be required to surmount the energy barrier shown in Figure 3.5, and so the rate of ion transfer will be proportional to \( \exp(-\Delta U/kT) \). For \( \Delta U \) equal to 51.3 \( kT \) this exponential factor has a value of \( 5 \times 10^{-23} \). A pure lipid bilayer therefore presents an impermeable barrier to biologically important ions such as Na\(^+\), K\(^+\) and Cl\(^-\), and even more so for a Ca\(^{2+}\) ion with its double charge. This demonstrates why the presence of ion channels, formed of water-filled proteinaceous pores, is of such significance in cell membranes. The water molecules in these pores maintain the relatively high \( \varepsilon_r \) environment normally experienced by an ion as it crosses the lipid membrane. The fact that some

![Figure 3.7](image)

**Figure 3.7** A large energy barrier (~51 kT units in height) confronts the passage of a sodium ion across a pure bilipid membrane structure. The membrane structure effectively acts as an impermeable barrier to electrically charged particles.
membrane ion channels exhibit selectivity differences of more than 1000 towards different ions also indicates that variations in the molecular structure of the proteins forming the walls are of great importance. Differences in ionic radii and the physical diameter of a particular ion channels type are also considerations of importance.

The effective permittivity of the hydrophobic interior of a protein molecule can be estimated to have the same value as the inner region of a cell membrane [6]. The energy barrier presented to an ion wishing to penetrate into the protein from an external aqueous environment will thus be of the same order as that required of an ion entering a cell membrane. So, unless the protein contains a small pocket of water molecules that are accessible to the surrounding aqueous medium, it is very difficult indeed to place a charge inside a protein. A bare ion lacking an extensive hydration shell would represent a highly disruptive influence if located within a protein. This is why the ionisable amino-acid residues in a protein structure are located on the outside of the molecule and make contact with the surrounding aqueous medium.

3.3 Hydrophobic and Hydration Forces

3.3.1 Hydrophobic Forces

The familiar saying that ‘oil and water do not mix’ is basically a description of the hydrophobic force. This is commonly observed as oily liquids aggregating to form a separate phase from water, or the formation of beads of water on a waxy leaf surface, for example. The aversion (phobia) that nonpolar substances have to water explains the creation of the term ‘hydrophobic’.

At the molecular level the hydrophobic effect is an important driving force in the folding of protein structures. Water soluble proteins have structures in which the side groups of hydrophobic amino acids such as alanine, tryptophan and valine, are brought together to form a hydrophobic core situated as far as possible from the surrounding solvent. Hydrophilic (water-liking) amino acids such as arginine, aspartic acid and lysine, are situated on the protein surface such that their polar or charged side groups interact with surrounding water molecules. The principal driving force behind the protein folding process is the minimising of the number of hydrophobic side groups exposed to water. Hydrophobic forces also drive the formation of cell membranes and the insertion of membrane proteins into their nonpolar lipid interiors. They are also important energetic factors in the tertiary structures of DNA through stacking interactions between hydrocarbon bases.

The hydrophobic effect is entropy driven. It involves neither a repulsive force between nonpolar molecules and water, nor an attractive force between nonpolar molecules. A decrease in entropy results from a hydrocarbon molecule causing a disruption of the normal hydrogen-bond network between water molecules. Each hydrogen-bond has a strength of around 20 kJ/mol. As pictured in Figure 3.8 the nonpolar hydrocarbon is unable to form hydrogen bonds, and so the hydrogen bonding of water molecules at its surface is disrupted and partially reconstructs to form a solvation shell. The water molecules in this shell have restricted rotational and translational mobilities, and this represents an unfavourable free energy of the system. The overall disruptive effect to the system is reduced by hydrophobic molecules aggregating together so as to reduce their surface area exposed to water.
3.3.2 Hydration Forces

Surfaces that consist of polar molecules or ionisable acidic and basic groups attract water dipoles or can form hydrogen bonds with them. They are hydrophilic surfaces. An example is the surface of the phospholipid bilayer structure shown in Figure 2.2, where the polar phosphate head groups also carry a negative charge. As two hydrated lipid bilayers come closer together they experience a repulsive force. Depending on the chemical composition of the phospholipids head group, this force increases exponentially with decreasing separation distance, and prevents them from approaching closer than around 2–4 nm. Measurements of this effect are not performed by physically pushing membrane surfaces together, but by osmotically taking away water and thus reducing the chemical potential of the water [7]. Measurement of the membrane separation as a function of the water potential leads to a value of the hydration force, which is related to the work required to remove the hydration layer on each membrane surface. The water molecules are strongly attracted to the polar and charged head groups and rearrange themselves around these groups as the membrane surfaces approach each other. At very close separation the chemical potential is similar to that of the water molecules shown nearest to an ion in Figure 3.3.

A hydrophilic surface, and especially a cell membrane surface, will carry a net surface charge. Electrostatic interactions should therefore also be involved as membrane surfaces approach each other. The evidence is, however, that such interactions are overcome by the hydration forces for separation distances below around 2.5 nm. The hydration force can be overcome by protein-protein interactions between adjacent membranes, or by divalent cations (e.g. Ca$^{2+}$, Mg$^{2+}$) that can link between two adjacent negative phosphate head charges and also disrupt water molecule binding. The coming together and subsequent fusing of membranes is an important process in cell biology. Hydration repulsion is therefore a key factor in ensuring that membrane fusion is a highly controlled and not a random process.

3.4 Osmolarity, Tonicity and Osmotic Pressure

3.4.1 Osmoles

This refers to the number of impermeable particles dissolved in a solution, regardless of charge. This is important for determining the diffusional movement of water, as for example
across a cell membrane. For substances that maintain their molecular structure when they
dissolve (e.g. a sugar molecule such as glucose, proteins, DNA), the osmolarity and the molarity are essentially the same. For substances that dissociate (e.g. an ionic salt such as NaCl) when they dissolve, the osmolarity is the number of free particles times the molarity. Thus for a pure NaCl solution, a 1 mM solution would be 2 mOsmolar (1 mOsm each for Na and Cl). A 1 mM MgCl$_2$ solution would have an osmolarity of 3 mOsm (for simplicity we are assuming unity activity coefficients). When measured as osmoles per litre, one obtains the osmolarity. For osmoles per kg water, one obtains osmolality.

3.4.2 Calculating Osmolarity for Complex Solutions

As described above, the osmolarity of a simple solution is equal to the molarity times the
number of particles per molecule. However, real solutions can be much more complex. For example:

- proteins with many equivalents/L may only contribute a small amount to the osmolarity, since they consist of a few very large ‘particles’;
- not all the solution volume is aqueous; for example, blood plasma has 7% dissolved proteins and lipids;
- not all ions are free in a solution; cations may be bound to other anions or to proteins.

For complete accuracy, all constituents should be included in the calculation. However, such aspects as those given above can leave many uncertainties when calculating the osmolarity of a solution like blood plasma. Therefore, we sometimes have to take shortcuts that provide us with good approximations.

For example, we can obtain a good estimate for plasma osmolarity by taking the reported Na concentration (mEq/Litre plasma) and doubling this value. This obviously erroneous calculation (given all of the above) gives a result close to the correct one, since the errors tend to cancel each other! In some clinical settings, one must also account for the effects of elevated plasma glucose or urea.

3.4.3 Osmolarity Versus Tonicity

Consider the situation shown in Figure 3.9, where a protein solution is separated from a protein-free buffer solution by a semi-permeable membrane. If buffer components (e.g. salts, small sugars, amino-acids) and solvent may pass through the membrane, but the protein cannot (i.e. is impermeable), then we have a nonequilibrium situation. The chemical activity (effective concentration) of water in the protein solution will be lower than that in the solution on the other side of the membrane; consequently, water will tend to flow into the protein solution. The strength of this tendency is termed osmotic pressure, and can be measured by the amount of excess pressure required to prevent water flow across the membrane. (This device is called an osmometer.)

Osmotic pressure $P_{osm}$ is proportional not only to the concentration $C$ of the solute but also to the absolute temperature $T$:

$$P_{osm} = RTC = \frac{nRT}{V},$$ (3.47)
where \( n \) is the number of mole equivalents of solute, \( R \) is the molar gas constant and \( V \) is the volume in litres (solute molecules in solution behave thermodynamically like gas molecules). The molar gas constant has the value 0.082 \( \text{L atm K}^{-1} \text{mol}^{-1} \) (or 8.314 \( \text{J K}^{-1} \text{mol}^{-1} \)). Like the gas laws, however, this expression for osmotic pressure holds true only for dilute solutions, and so corrections must be made for concentrated solutions and the activity coefficients of electrolytes. For example, to calculate the osmotic pressure of a 100 mM aqueous solution of NaCl we note from Table 1.3 that at 298 K the activity coefficient is 0.78. Thus, the mole equivalent/litre of a 100 mM NaCl salt solution is 

\[
\frac{2}{0.1} \times 0.78 = 0.156 \text{ equiv/L}.
\]

According to Equation (3.47) the osmotic pressure is:

\[
P_{\text{osm}} = \left( \frac{0.156 \text{ mol}}{1 \text{L}} \right) \left( 0.082 \frac{\text{L atm}}{\text{K mol}} \right) (298 \text{ K}) = 3.81 \text{ atm}.
\]

Osmolarity measures the effective gradient of water, assuming that all the osmotic solute is completely impermeant. It is simply a count of the number of dissolved particles. Therefore a 300 millimolar solution of glucose, a 300 millimolar solution of urea, and a 150 millimolar solution of NaCl each have the same osmolarity.

However, a cell placed in each of these solutions would behave very differently. In a 150 mM NaCl solution there would be equal osmotic strengths on both sides of the cell membrane, so that the cell should maintain the same volume. NaCl in its ionic dissociated form cannot cross the membrane, and the cytoplasm is mostly equivalent to a 150 mM NaCl solution. On the other hand urea is very permeable through most cell membranes and so it exerts little osmotic force against a real cell and its membrane. A cell placed in 300 mM urea would rapidly swell because urea would enter the cell down its concentration gradient, followed by water down its activity gradient.

Tonicity is a functional term that describes the tendency of a solution to resist expansion of the intracellular volume. Two solutions are isosmotic when they have the same number of dissolved particles, regardless of how much water would flow across a given membrane barrier. In contrast, two solutions are isotonic when they would cause no water movement across a membrane barrier, regardless of how many particles are dissolved. In the example given

![Figure 3.9](image-url)
above, a 150 mM NaCl solution would be isosmotic to the inside of a cell, and it would also be isotonic – the cell would not swell or shrink when placed in this solution. On the other hand, a 300 mM urea solution, while still isosmotic would cause the cell to swell and burst (due to its permeability). This isosmotic urea solution is not isotonic. Instead, it has a lower tonicity and is termed as being hypotonic. (A solution of higher tonicity is called hypertonic.) These various situations are cartooned in Figure 3.10.

### 3.5 Transport of Ions and Molecules across Cell Membranes

The main function of the plasma membrane is to protect the interior of the cell from the outside world. It controls the passage of incoming and outgoing substances, and maintains the ionic concentrations of various substances. It is also selectively permeable, allowing some molecules into the cell and keeps others out. A good example is the blood–brain barrier, which allows the passage of some substances into the brain, but screens out toxins and bacteria (although HIV and bacterial meningitis can cross this barrier). Substances allowed to cross this barrier include water, CO\(_2\), O\(_2\), glucose, amino acids and antihistamines.

Cells obtain the ions and molecules they need from their surrounding fluid. This involves their transport across membranes – the plasma membrane as well as those membranes that bound the nucleus, endoplasmic reticulum, and mitochondria. The principle modes of transport are diffusion, osmosis, facilitated diffusion and through active transport.

#### 3.5.1 Diffusion

Molecules move spontaneously down their concentration gradient (i.e. from a region of higher to a region of lower concentration) by diffusion, a process that is facilitated by their Brownian motion. As a result of diffusion molecules reach an equilibrium where they are evenly distributed and no net movement of molecules occurs across the membrane. The membrane is permeable to water molecules and other small ones such as oxygen and carbon dioxide. Oxygen is nonpolar and so diffuses very quickly. Carbon dioxide and water molecules are polar, but are also very small and so diffuse freely in and out of the cell. Other substances do not.

If a solute molecule comes into contact with the lipid layer of the membrane, it may enter the lipid phase by virtue of its thermal energy and cross the lipid bilayer, to emerge into the aqueous phase on the other side of the membrane. To leave the aqueous phase and enter the

![Figure 3.10](image-url)  
**Figure 3.10**  
Cells suspended in various solutions. (a) Cells swell and burst in a hypotonic solution such as pure water. (b) Cells are ‘happy’ in an isotonic solution such as physiological strength saline. (c) Cells shrink and shrivel in a hypertonic solution such as a concentrated salt solution.
lipid phase, a solute must first break all its hydrogen bonds with water. This activity requires kinetic energy of ~5 kcal per hydrogen bond. Moreover, the solute molecule crossing the lipid phase of the membrane must dissolve in the lipid bilayer. Its lipid solubility will therefore play a role in determining whether or not it will cross the membrane by simple diffusion.

It is therefore evident that those molecules with a minimum of hydrogen bonding with water will most readily enter the lipid bilayer, whereas the probability is low that polar molecules such as water and inorganic ions will dissolve in the bilayer. Consider for example the structures of two 6-carbon molecules – hexanol and D-mannitol:

\[
\begin{align*}
\text{Hexanol:} & \quad \text{OH} & \text{H} & \text{H} & \text{H} & \text{H} & \text{H} \\
& \quad \text{C} & - & \text{C} & - & \text{C} & - & \text{C} & - & \text{C} & - & \text{H} \\
& \quad \text{H}_2 & \text{H} & \text{H} & \text{H} & \text{H} & \text{H} \\

\text{D-mannitol:} & \quad \text{OH} & \text{H} & \text{OH} & \text{OH} & \text{OH} \\
& \quad \text{C} & - & \text{C} & - & \text{C} & - & \text{C} & - & \text{C} \\
& \quad \text{H}_2 & \text{OH} & \text{OH} & \text{H} & \text{H} & \text{H}_2
\end{align*}
\]

Note the difference in the number of hydroxyl (OH) groups. Hexanol is poorly soluble in water and highly soluble in lipids, whereas mannitol is highly soluble in water and poorly soluble in lipids owing to its hydrogen-bonding capacity. Thus, even though they are of the same size, hexanol diffuses across membranes much more readily than mannitol.

The probability $P$ with which a molecule will cross a membrane can be expressed as:

\[ P = \frac{D_m K}{d}, \]

in which $D_m$ is the diffusion coefficient of the molecule within the membrane (the more viscous the membrane or the larger the molecule the lower this value), $K$ is the partition coefficient of the molecule, and $d$ is the thickness of the membrane. A simple way to determine the partition coefficient is to shake the test substance in a closed tube containing equal amounts of water and olive oil. The partition coefficient $K$ is determined from the relative solubilities in water and oil at equilibrium by the equation:

\[ K = \frac{\text{solute concentration in oil}}{\text{solute concentration in water}}. \]

The diffusion of water through the plasma membrane is of such importance to the cell that it is given a special name: osmosis.

### 3.5.2 Osmosis

Osmosis is a special term used for the diffusion of water through cell membranes. Although water is a polar molecule, it is able to pass through the lipid bilayer of the plasma membrane. Selective transport of water molecules, in single file, also takes place through pores formed by transmembrane proteins called aquaporins (Peter Agre received the 2003 Nobel Prize in Chemistry for their discovery).

Water passes by diffusion from a region of higher to a region of lower water concentration. Osmosis through a selectively permeable membrane is illustrated in Figure 3.11. Water is never transported actively – it never moves against its concentration gradient. However, the concentration of water can be altered by the active transport of solutes, and thus its movement in and out of the cell can be controlled.
3.5.2.1 Hypotonic Solutions

If the concentration of water in the medium surrounding a cell is greater than that of the cytosol, the medium is said to be hypotonic. Water enters the cell by osmosis. A simplified depiction of this osmotic process is shown in Figure 3.12. As shown in Figure 3.10 a red blood cell placed in a hypotonic solution (e.g. 0.1% salt solution) will burst (haemolysis) as a result of the influx of water. White blood cells with their nucleus and more extensive cytoskeleton will expand but are less likely to burst. Bacteria and plant cells avoid bursting in hypotonic solutions because of strong cell walls. These allow the buildup of turgor pressure within the cell, until it equals the osmotic pressure and osmosis ceases.

As depicted in Figure 3.10 when red blood cells are placed in a 0.9% salt solution, they neither gain nor lose water by osmosis. Such a solution is said to be isotonic. The extracellular fluid of mammalian cells is isotonic to their cytoplasm. This balance must be actively maintained because of the large number of organic molecules dissolved in the cytosol but not present in the external fluid. These organic molecules exert an osmotic effect that, if not compensated for, would cause the cell to take in so much water that it would swell and might even burst. This fate is avoided by pumping sodium ions out of the cell with the sodium-potassium pump.

Figure 3.11 Osmosis is the term given to the diffusion of water from a region of high concentration (high potential) of water molecules to a region of low concentration (low potential) across a partially permeable membrane. Water is shown here passing from a dilute to a high concentration of impermeable sugar molecules.

Figure 3.12 Water passing by osmosis across a selectively permeable membrane from a hypotonic to a hypertonic solution.
If red blood cells are placed in sea water (~3% salt) they lose water by osmosis and the cells shrink and shrivel up. Sea water is hypertonic to the cytosol of the red cells. Water will diffuse from the cytoplasm down its activity gradient in an attempt to dilute out the salt solution, following the same basic process shown in Figure 3.12. Sea water is also hypertonic to the external fluid of most marine vertebrates. To avoid fatal dehydration these animals must continuously drink sea water and then desalt it by pumping ions out of their gills by active transport. Marine reptiles (turtles and snakes) use special salt glands for the same purpose. If a plant tissue is placed in sea water, the cell contents shrink away from the rigid cell wall in a process called plasmolysis.

However, lipid bilayers are impermeable to most essential ions and molecules, such as: $K^+$, $Na^+$, $Ca^{2+}$ (cations); $Cl^-$, $HCO_3^-$ (anions); small hydrophilic molecules like glucose and mannitol; macromolecules like proteins and RNA. Cells solve this problem by means of facilitated diffusion and active transport.

### 3.5.3 Facilitated Diffusion

Large polar molecules, such as glucose and amino acids, cannot diffuse across the plasma membrane of a cell. Ions such as $Na^+$ and $Cl^-$ also cannot pass through by simple diffusion. These molecules and ions pass through transmembrane protein channels instead by a process known as facilitated diffusion. These proteins, or assemblies of proteins, are embedded in the plasma membrane to form a water-filled channel through which an ion or molecule can pass down its concentration gradient into or out of the cell. This is depicted in Figure 3.13. Molecules will randomly move through the channel or pore by diffusion. This requires no energy. It is a passive process, just as for osmosis. The transmembrane channels that permit facilitated diffusion can be opened or closed. They are said to be gated. Many ion channels open or close in response to binding a small signalling molecule or ligand. Apart from ligand-gated ion channels, there are also mechanically-gated, voltage-gated, and light-gated channels.

### 3.5.4 Active Transport

Cells must maintain ion concentration gradients across their plasma membrane (see Table 3.2). Passive transport cannot achieve this. Active transport is the pumping of molecules or
ions through a membrane against their concentration gradient. It requires a transmembrane protein (usually a complex of them) called a transporter, as well as energy in the form of ATP. The energy of ATP may be used directly or indirectly. Some transporters bind ATP directly and use the energy of its hydrolysis as described in Chapter 1 to drive active transport. Other transporters use the energy already stored in the gradient of a directly-pumped ion. Direct active transport of the ion establishes a concentration gradient. When this is relieved by facilitated diffusion, the energy released can be harnessed to the pumping of some other ion or molecule.

### 3.5.4.1 Sodium-Potassium Pump

The cytosol of animal cells contains a concentration of potassium ions as much as 20-times higher than that in the extracellular fluid. Conversely, the extracellular fluid contains a concentration of sodium ions as much as 10-times greater than that within the cell. These concentration gradients are established by the active transport of both ions, using the same transporter called the sodium-potassium pump (or Na\(^+\)/K\(^+\) ATPase). This pump uses the energy from the hydrolysis of ATP described in Chapter 1 to actively transport 3 Na\(^+\) ions out of the cell for each 2 K\(^+\) ions pumped into the cell. This result is shown schematically in Figure 3.14. Almost one third of all the energy (ATP) generated by the mitochondria in animal cells is used solely to run this pump!

As will be discussed later in this chapter, the action of the sodium-potassium pump accomplishes several vital functions:

(a) Accumulation of sodium ions outside of the cell draws water out of it and thus enables the cell to maintain osmotic balance.
(b) The gradient of sodium ions is harnessed to provide the energy to run several types of indirect pumps.
(c) It helps establish a net charge across the plasma membrane – producing a resting membrane potential that prepares nerve and muscle cells for the propagation of action potentials leading to nerve impulses and muscle contraction.

![Figure 3.14](image_url) The sodium-potassium pump uses the free energy released by the hydrolysis of ATP to pump out 3 sodium ions for 2 potassium ions pumped into the cell. This generates a membrane potential of around \(-70\) mV (negative with respect to the reference zero potential of the extracellular medium).
A summary of the methods of transport of molecules and ions across the cell membrane is given in Table 3.1.

3.6 Electrochemical Gradients and Ion Distributions Across Membranes

If a molecule bears an electric charge its net ionic flux across a membrane will be determined not only by the permeability of the membrane to that ion and the concentration gradient of the ion, but also by the electric potential difference between the two sides of the membrane. The following are the main factors of importance:

For charged molecules (e.g. Na\(^+\), K\(^+\), Cl\(^-\), Ca\(^{2+}\), amino acids) two forces act to produce net passive diffusion of the species across a membrane:

(a) The chemical gradient arising from differences in the concentration of the substance on the two sides of the membrane, and
(b) The electrical field (i.e. difference in potential across the membrane divided by membrane thickness) experienced by the ion as it enters the membrane.

A positively charged ion will tend to move in the direction of increasing negative potential. The sum of the combined forces of concentration gradient and electrical gradient acting on an ion determine the net electrochemical gradient acting on the ion. It follows that there must be a potential difference just sufficient to balance and counteract the chemical gradient acting on an ion, so as to prevent a net trans-membrane flux of the ion in question. The potential at which an ion is in electrochemical equilibrium is termed the equilibrium potential. The value of this potential depends on several factors, the most prominent being the ratio of concentrations of the ion in question. We will cover this in more detail in Section 3.10, but for the present we can state that for a monovalent ion at 298 K the equilibrium potential is equal to:

\[ 0.059 \times \log_{10} \text{(ratio of extracellular to intracellular concentration)} \text{ Volts.} \]
Thus, a 59 mV potential difference across the membrane has the same effect on the net diffusion of that ion as a transmembrane concentration ratio of 10:1 for that ion.

Passive diffusion of an ion species will therefore take place against its chemical concentration gradient if the electrical gradient (i.e., potential difference) across the membrane opposes, and exceeds, the concentration gradient. For example, if the interior of a cell is more negative than the equilibrium potential for K$^+$, potassium ions will diffuse into the cell even though the intracellular concentration of K$^+$ is much higher than the extracellular concentration. Electrical forces, of course, do not act directly on uncharged molecules such as sugars.

3.6.1 Donnan Equilibrium

In 1911, the physical chemist Frederick Donnan examined the distribution of diffusible solutes separated by a membrane that is freely permeable to water and electrolytes, but totally impermeable to one species of ion confined to one of two compartments. In this situation, as Donnan discovered, the diffusible solutes become unequally distributed amongst the two compartments. We will consider the experiment outlined in Figures 3.15 and 3.16.

The experiment involves four stages, the first two of which are shown in Figure 3.15.

(i) Pure water is placed in the two chambers separated by a semipermeable membrane. KCl is dissolved into chamber 1.
(ii) The dissolved salt (K$^+$ and Cl$^-$) will diffuse through the membrane until the concentration of K$^+$ and Cl$^-$ become equal on both sides.

The third and fourth stages are shown in Figure 3.16.
(iii) The potassium salt of a nondiffusible anion (a macromolecule such as a protein P$^-$ (which may have multiple negative charges) is added to the solution in chamber 1.
(iv) The K$^+$ and Cl$^-$ ions redistribute until a new equilibrium is established by movement of some K$^+$ from chamber 2 into chamber 1 to compensate for the nondiffusible P$^-$ ions, as well as a net transfer of Cl$^-$ into chamber 2.

![Figure 3.15](image)

Figure 3.15  (i) KCl is dissolved in chamber 1, the dissociated ions diffuse through the semi-permeable membrane into chamber 2. (ii) At equilibrium the concentrations of the ions are equal on both sides of the membrane.
The Donnan equilibrium is characterised by a reciprocal distribution of the anion and cation such that:

\[
\frac{[K^+]_1}{[K^+]_2} = \frac{[Cl^-]_2}{[Cl^-]_1} \quad \text{(square brackets indicate concentrations).} \tag{3.48}
\]

At equilibrium, the diffusible \( K^+ \) is more concentrated in the compartment in which the nondiffusible anion \( P^- \) is confined than in the other, whereas the diffusible anion \( Cl^- \) becomes less concentrated in that compartment than in the other.

This equilibrium situation arises from the following physical requirements:

(a) There must be electroneutrality in both compartments. Within each compartment the total number of positive charges must equal the total number of negative charges. In this example, \([K^+] = [Cl^-]\) in compartment 2.

(b) The diffusible ions \( K^+ \) and \( Cl^- \) must, statistically, cross the membrane in pairs to maintain electrical neutrality. The probability that they will cross together is proportional to the product \([K^+][Cl^-]\).

(c) At equilibrium the rate of diffusion of KCl in one direction through the membrane must equal the rate of KCl diffusion in the opposite direction. Thus, at equilibrium the product \([K^+][Cl^-]\) in one compartment must be equal to the same product in the other compartment. This is the relationship given by Equation (3.48).

An algebraic expression for the equilibrium condition can be derived by assigning to the chamber 2 the concentrations \([K^+]_2 = [Cl^-]_2 = x\). We also let \([Cl^-]_1 = y\), and for the added protein salt we assign \([KP] = z\). The protein anion \( P^- \) will remain in chamber 1, and so at final equilibrium \([K^+]_1 = (y + z)\). The equality of the product \([K^+][Cl^-]\) in the two compartments at final equilibrium can thus be expressed as:

\[
y(y + z) = x^2. \tag{3.49}
\]

This equation holds if \( P^- \) is not present, for in that case \( K^+ \) and \( Cl^- \) are equally distributed and \( z = 0 \) and \( x = y \).
Rearrangement of Equation (3.49) gives, at equilibrium:

\[
\frac{x}{y} = \frac{y + z}{x}, \quad (3.50)
\]

to show that as the concentration \( z \) of the nondiffusible anion \( P^- \) is increased, the concentrations of the diffusible ions will become increasingly divergent.

Example 3.1

Figure 3.17 shows the initial (before equilibrium) situation of a dialysis bag, containing 300 mM of a sodium-protein salt in 1L of pure water, immersed into a vessel containing 200 mM NaCl in 1 litre of pure water. The dialysis bag membrane allows small ions to pass through it, but not protein molecules. Assuming that one sodium ion completely dissociates from the protein, determine the equilibrium concentrations of sodium and chloride ions within and outside the dialysis bag.

Solution:
Consider the two conditions that determine the final equilibrium:

(i) Chemical equilibrium for the mobile salt:

Activity of NaCl outside the bag = Activity of NaCl inside the bag:

\[
[\text{Na}^+]_{\text{out}} \cdot [\text{Cl}^-]_{\text{out}} = [\text{Na}^+]_{\text{in}} \cdot [\text{Cl}^-]_{\text{in}} \quad (i)
\]

(ii) Macroscopic Electroneutrality:

Outside:

\[
[\text{Na}^+]_{\text{out}} = [\text{Cl}^-]_{\text{out}} \quad (ii)
\]

Inside:

\[
[\text{Protein}^-] + [\text{Cl}^-]_{\text{in}} = [\text{Na}^+]_{\text{in}} \quad (iii)
\]

We know the following:

\[
[\text{Cl}^-]_{\text{out}} + [\text{Cl}^-]_{\text{in}} = 200 \text{ mM} \quad (iv)
\]

\[
[\text{Na}^+]_{\text{out}} + [\text{Na}^+]_{\text{in}} = 500 \text{ mM} \quad (v)
\]

Figure 3.17 Initial concentrations of protein and NaCl across a dialysis membrane.
Thus, we have 5 independent equations and 4 unknowns – implying that there is more than one way to solve this problem.

Step 3: Steps to a solution (here is one route):
In (i) substitute for $[\text{Cl}^-]_{\text{out}}$ using (ii):

$$\left\{ [\text{Na}^+]_{\text{out}} \right\}^2 = [\text{Na}^+]_{\text{in}} [\text{Cl}^-]_{\text{in}}$$

(vii)

In (vii) substitute for $[\text{Cl}^-]_{\text{in}}$ using (iii) and (vi):

$$\left\{ [\text{Na}^+]_{\text{out}} \right\}^2 = [\text{Na}^+]_{\text{in}} \{[\text{Na}^+]_{\text{in}} - 300\}$$

Finally, on substituting for $[\text{Na}^+]_{\text{in}}$ using (v) we obtain:

$$\left\{ [\text{Na}^+]_{\text{out}} \right\}^2 = (500 - [\text{Na}^+]_{\text{out}})(200 - [\text{Na}^+]_{\text{out}}).$$

Leading to: $700[\text{Na}^+]_{\text{out}} = 10,000$

To give $[\text{Na}^+]_{\text{out}} = 143 \text{ mM}.$

From (v) : $[\text{Na}^+]_{\text{in}} = 357 \text{ mM};$

From (ii) & (iv) : $[\text{Cl}^-]_{\text{out}} = 143 \text{ mM}, [\text{Cl}^-]_{\text{in}} = 57 \text{ mM}.$

### 3.7 Osmotic Properties of Cells

We will now begin to consider the properties of the cell membrane that are responsible for the different concentrations of ions that are maintained inside and outside the cell – and for the regulation of cell volume.

Ionic Steady State:

Although the intracellular concentrations of inorganic solutes at ionic steady state conditions differ somewhat amongst different cell types and different organisms, certain generalisations can be made:

- The most concentrated inorganic ion in the cytosol is $\text{K}^+$, which is typically 10–30 times as concentrated in the cytosol as in the extracellular fluid. Conversely, the internal concentrations of free $\text{Na}^+$ and $\text{Cl}^-$ are typically less ($\sim$ one-tenth or less) than the external concentrations.
- The intracellular concentration of $\text{Ca}^{2+}$ is maintained several orders of magnitude below the extracellular concentration. This situation is due in part to active transport of $\text{Ca}^{2+}$ out of the cell, across the membrane, and in part to the sequestering of this ion within such organelles as the mitochondria and cytoplasmic reticulum. As a result, the activity of $\text{Ca}^{2+}$ in the cytosol is generally well below 1 $\mu$M.
Cell membranes are typically far more permeable (~30 times) to $K^+$ than to $Na^+$. Membrane permeability to chloride varies. In some cells it is similar to potassium, whilst in others it is lower. The permeability to $Na^+$ is low – but not low enough to prevent sodium from leaking steadily into the cell.

The steady state internal and extracellular concentrations of ions for a typical mammalian muscle cell are given in Table 3.2.

In view of the general leakiness of the cell membrane, the question arises as to what degree the Donnan equilibrium described in Section 3.6 contributes to the steady-state ionic distributions between the cell interior and cell exterior. Three related factors are involved:

1. A preponderance of net negative charge resides in the form of anionic sites such as carboxyl on peptide and protein molecules that are nonpermeant and thus trapped within the cell. These charges must be balanced by positively charged counterions such as $Na^+$, $K^+$, $Mg^{2+}$, and $Ca^{2+}$.

2. Because such ‘immobile’ anionic sites are trapped within the cell by the inability of the parent peptides and proteins to cross the outer cell membrane, we have a natural situation similar in some respects to the artificial situation we considered in Section 3.6 to illustrate Donnan Equilibrium. If $K^+$ and $Cl^-$ were the only diffusible ions, an equilibrium situation in the cell would indeed develop similar to that shown in Figure 3.16. However, the cell membrane is leaky to $Na^+$ and other inorganic ions. With time the cell would load up with these ions if they were simply allowed to accumulate. This in turn would cause osmotic movement of water into the cell, causing it to swell.

3. Such osmotic disasters are avoided by the ability of the cell membrane to pump out $Na^+$, $Ca^{2+}$, and some other ions at the same rate they leak in, keeping the intracellular $Na^+$ concentration about an order of magnitude lower than the extracellular concentration. This active pumping confers on the membrane an effective impermeability to $Na^+$ and $Ca^{2+}$. As a result, the concentrations of these ions are not allowed to come into equilibrium, and the cell in fact behaves very much on the surface as if it were in a state of Donnan Equilibrium. In spite of this resemblance, the unequal distribution of ions represents a steady state requiring the continual expenditure of energy (to pump ions) rather than a true equilibrium.

Since $K^+$ and $Cl^-$ are by far the most concentrated and most permeant ions in the tissue, they distribute themselves in a way similar to that in an ideal Donnan Equilibrium – namely that the KCl concentration product ($[K^+] \times [Cl^-]$) of the cell interior will approximately equal the KCl concentration product of the extracellular solution, providing the membrane permeabilities of chloride and potassium are both high relative to those of other ions present. This is shown in Figure 3.18.

<table>
<thead>
<tr>
<th>Internal ion concentration (mM)</th>
<th>Extracellular ion concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Na^+$: 10; $K^+$: 140; $Ca^{2+}$: $&lt;10^{-6}$; $Cl^-$: 3~4; $A^-$: 140</td>
<td>$Na^+$: 120; $K^+$: 2.5; $Ca^{2+}$: 2.0; $Cl^-$: 120</td>
</tr>
</tbody>
</table>
The combination of an asymmetric distribution of ions between the intra- and extracellular fluids, together with the selective ion permeability of the cell membrane, give rise to the membrane equilibrium potentials listed in Table 3.3. This will be discussed in more detail later in this chapter.

### 3.8 Probing the Electrical Properties of Cells

Electrical phenomena in living tissues can be detected by placing two electrodes in the tissue to measure the field set up by electric currents flowing through the extracellular fluids. Since these currents originate across cell membranes, a more direct and quantitative approach is to measure electrical events across the membrane of a single cell. This measurement is done by comparing the electric potential of one side of the membrane with that of the other side. One sensing electrode is placed in electrical continuity with the outside of the cell, and another is inserted inside the cell. The difference between these two potentials is the membrane potential $V_m$ and is always given as the intracellular potential relative to the extracellular potential, which is arbitrarily defined as zero. A simple electrical stimulating and recording arrangement is shown in Figure 3.19.

### Table 3.3 Ratios of the external and internal cell concentrations of important ions for a typical mammalian muscle cell and human red blood cell

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Ion</th>
<th>Conc. ratio (out/in)</th>
<th>Equil. potential mV (calc.)</th>
<th>Measured potential mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>Na$^+$</td>
<td>12</td>
<td>+67</td>
<td>$-90$ mV</td>
</tr>
<tr>
<td></td>
<td>K$^+$</td>
<td>0.026</td>
<td>−98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ca$^{2+}$</td>
<td>15 000</td>
<td>+123</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl$^-$</td>
<td>30</td>
<td>−90</td>
<td></td>
</tr>
<tr>
<td>Red blood cell</td>
<td>Na$^+$</td>
<td>18</td>
<td>+74</td>
<td>$-10$ to $-14$</td>
</tr>
<tr>
<td></td>
<td>K$^+$</td>
<td>0.05</td>
<td>−77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ca$^{2+}$</td>
<td>52 000</td>
<td>+139</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl$^-$</td>
<td>1.6</td>
<td>−12</td>
<td></td>
</tr>
</tbody>
</table>

The calculated equilibrium and measured membrane potential is also given. (Derived from Wilfred D. Stein, *Channels, Carriers, and Pumps*, Academic Press, p. 37, 1990.)
As shown in Figure 3.19 the cell is immersed in a physiological saline solution containing a reference electrode. Glass capillary microelectrodes, with tip diameters less than 0.1 micron and filled with an electrolyte such as 3 M KCl, can be inserted into cells with negligible damage to their membranes. The tip resistance of such microcapillary electrodes can approach values of $20 \sim 50 \, \Omega$ and so will not act as a short-circuit across the membrane and can serve as a voltage probe. The first step is to insert the tip of such a recording electrode through the membrane of the cell. Before the tip of this microelectrode enters the cell, it and the reference electrode are at the same potential (taken to be reference zero). When the fine capillary tip penetrates the membrane, the cytoplasm is in continuity with the electrical connection to a voltage amplifier via a fine column of electrolyte that fills the inside of the capillary electrode (e.g. a 3 M solution of KCl). As the tip of the recording microelectrode is advanced, penetration of the plasma membrane is indicated by the sudden appearance of a negative potential shift of the voltage trace (see Figure 3.20). The steady negative potential recorded by the electrode tip in the cytoplasm is the resting potential $V_{\text{rest}}$ (mV). All cells that have been investigated have a negative resting potential, which can be as high as $-70$ mV.

Figure 3.19 A basic system for the stimulation and recording of the electrical properties of a cell membrane. Ingoing or outgoing current pulses are applied to the cell. The difference between the potential of an electrode inserted into the cytosol and an external electrode gives the membrane potential $V_m$.

As shown in Figure 3.19 the cell is immersed in a physiological saline solution containing a reference electrode. Glass capillary microelectrodes, with tip diameters less than 0.1 micron and filled with an electrolyte such as 3 M KCl, can be inserted into cells with negligible damage to their membranes. The tip resistance of such microcapillary electrodes can approach values of $20 \sim 50 \, \Omega$ and so will not act as a short-circuit across the membrane and can serve as a voltage probe. The first step is to insert the tip of such a recording electrode through the membrane of the cell. Before the tip of this microelectrode enters the cell, it and the reference electrode are at the same potential (taken to be reference zero). When the fine capillary tip penetrates the membrane, the cytoplasm is in continuity with the electrical connection to a voltage amplifier via a fine column of electrolyte that fills the inside of the capillary electrode (e.g. a 3 M solution of KCl). As the tip of the recording microelectrode is advanced, penetration of the plasma membrane is indicated by the sudden appearance of a negative potential shift of the voltage trace (see Figure 3.20). The steady negative potential recorded by the electrode tip in the cytoplasm is the resting potential $V_{\text{rest}}$ (mV). All cells that have been investigated have a negative resting potential, which can be as high as $-70$ mV.

Figure 3.20 When a high resistance glass capillary voltage probe is inserted into a cell it records a negative potential with respect to the outside of the cell. This is the resting membrane potential ($V_{\text{rest}}$).
The potential sensed by the intracellular electrode does not change as the tip is advanced further into the cell. Thus, the entire potential difference between the cell interior and cell exterior exists across the surface membrane and in the regions immediately adjacent to the inner and outer membrane surfaces.

The electrical properties of the cell membrane can be examined by causing a pulse of current to pass through the membrane so as to produce a perturbation in the membrane potential. A second microelectrode, the current electrode shown in Figure 3.19, can deliver such a current. The current from this electrode, in the form of a current pulse generated by applying a step voltage in series with a high value resistance (>1 GΩ), flows across the membrane in either the inward (bath to cytoplasm) or the outward direction depending on the polarity of the step voltage. When current pulses are passed so that positive charge is removed from inside the cell via the current electrode, the potential difference across the membrane increases (hyperpolarises). The intracellular negative potential is increased (e.g. from −60 to −70 mV). With hyperpolarisation, the membrane (with some exceptions) produces no response other than a positive potential change due to the applied current. If a current pulse is passed from the electrode into the cell, positive charge will be added to the inner surface of the cell membrane. This charge causes the potential difference across the membrane to decrease, and the cell is then said to become depolarised (e.g. from −60 to −50 mV). These two types of response are shown in Figure 3.21.

As the strength of the outward pulse is intensified, depolarisation will increase, as shown in Figure 3.21. Excitable cells, such as nerve, muscle, and many receptor cells, exhibit a threshold potential at which the membrane will produce a strong active response. This is known as the action potential shown in Figure 3.22. The action potential is caused by the activation of membrane channels permeable to sodium, which themselves are activated by the reduction in voltage difference between the two sides of the cell membrane. The opening of the sodium channels in response to depolarisation and the resulting flow of sodium ions into the cell provide an example of membrane excitation. The mechanisms underlying the action potential and other instances of membrane excitation will be considered later in this chapter.
We can now appreciate that cell membranes respond to stimuli with two quite different classes of electrical behaviour – namely, passive and active behaviour:

3.8.1 Passive Electrical Response

This is always produced when an electric current is forced across a biological membrane, because of the electrical capacitance and conductance properties of the membrane. Passive responses occur independently of any molecular changes that open or close gated ion channels in the membrane. The resistance (reciprocal of conductance) of a cell membrane is associated with leakage pathways that allow inorganic ions to cross the membrane. The capacitance of a membrane is a measure of the extent to which the ion impermeability of the membrane leads to separation of electrical charges across the membrane.

3.8.2 Active Electrical Response

Such responses, known as membrane excitations, are found in excitable tissue such as nerve, muscle, and sensory receptors. They depend on the opening and/or closing of numerous ion channels (also called membrane channels) in response to a stimulus. Some ion channels are gated (i.e. opened and shut) by changes in voltage across the membrane, while others are opened by the binding of transmitter or messenger molecules. Other channels, primarily in sensory receptor cells, are activated by specific stimulus energies such as light (photoreceptors) or mechanical strain (mechanoreceptors). When a certain group of channels selectively permeable to a certain species of ion is opened, a current may be carried across the membrane. As in the case of sodium channels, such a current normally produces a voltage signal across the membrane. As we will later in this chapter, the gating of ion channels is the immediate cause for nearly all electrical activity in living tissue.

3.8.3 Membrane Resistance

The passive resistance of a membrane is a measure of its permeability to ions. In saline solutions the resistivity of pure phospholipids is as high as $10^{13}\,\Omega\,\text{m}$. This can
be compared to 298 K values that range from 0.6 to 0.8 Ω m for prepared physiological solutions (buffers). The value for sea water is \( \sim 0.2 \Omega \text{ m} \). A 4 nm thick lipid bilayer can be estimated to have a specific resistance of 40 kΩ m². The significantly lower resistivities of biological membranes (typically 0.01~1 Ω m²) therefore can be assumed to arise from structures other than the lipid bilayer itself. These structures are protein-bound aqueous pores (aquaporins) and various ion channels embedded in the lipid. The density of different channels typically range from 50 to 500 per µm², with conductances of 1 ~ 100 pS. However, many of these channels may not be ‘open’ at any given time.

If a step pulse of steady current is applied across the membrane, the membrane potential shifts by \( \Delta V_m \) from the resting value. \( \Delta V_m \) depends on the magnitude of the applied current \( \Delta I \) and the membrane resistance \( R \), which can be determined from Ohm’s Law:

\[
\Delta V_m = R \Delta I
\]

Consider two spherical cells, one small and the other large and both with membranes having the same specific resistance \( R_m \) to electric current (i.e. the same resistance per unit square area of membrane). For a given increment of current \( \Delta I \) inserted into the cells, the large cell will show a smaller increment of voltage \( \Delta V_m \) because the same current will flow through a larger area of membrane. Because the input resistance of a cell (i.e. the total resistance encountered by current flowing into or out of a cell) is a function of both membrane area \( A \) and specific resistance \( R_m \) of a cell, it is useful when comparing membranes of different cells to correct for the effect of membrane area on the current density. Thus, the specific membrane resistance is calculated as:

\[
R_m = RA = \frac{\Delta V_m}{\Delta I} A \quad (\text{ohms m}^2).
\]

### 3.8.4 Membrane Capacitance

Because they are very thin (\( \sim 4 \text{ nm} \)) and virtually impermeable to ions over most of their surface area, cell membranes can violate the principle of electroneutrality at the microscopic scale. Negative charges accumulated at or near one surface of a membrane will interact electrostatically over the short distance of the membrane thickness, with positive charges on the other side of the membrane. The ability of the cell membrane to accumulate and separate electric charge is called its membrane capacitance. Electronic engineers can view this situation as a very thin dielectric (the lipid bilayer) sandwiched between two conductors (electrolytes) representing the basic form of a capacitor. Cell membranes contain a lipid bilayer of about 3 nm in thickness (verified by electron microscopy) with proteins protruding on each side. Assuming a total thickness of 4 nm for the insulating region of the membrane, and that the lipid content has a relative permittivity of 2.35 (as estimated in Section 3.2.4), the membrane capacitance for a smooth cell surface can be calculated to be \( \sim 5.2 \text{ mF/m}^2 \).

The equivalent circuit for a cell membrane to describe the charging and discharging of the membrane on application and then removal of a current pulse is shown in Figure 3.23.
relationship between potential $V$ and time during the charging of the membrane capacitance is given by:

$$V(t) = V_0 e^{-t/R_mC_m},$$

where the time to fall to $1/e$ of its initial value is the time constant given by $\tau = R_mC_m$. Having determined the membrane specific resistance $R_m$, the membrane capacitance can be determined from measurement of the membrane time constant (typically $0.1 \sim 10\text{ ms}$). Experimental values obtained for $C_m$ are normally larger than the theoretical value of $\sim 5\text{ mF/m}^2$ determined above for a smooth membrane structure. The experimental value (typically $10 \sim 30\text{ mF/m}^2$) obtained correlates closely with the extent to which the area of an otherwise smooth membrane surface is increased as a result of the presence of membrane folds and proturbances, such as blebs and microvilli, for example.

### 3.8.5 Extent of Ion Transfer Associated with the Membrane Resting Potential

We have learnt that cells have a negative resting membrane potential of about $-70\text{ mV}$. How many ions must be transferred across the membrane to produce this potential? We can derive an estimate of this by considering a cell of radius $10\mu\text{m}$ having a membrane capacitance of $10\text{ mF/m}^2$. The total surface area of this cell is $4\pi(10^{-10})^2$, to give a total membrane capacitance of $4\pi 10^{-12}\text{ F}$. To set up a potential of $70\text{ mV}$ will require a charge $Q$ given by $Q = VC = 70 \times 10^{-3} \times 4\pi 10^{-12} = 8.8 \times 10^{-13}\text{ C}$. Dividing by the Faraday constant ($9.65 \times 10^4\text{ C/mol}$) leads to the result that we require the equivalent of $9.1 \times 10^{-18}\text{ mol}$ of monovalent ions to be transferred across the membrane to generate a membrane potential of $-70\text{ mV}$. The cell volume is $(4\pi 10^{-15})/3 = (4\pi 10^{-12})/3\text{ litres}$. The potassium content of a cell of this volume, when the potassium is present at $150\text{ mM}$, is roughly $(4\pi 10^{-12})/3 \times 0.15 = 6.3 \times 10^{-13}\text{ moles}$ (we will assume an activity coefficient of unity). Thus, to charge the membrane to $-70\text{ mV}$ requires as little as $1.4 \times 10^{-3}\%$ of the cell’s total potassium to be transferred across the membrane. The rule of electroneutrality – that positive charges must equal negative charges remains essentially unviolated at the macroscopic scale. The imbalance of charges exists only at the microscopic scale across the membrane thickness.
3.9 Membrane Equilibrium Potentials

The electrical energies of the transmembrane potentials of cells are responsible for nearly all the electrical phenomena that occur in the animal body. These potentials originate from two features of biological membranes:

- asymmetrical distribution of ions between the intracellular and extracellular compartments;
- selective permeability of the membrane.

Consider the chamber shown in Figure 3.24. It is divided into two compartments by a membrane that is selectively permeable to potassium ions. We will assume that both compartments initially contain a 1 mM KCl solution. Electrodes inserted into the compartments would record that no potential difference occurs across the membrane. The hypothetical membrane is permeable to K\(^+\) but not to Cl\(^-\), and so it is possible for the K\(^+\) ions to diffuse across the membrane on their own. On average for every potassium ion that passes in one direction through the membrane another potassium ion will pass through in the opposite direction. As long as the two compartments contain the same concentration of KCl the net flux of K\(^+\) ions is zero and the potential difference across the membrane remains zero.

If the concentration of KCl in compartment 1 is suddenly increased to 10 mM, as shown in Figure 3.24b, a net diffusion of K\(^+\) ions will take place through the potassium-selective membrane from compartment 1 to compartment 2. This net transfer of positive ions will create a potential difference across the membrane such that compartment 1 is more negative than compartment 2. The K\(^+\) concentration gradient across the membrane represents a chemical potential difference that initially drives diffusion of K\(^+\) from compartment 1 to 2. Each additional K\(^+\) that diffuses from 1 to 2 adds its positive charge to that side, and Cl\(^-\) is left behind since it cannot cross this hypothetical membrane. This generates an electrical potential difference (a back emf). Thus, each K\(^+\) ion now entering the membrane has two forces acting on it: a chemical potential difference, favouring net K\(^+\) flux from 1 to 2, and an electrical potential difference, favouring net K\(^+\) flux from 2 to 1. These two opposing forces

**Figure 3.24** (a) A chamber divided into two compartments by a membrane permeable only to K\(^+\) ions. Each compartment contains 1 mM KCl. The net flux of K\(^+\) ions across the membrane is zero and no potential difference occurs across the membrane. (b) The concentration of KCl in compartment 1 is increased to 10 mM. K\(^+\) ions diffuse across the membrane down their chemical gradient \(\nabla(\text{chem.})\) and initiate a back emf across the membrane. (c) At electrochemical equilibrium the electrical potential difference across the membrane that exactly opposes diffusion down the chemical gradient is termed the equilibrium potential for the K\(^+\) ion.
come into equilibrium and remain balanced, as depicted in Figure 3.24c. The potassium ion is then said to be in electrochemical equilibrium. The potential difference that is established across a membrane in this way is termed the equilibrium potential for the ion in question (in this case, the potassium equilibrium potential, \( E_K \)). In our hypothetical situation here this potential difference \( E_K \) will be maintained indefinitely provided there is no leakage of \( \text{Cl}^- \) ions across the membrane. As discussed in Section 3.8.5, when considering the development of a potential across a cell membrane, very few ions actually diffuse across a unit area of the membrane before the equilibrium potential \( E_K \) is established. The concentrations of potassium ions in compartments 1 and 2, 10 mM and 1 mM respectively, therefore remain virtually unchanged during the overall process shown in Figure 3.24.

If for any reason the membrane potential \( V_m \) is not at the equilibrium potential \( E_X \) for an ion \( X \), there will exist an emf acting on that ion, \( \text{emf}_X \), equal to the difference between \( V_m \) and \( E_X \):

\[
\text{emf}_X = V_m - E_X.
\]

Clearly, when \( V_m = E_X \), ion \( X \) will experience no emf and will be in electrochemical equilibrium across the membrane.

An equivalent electrical circuit is shown in Figure 3.25 for the process shown in Figure 3.22 of the development of the membrane potential. Positive charge (in the form of potassium ions) driven by the emf acting on potassium (i.e. \( V_m - E_K \)) leaks through the potassium conductance (i.e. \( R_K \)) of the membrane so as to accumulate on the other side of the membrane capacitance \( C_m \). When the voltage across the capacitance of the membrane equals the potassium equilibrium potential (i.e. when \( V_m - E_K = 0 \)) net diffusion of \( K^+ \) ions ceases and the system is at equilibrium – side 2 positive with respect to side 1. Although the electrochemical gradient for the chloride ion is in the opposite direction, it has no effect because our hypothetical membrane is impermeable to chloride ions (i.e. \( R_{Cl} \) in Figure 3.25 is effectively an open circuit).

### 3.10 Nernst Potential and Nernst Equation

The Nernst equation is one of the most widely used mathematical relationships in studies of bioelectric phenomena. Its derivation is based on the concept of a thermodynamic
equilibrium between the osmotic work that is required to move a given number of ions across a membrane in one direction, and the electrical work required to move the same number of charges back across the membrane in the opposite direction. The potential across the cell membrane that exactly opposes net diffusion of a particular ion through the membrane is called the Nernst potential for that ion. The magnitude of this potential is determined by the ratio of the concentrations of that specific ion on the two sides of the membrane. The greater this ratio the greater is the tendency for that ion to diffuse in one direction, and thus the greater the potential \( V \) required to prevent its diffusion.

The free energy \( G \) of a system is only really of interest when that system undergoes some kind of change that results in a change \( \Delta G \) of the free energy. Free energy has been defined such that \( \Delta G \) directly measures the amount of disorder created. According to the 2nd law of thermodynamics a physico-chemical reaction can proceed spontaneously only if this results in a net increase of disorder (entropy) of the total system. Energetically favourable processes are therefore those that decrease free energy and have a negative \( \Delta G \), and the relevant example of this here is the diffusion of an ion from a region of high to one of a low ionic concentration.

Movement of an ion down its concentration gradient across the plasma membrane and into a cell is accompanied by a favourable free-energy change per mole:

\[
\Delta G_{\text{conc}} = -RT \ln \left( \frac{\text{Conc outside}}{\text{Conc inside}} \right), \tag{3.51}
\]

where \( R \) is the universal gas constant: \( R = 8.31 \text{ J K}^{-1} \text{ mol}^{-1} \) and \( T \) is the absolute temperature (Kelvin). (As noted earlier in this chapter, solute molecules in solution behave thermodynamically like gas molecules. We can therefore use the ideal gas law \( PV = nRT \), with \( n \) the number of moles of the solute molecule.) Moving the ion into a cell across a membrane whose inside is at a potential \( V \) relative to the outside will cause an additional free-energy change (per mole of ion moved) given by:

\[
\Delta G_V = zFV, \tag{3.52}
\]

where \( F \) is the Faraday Constant \( (F = 9.648 \times 10^4 \text{ C mol}^{-1}) \) and \( z \) is the number of charges on the ion. At the point where the concentration and potential gradients just balance \( \Delta G_{\text{conc}} + \Delta G_V = 0 \). From Equations (3.51) and (3.52) this leads to:

\[
zFV - RT \ln \left( \frac{\text{Conc outside}}{\text{Conc inside}} \right) = 0
\]

and from this we obtain the Nernst Equation:

\[
V = \frac{RT}{zF} \ln \left( \frac{\text{Conc outside}}{\text{Conc inside}} \right) = 2.3 \frac{RT}{zF} \log_{10} \left( \frac{\text{Conc outside}}{\text{Conc inside}} \right). \tag{3.53}
\]

For a univalent cation, \( z = +1 \), and \( 2.3 \frac{RT}{zF} = 59.1 \text{ mV at 298 K} \). At 298 K, we obtain a value for the Nernst Potential \( V \) of \(-59.1 \text{ mV}\) for the case where \( \text{C}_{\text{in}}/\text{C}_{\text{out}} = 10 \). If a univalent cation diffuses down this concentration gradient to the outside of the cell, \( V \) will become less negative. Thus, for the situation \( \text{C}_{\text{in}}/\text{C}_{\text{out}} = 1 \), \( V = 0 \).
The intra- and extracellular ion concentrations, together with the equilibrium potentials and ion channel conductance values, are given for frog muscle membranes in Table 3.4.

From Table 3.4 we obtain values for $[K^+]_{\text{out}}$ and $[K^+]_{\text{in}}$ of 2.5 mM and 138 mM, respectively, for a frog muscle cell. From the Nernst Equation we calculate that the $K^+$ equilibrium potential ($V_K$) at 298 K is $-103\,\text{mV}$ (agreeing with the value given in Table 3.4). At this potential difference of $-103\,\text{mV}$ across the membrane there is no net flow of $K^+$ across the membrane. For any particular membrane potential $V_m$ the net force tending to drive a particular type of ion out of the cell through that ion-specific channel is proportional to the difference between $V_m$ and the equilibrium potential for the ion. For $K^+$ it is $V_m - V_K$. The ion current through the potassium channel will be $(V_m - V_K)G_K$ where $G_K$ is the conductance represented by the number of potassium channels per unit area of membrane.

In the situation where the membrane potential $V_m$ is $-103\,\text{mV}$, from Table 3.4 we can see that, although the potassium ion current through the membrane would be zero, there would be a sodium ion current of $(V_m - V_{Na})G_{Na} = -158 \times 0.8 \times 10^{-2} = -1.3\,\text{mA/m}^2$, as well as a chloride ion current $= -17\,\text{mA/m}^2$. This gives a net membrane current of $-18.3\,\text{mA/m}^2$ (i.e. current flowing out of the cell across the membrane).

How then, in the presence of these various ion concentrations, and ion channels with their different equilibrium potentials and conductance values, does the membrane potential ever attain an equilibrium value? Can we calculate such an equilibrium membrane potential? The answers to these questions form our next subject matter.

### 3.11 The Equilibrium (Resting) Membrane Potential

It is clear from Section 3.10 that the relative trans-membrane concentrations, together with the ease with which different ions can cross the membrane, determine their relative contributions to the potential they produce in diffusing across the membrane. On this basis, and by making the assumption that there is a uniform gradient of potential in going from one side of the membrane to the other side, Goldman [8] derived the following equation for the steady state resting potential $V_m$ across a cell’s membrane (taking into account the dominant ions that can permeate through that membrane):

$$V_m = \frac{RT}{F} \ln \left( \frac{P_{Na}[Na^+]_{\text{out}} + P_{K}[K^+]_{\text{out}} + P_{Cl}[Cl^-]_{\text{in}}}{P_{Na}[Na^+]_{\text{in}} + P_{K}[K^+]_{\text{in}} + P_{Cl}[Cl^-]_{\text{out}}} \right),$$  \hspace{1cm} (3.54)

where $P_{\text{ion}}$ is the permeability for that ion (m/sec). This is commonly referred to as the Goldman equation, but is also known as the Goldman-Hodgkin-Katz or GHK equation. Values for

<table>
<thead>
<tr>
<th>Ion species</th>
<th>Intracellular conc. (mM)</th>
<th>Extracellular conc. (mM)</th>
<th>Equilibrium potential (mV)</th>
<th>Ion channel conductance G (S/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>13</td>
<td>110</td>
<td>+55</td>
<td>$0.8 \times 10^{-2}$</td>
</tr>
<tr>
<td>K⁺</td>
<td>138</td>
<td>2.5</td>
<td>−103</td>
<td>$85 \times 10^{-2}$</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>3</td>
<td>112.5</td>
<td>−93</td>
<td>$170 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

The intra- and extracellular ion concentrations, together with the equilibrium potentials and ion channel conductance values, are given for frog muscle membranes in Table 3.4.
the intra- and extracellular ion concentrations, together with the membrane permeabilities, are given in Table 3.5 for the frog muscle membrane.

Inserting the values from Table 3.5 into Equation (3.54):

$$V_m = \frac{RT}{F} \ln \frac{0.02 \times 10^{-8} \cdot 110 + 2.0 \times 10^{-8} \cdot 2.5 + 4.0 \times 10^{-8} \cdot 3.0}{0.02 \times 10^{-8} \cdot 13 + 2.0 \times 10^{-8} \cdot 138 + 4.0 \times 10^{-8} \cdot 112.5} = -93.4 \text{ mV}.$$  

Another way to evaluate the total current and membrane potential for a membrane is to consider the membrane as a heterogeneous structure consisting of a variety of distinct elements that are arranged in parallel in the membrane. This so-called \textit{mosaic} membrane model consists of a matrix into which are embedded numerous, closely packed, minute cylinders that all penetrate the membrane in its entirety. These cylinders (\textit{ion channels}) have the unique property of displaying \textit{ion-specific permeability}.

Consider the mosaic membrane, shown in Figure 3.26, equipped with three different types of ion channel specifically permeable to Na$^+$ ions, K$^+$ ions, and Cl$^-$ ions. On each side of the membrane are different concentrations of Na$^+$, K$^+$ and Cl$^-$ ions, which will strive by the diffusional forces to attain equal concentrations on both sides for all three kinds of ion. However, before this situation can arise this diffusion process will have established a \textit{diffusion potential} across the membrane and also across all three types of ion channel. We will now derive the magnitude of the equilibrium \textit{membrane potential} $V_m$.

![Figure 3.26](image-url)  

\textbf{Figure 3.26}  Equivalent circuit for a mosaic membrane possessing three different ion channels. The conductances $G$ are represented by the number of ion channels per unit area for each ionic species. The battery $V_L$ represents the contribution to the membrane current from other ionic sources. These other sources are relatively insignificant.
As discussed in Section 3.10, the currents that each type of ion carry are given by 
\( (V_m - V_{ion})G_{ion} \) where \( G_{ion} \) is the membrane conductance for that ion channel type:

\[
\begin{align*}
I_{Na} &= G_{Na}(V_m - V_{Na}) \\
I_{K} &= G_{K}(V_m - V_{K}) \\
I_{Cl} &= G_{Cl}(V_m - V_{Cl})
\end{align*}
\]

where \( V_m \) is the equilibrium membrane potential.

When the equilibrium membrane potential \( Vm \) is established, the sum of these ion channel currents will be zero (Kirchoff’s 2nd law):

\[
G_{Na}(V_m - V_{Na}) + G_{K}(V_m - V_{K}) + G_{Cl}(V_m - V_{Cl}) = 0.
\]

Solving for \( V_m \) leads to the result:

\[
V_m = \frac{G_{Na}V_{Na} + G_{K}V_{K} + G_{Cl}V_{Cl}}{G_{total}} = \frac{G_{Na}}{G_{total}}V_{Na} + \frac{G_{K}}{G_{total}}V_{K} + \frac{G_{Cl}}{G_{total}}V_{Cl}.
\] (3.55)

The steady state membrane potential can thus be described as the sum of the products of the equilibrium potential and membrane conductance for each participating ion divided by the total conductance of the membrane for the ions that cross the membrane. We can define the Transferance Number or Transport Number \( T_j \) of an ion as \( T_j = G_j/G_{total} \). These quantities will obey the relation:

\[
\sum \frac{G_j}{G_{total}} = \sum T_j = 1.
\] (3.56)

Thus, although the equilibrium potential \( V_j \) for a particular ion may be very large, its contribution to the membrane potential \( V_m \) may be insignificant if the permeability to that particular ion is much less than those of the other ions (\( T_j \ll 1 \)). Although the procedures for formulating Equations (3.54) and (3.55) are fundamentally different, they both express the same result, namely that the magnitude of the membrane potential is determined by that ion whose flux through the membrane is dominant.

From Table 3.4 for frog muscle, \( G_{total} = G_{Na} + G_{K} + G_{Cl} = 2.56 \text{ S/m}^2 \). From Equation (3.55):

\[
V_m = 0.003 \times 55 + 0.332(-103) + 0.665 \times (-93) = -95.9 \text{ mV}.
\]

This result is close to the value \(-93.4 \text{ mV}\) obtained using Equation (3.54) and to the experimental result obtained by Hodgkin and Horowicz [9] of \(-95 \text{ mV}\).

### 3.12 Membrane Action Potential

In Section 3.8 we learnt that in response to an appropriate (electrical) stimulus the membranes of excitable cells, such as nerve and muscle, exhibit a strong active response known as the action potential.
3.12.1 Nerve (Axon) Membrane

A schematic of an action potential generated by a nerve axon is shown in Figure 3.27. The action potential takes the form of a depolarisation from its resting state (\(-70\) mV) followed by repolarisation to the resting state. In other words, the potential of the membrane’s inner surface briefly goes from a negative to a positive potential with respect to the outer medium, before reestablishing the rest potential. Hodgkin and Huxley [10] in work that led to the award of the Nobel Prize in 1963 demonstrated that the action potential involves an early inward current of Na\(^+\) ions (down its electrochemical gradient) into the cell followed by a later outward current of Na\(^+\) ions (also down its electrochemical gradient).

The process involves several steps:

1. The condition at the resting membrane potential of around \(-70\) mV. (Na\(^+\) is more concentrated on the outside and K\(^+\) on the inside of the axon membrane.)
2. An electrical stimulus causes ‘fast’ voltage-gated Na\(^+\) channels to open. If the opening is sufficient to drive the interior potential from \(-70\) to \(-55\) mV the process of activation continues. In other words there is an action threshold potential corresponding to where the membrane is depolarised by a stimulus of \(-15\) mV.
3. Having reached the action threshold more Na\(^+\) channels open. The Na\(^+\) influx drives the interior of the cell membrane up to about \(+30\) mV. The process to this point is called depolarisation.
4. After \(~1\) ms, the Na\(^+\) channels close and ‘slow’ K\(^+\) channels open. Because the K\(^+\) channels are much slower to open the depolarisation has time to be completed. (Having both Na\(^+\) and K\(^+\) channels open at the same time would drive the membrane potential towards its resting value and prevent the creation of the action potential.)
5. With the voltage-gated K\(^+\) channels open the membrane begins to repolarise back towards its resting potential.
6. The repolarisation typically overshoots the resting potential to about \(-90\) mV. This is called hyperpolarisation and is important for the transmission of signals (at \(~100\) m/sec) along the neuron’s axon. Hyperpolarisation prevents the neuron from receiving another stimulus during this time, or at least raises the threshold for any new stimulus. Part of the importance of hyperpolarisation is in preventing any stimulus already sent along an axon...
from triggering another action potential in the opposite direction. In other words, hyperpolarisation assures that the signal is proceeding in one direction.

7. After hyperpolarisation the sodium-potassium pump eventually brings the membrane back to its resting state of $-70\ mV$.

The equivalent circuit to describe the contributions of the sodium and potassium pumps in producing an action potential in an axon membrane is shown in Figure 3.26. Included in this figure are the approximate values for the Na and K conductances ($g_{Na}$ and $g_{K}$) derived from Hille ([11], pp. 329–30) and the equilibrium potentials ($E_{Na}$ and $E_{K}$) for these two ions.

![Figure 3.28](image)

**Figure 3.28** The equivalent circuit to describe the contributions of the sodium and potassium pumps in producing an action potential in an axon membrane is shown in Figure 3.26. Included in this figure are the approximate values for the Na and K conductances ($g_{Na}$ and $g_{K}$) derived from Hille ([11], pp. 329–30) and the equilibrium potentials ($E_{Na}$ and $E_{K}$) for these two ions.

The voltage $V$ appearing across the membrane will change at a rate dictated by this current and the membrane capacitance according to the relationship:

$$C_m dV/dt = -g_{Na}(V - E_{Na}) - g_{K}(V - E_{K})$$.

The way in which action potentials are propagated along the axon of a neuron will be described in Section 3.13. For now it is sufficient to state that the action potential does not decrease in strength with distance propagated along an axon. It is an *All-or None* phenomenon – action potentials either happen completely or not at all.

### 3.12.2 Heart Muscle Cell Membrane

The action potentials in an axon membrane are produced by voltage-gated sodium and potassium channels. For heart muscle membranes voltage-gated calcium ($\text{Ca}^{2+}$) channels, instead of sodium channels, play important roles. For example, in ventricular myocytes, high-voltage activated $\text{Ca}^{2+}$ channels contribute towards keeping the cell depolarised for several hundred milliseconds. Cardiac pacemaker cells possess no functional $\text{Na}^{+}$ channels, and so their entire action potentials are generated by voltage-gated opening of $\text{Ca}^{2+}$ channels. Electrical stimulations arising from action potentials of a heart muscle cell are conducted from one cell
to all the cells that are adjacent to it, and thence to all the cells of the heart. A typical action potential exhibited by a cardiac muscle cell and a pacemaker cell are shown in Figure 3.29.

The resting membrane potential phase (\(~-85\,\text{mV}\)) of the cardiac action potential is associated with diastole of the chamber of the heart, the period of time when the heart fills with blood after systole (heart contraction). If the resting membrane potential becomes too positive, the cell may not be excitable, and conduction through the heart may be delayed, increasing the risk for arrhythmias (irregular heart beats). The ‘plateau’ phase of the cardiac action potential is sustained by a balance between inward movement of \(\text{Ca}^{2+}\) through calcium channels and outward movement of \(\text{K}^+\) through the \(\text{K}^+\) channels. During the rapid repolarisation phase the \(\text{Ca}^{2+}\) channels close, while the \(\text{K}^+\) channels remain open, ensuring a net outward current and a return to the resting potential.

Two voltage-dependent calcium channels play critical roles in the physiology of cardiac muscle, namely the L-type (‘L’ for Long-lasting) and T-type (‘T’ for Transient) voltage-gated calcium channels. These channels respond differently to voltage changes across the membrane. L-type channels respond to higher membrane potentials, open more slowly, and remain open longer than T-type channels. Because of these properties, L-type channels are important in sustaining an action potential, while T-type channels are important in initiating them. Because of their rapid kinetics, T-type channels are commonly found in cells undergoing rhythmic electrical behaviour. T-type calcium channels are also found in the so-called pacemaker cells of the heart, which control the heart beat. (T-type channels are also commonly found in some neuron cell bodies involved in rhythmic activity such as walking and breathing.) L-type channels are the targets of a class of drugs called dihydropyridines, which block the currents produced by these channels.

In addition to stimulus from adjacent cells, certain cells (pacemaker cells) of the heart have the ability to undergo spontaneous depolarisation, in which an action potential is generated without any electrical stimulation from nearby cells. This is called cardiac muscle automaticity. The cells that can undergo spontaneous depolarisation the fastest are the primary pacemaker cells of the heart, and set the heart rate. This spontaneous depolarisation is due to the plasma membranes within the heart that have reduced permeability to potassium ions but still allow passive transfer of calcium ions, allowing a net charge to build. The normal activity of the pacemaker cells of the heart is to spontaneously depolarise at a regular rhythm, whilst abnormal automaticity involves the abnormal spontaneous depolarisation of cells of the heart. This abnormality typically causes arrhythmias (irregular rhythms) in the heart.
3.13 Channel Conductance

Conductance values are given in Figure 3.28 for the sodium and potassium selective pumps. These pumps are envisaged to take the form of aqueous pores or channels through the cytoplasmic membrane. Supporting evidence for this concept is the high permeability and high ionic throughput rates measured for single channels. A simple model for a membrane channel is shown in Figure 3.30. It takes the form of a cylinder that spans across the membrane, and is open to the solutions on either side of the membrane. The overall effective electrical resistance of the channel consists of three components, namely the resistance of the cylindrical pore itself together with the resistance of the regions adjacent to the open ends of the channel ([11] Chapter 11).

A well-studied example of the physical end electrical properties of a channel is that of the mechanically gated MscL channel located in the cell envelope of bacteria. This channel enables fast adjustments of turgor pressure in response to a sudden reduction of osmotic pressure. When the tension forces acting on the membrane approach the point where lysis of the membrane can occur, the MscL channel forms a large nonselective pore and acts as a safety valve by releasing osmolytes from the cell interior. The MscL channel has no selectivity towards anions and cations, and its effective conductance is directly proportional to the conductivity of the surrounding bulk electrolyte. This channel appears therefore to act as a classical ohmic resistor. The conductance $G$ of a cylindrical pore of the form shown in Figure 3.30 can therefore be calculated as:

$$G = \frac{\sigma A}{l} = \frac{\sigma \pi r^2}{l},$$

where $\sigma$ is the conductivity of the solution filling the pore, and $l$ and $r$ are the length and radius of the pore, respectively. For the MscL channel from E. coli values for $r$ and $l$ are approximately 1.6 nm and 4 nm, respectively [12]. The conductivity of a typical electrolyte for mammalian cells is 1.7 S m$^{-1}$. Using this conductivity value in Equation (3.56) gives a channel conductance of 3.4 nS. This conductivity is three-orders greater than the value of 4 pS given for the selective sodium channel in Figure 3.28 and leads to the question as to the relevance of Equation (3.56) for other ion channels. In Chapter 10 the dimensionless Knudsen number is introduced as the means for deciding whether or not a microfluidic system can be analysed using macroscopic concepts. Equation (3.56) represents a macroscopic

![Figure 3.30](image.png)

**Figure 3.30** A simple model for a membrane channel consists of an open cylinder that spans the membrane. Its overall electrical resistance comprises the resistance of the cylindrical pore when filled with electrolyte together with the resistances of the regions leading up to the open ends of the channel.
description of channel conductance. The Knudsen number (Kn) is the ratio of the inter-molecular spacing of the fluid (i.e. the diameter 0.28 nm of a water molecule) to the characteristic dimension of the fluidic system, which for a cylindrical channel is its diameter. For the MscL channel Kn = 0.28/3.2 = 0.09. In Chapter 10 we find that this places the MscL at close to the limit where a continuum, macroscopic, model is appropriate. This is consistent with the finding that the conductance of the MscL channel is proportional to a wide range of electrolyte conductivity values (see [12]).

Two other well studied channels are the acetylcholine receptor (AChR) and the gramicidin A channel. The AChR channel has a radius of 0.3 nm and a length of 0.6 nm, and its structure contains rings of negatively charged amino acids at the extracellular and the cytoplasmic portions of the channel [13]. Equation 3.56 predicts an AChR channel conductance value of 0.6 nS in a 100 mM KCl solution (1.29 S m⁻¹). The actual measured conductance is 0.08 nS [13]. This near eightfold discrepancy will arise from the fact that the corresponding Kn value of 0.47 places the problem of calculating the conductance of the AChR channel into the meso-scale region between the continuum approximation and a model involving discontinuous, dynamic, molecular physics (see Chapter 10). The presence of the rings of negative charges at the openings of the AChR channel also indicate that its effective conductance will also be determined by the rates at which ions can diffuse towards (or be repelled from) the channel openings. The gramicidin A channel has an effective radius of 0.4 nm, a length of 2.5 nm and a measured conductance for K⁺ ions in 0.1 M KCl of 21.6 pS ([11], Chapter 11). The theoretical conductance based on Equation (3.56) is 0.26 nS, a value some 12-times larger than the measured one. The Kn value for the gramicidin channel is 0.7, which like the AChR channel places it in the meso scale and explains why a macroscopic model is not appropriate for calculating its conductance. Some of the molecular models that have been explored to understand the conductance of the smallest diameter ion channels are described by Hille ([11], Chapter 11).

### 3.14 The Voltage Clamp

To explain the ‘overshoot’ shown in Figure 3.27 of the membrane potential to +30 ~ +40 mV observed at the peak of the action potential, Hodgkin et al. [14] formulated the so-called sodium hypothesis. Basically, this assumes that the initial change in the membrane potential only consists of a selective increase in the permeability to sodium that is large enough to dominate the diffusion regime for a short time. In the extreme case one might expect an overshoot of ~ 60 mV (the equilibrium potential \(V_{Na}\) for the sodium ions) but never a substantially higher value. They demonstrated that replacement of the extracellular NaCl by choline chloride, glucose or sucrose, molecules which do not penetrate the membrane, resulted in a reduction of the action potential in proportion to the reduction of the extracellular Na⁺ concentration, whereas the resting membrane potential remained unchanged. Replacement of the normal extracellular fluid by a hypertonic solution having an excess of sodium resulted in an increase in the overshoot of a magnitude that fitted with that predicted by the Nernst equation.

The amounts of Na⁺ and K⁺ entering or leaving the axoplasm during the activity of the membrane were determined using of radioactive tracers. Measurements were performed on the squid axon because its diameter of 500 ~ 1000 µm allows capillary electrodes to be readily inserted into the axoplasm (mammalian nerve axons have much smaller diameters of...
less than 10 \( \mu \text{m} \)). It was found that in the course of an action potential there was a net \( \text{Na}^+ \) entry of about 4 pmol/cm\(^2\) (~20,000 ions across 1 \( \mu \text{m}^2 \)) and a \( \text{K}^+ \) loss from the cytoplasm of the same amount. However, these experiments did not provide any information on the temporal course of the inward and outward flows of sodium and potassium. If these two oppositely directed ionic movements do generate a change in the membrane potential of the form of the action potential, the first event must be a charging of the membrane’s inside by an inwards-directed current to a positive value (e.g. +40 mV) that is followed by an outward-directed current that leads to the repolarisation of the membrane back down to around −70 mV. If these currents are carried by \( \text{Na}^+ \) and \( \text{K}^+ \) ions there must be a time lag between the \( \text{Na}^+ \) entry and the \( \text{K}^+ \) outflow.

Any attempt to demonstrate the separate contributions of these two currents on the propagating action potential would face the problem that the membrane potential changes with time along the length of the axon (in the manner of a wave packet). Consequently, the membrane current is composed partly of ionic currents crossing the membrane and partly of a component used to charge the membrane capacitance \( C_m \) to a changing membrane potential \( (i = dq/dt = C.dV/dt) \). Therefore, to measure the ionic currents a method was required to eliminate the complications arising from the charging of the membrane capacitance. This method is called the Voltage Clamp technique [14].

By means of the voltage clamp technique (described in more experimental detail in Chapter 8) the membrane potential can be maintained (clamped) at an arbitrary prechosen value and then changed and clamped almost instantaneously to a new chosen value. This can be achieved irrespective of the changes in the ionic currents that might follow as a result of changes in the driving force on the ions in the membrane, and changes in the membrane’s permeability to one or several ionic species. Because of the instantaneous potential displacement from one level to another, the membrane capacitance changes charge only at the instant of the membrane potential change. Therefore, the currents that may be measured during the voltage clamp (where \( dV/dt = 0 \)) are exclusively ionic currents that flow through the membrane. This is equivalent to connecting the axon’s inside and outside with a controllable constant voltage generator. As described in Chapter 8, this is achieved by inserting into the axoplasm an extra electrode (a so-called current electrode) that is connected to an electronic feedback circuit that, despite changes in membrane permeabilities, supplies a current of just the strength and direction to ensure that the membrane potential remains at a given predetermined level. Changes in the membrane current with time at this clamped membrane potential will provide information about the changes in the membrane permeability to the surrounding ions.

3.15 **Patch-Clamp Recording**

The direct way to investigate the functioning of membrane ion channels is to record the current which flows through an open channel, or to measure the changes in membrane potential produced by an imposed current. As depicted in Figure 3.20, the potential across a cell membrane can be measured by inserting a glass microelectrode into the cell and measuring the difference between its recorded potential and one registered by a reference electrode located in the extracellular medium. The voltage-clamp technique allows the membrane potential to be held at a constant value so that the current that flows through the membrane at any particular potential can be measured. However, some cells are too small to allow their penetration.
by a microelectrode. Also, the plasma membranes of neurons and muscle cells contain a very large number of voltage-gated ion channels, and the membranes of cells that do not exhibit electrical excitations contain a variety of other types of gated channel. The total current crossing a cell membrane is the algebraic sum of the currents flowing through all of these channels, which means that the functioning of a single ion channel is not possible using the conventional voltage-clamp technique. These issues can be overcome using the patch-clamp technique developed by Neher and Sakmann [15,16].

This technique (described further in Chapter 8) employs the fact that a clean, fire-polished, glass micropipette pressed against a cell can fuse to its membrane to form a very high resistance seal ($\geq 10^9$ $\Omega$) of good mechanical stability. This isolates a small patch of the membrane on the cell and the ion channels it contains can then be investigated through either electrical or chemical manipulation. The high resistance seal means that current can only enter or leave the micropipette through open channels in the isolated patch of membrane. Neher and Sakmann [15] were thus able to report the first recording of the activity of a single-channel (an acetylcholine-activated channel). By applying mild suction, a patch of membrane can be removed from a cell, so that the current through a single channel can be recorded as a function of different compounds exposed to the inside (cytoplasmic) membrane surface of a cell. By increasing the suction, an excised patch can also be prepared having its external membrane surface (outside-out) exposed to an outside solution. Measurements can be made of the millisecond kinetics for membrane ion currents as low as $10^{-12}$ A ($\sim 10^7$ ions/sec), with voltage clamping to give precise control over channel voltage-gating.

### 3.15.1 Application to Drug Discovery

The specific and regulated functioning of membrane ion channels plays important roles in many physiological processes. These include electrical signalling in the brain and heart, the secretion of hormones into the bloodstream, the transduction of sensory signals, the regulation of blood pressure and immune responses. Defects in ion-channel function can therefore result in profound physiological effects, and more than 55 different inherited ion channel diseases, termed as ‘channelopathies’, have in fact been identified [17]. Ongoing patch-clamp studies of ion-channel function and modulation, coupled with the identification of specific genetic defects that lead to ion-channel related diseases, are providing insights into the relationship between ion-channel structure and function. A well studied example is the potassium-ATP ($K_{ATP}$) channel, where mutations of its pore-forming proteins lead to an impaired ability of ATP to bind to the channel and thus to inhibit the channel’s transport of potassium ions. This can increase $K_{ATP}$ channel currents sufficiently enough to reduce electrical activity in nerves and muscles, leading to such diseases as diabetes, epilepsy and muscle weakness. This understanding has led to the development of drugs that specifically block ($K_{ATP}$) channels. The therapeutic action of many existing drugs (e.g. local anaesthetics, sedatives, antianxiety and antidiabetic) is through their interaction with membrane ion channels. Table 3.6 summarises some of diseases that are related to ion channel dysfunction.

Pharmaceutical companies have in the past faced problems in developing high-throughput assays able to randomly screen their large libraries (typically > 25 000) of potential ion-channel drugs. Many existing ion-channel drugs were developed without knowledge of the precise drug target and mode of action at the molecular level. Although molecular-induced modulation of ion channels can be observed using ion-sensitive or voltage-sensitive
fluorescent dyes, for example, this lacks the precision, temporal resolution, and voltage control that can be obtained using patch-clamp measurements. However, conventional patch-clamp studies are too technically demanding and laborious for the primary screening of potential ion-channel drugs. Recently, automated and medium-throughput techniques have been developed and are beginning to have an impact on the drug discovery ‘pipeline’ by providing high quality, information-rich, and biologically-relevant assays [17]).

3.16 Electrokinetic Effects

3.16.1 Electrophoresis

Electrically charged particles are induced to move in an electrical field – an effect called electrophoresis. In most practical situations the field is DC and spatially uniform. In this situation the force exerted on a particle carrying a net charge \( Q \) by an applied electric field \( E \) is given by:

\[
F_e = QE. \tag{3.57}
\]

The particle will accelerate until there is a balance between the electrophoretic force and the frictional force that opposes the particle’s motion through the fluid. To a first approximation we can consider the main resistive force to motion to be the Stokes viscous force:

\[
F_S = 6\pi \eta av \tag{3.58}
\]

where \( a \) and \( v \) are the particle’s radius and steady-state velocity, respectively, and \( \eta \) is the dynamic viscosity of the fluid medium. The steady-state velocity attained when the forces balance is:

\[
v = \frac{Q}{6\pi \eta a}. \tag{3.59}
\]

The electrophoretic mobility \( \mu_e \) can then be defined as:

\[
\mu_e = \frac{v}{E}. \tag{3.60}
\]

However, this analysis does not take into account the fact that the moving particle will carry a thin layer of fluid along with it. We will assume that this fluid flow behaves in the same way
as the Couette flow described in Section 9.3.5 and Figure 9.6 of Chapter 9. The fluid layer immediately in contact with the particle’s surface will match the velocity of that surface – we will have the condition known as zero slip at the interface between the particle surface and the fluid. This fluid layer will contain a distribution of counterions $r(x)$ of the form shown in Figure 3.4. Because, by definition, these counterions carry a charge of opposite polarity to that at the particle surface, they will experience a force given by Equation (3.57) but in the opposite sense to that experienced by the particle. This effect will produce a retarding force on the particle, so that the effective electrophoretic mobility will be less than that predicted by Equation (3.60). We have the overall scheme of forces acting on the charged particle as shown in Figure 3.31.

We will approximate the case shown in Figure 9.6, by assuming that the spatial gradient $dv/\text{dx}$ of velocity of the fluid associated with the particle decreases linearly as a function of distance $x$ from this interface. The sheer stress $\tau$ exerted on each fluid layer is thus given by Equation (9.14) of Chapter 9:

$$\tau = \eta \frac{dv}{dx} \cdot s,$$

where $\eta$ is the dynamic viscosity of the fluid. At the steady-state condition of electrophoresis, the velocity of each fluid layer will be constant. The sheer force and electric force acting on each volume element $Adx$ of the layer must therefore be equal and opposite:

$$\eta A \frac{dv}{dx} = E \rho(x) Adx$$

or

$$E \rho(x) = \eta \frac{d^2v}{dx^2}.$$

Replacing $\rho(x)$ using the Poisson Equation (3.15) we obtain

$$-\varepsilon_0 \varepsilon_r E \frac{d^2\phi(x)}{dx^2} = \eta \frac{dv}{dx}.$$
As boundary conditions for the integration of this equation, we can assume that as \( x \) tends to infinity the charged particle is effectively screened by the counterions (i.e. \( \phi(x) \) tends to zero, and that the velocity of a fluid layer is zero (the charged particle is moving relative to the bulk fluid). We will also depart from the Gouy-Chapman model of the electrical double layer by defining the potential \( \zeta \) as the potential at the boundary between the surface layer of fluid moving at the same velocity as the particle, rather than the potential exactly at the surface of the charged particle. On integration we thus obtain the result

\[-\varepsilon_0 \varepsilon_r E_\zeta = \eta v(0)\].

Thus, by adopting the definition of electrophoretic mobility given in Equation (3.60), from the above equation we obtain the following relationship:

\[\mu_e = \frac{v}{E} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta}\]  

(3.62)

This is known as the Helmholtz-Smoluchowski equation, and represents the improvement made by Smoluchowski in 1903 to Helmholtz’s original theory. The potential \( \zeta \) is known as the zeta potential. No adequate theory appears to definitely relate the zeta potential at the fluid slip plane to the electrostatic potential \( \phi(0) \) used in Equation (3.31) for the Gouy-Chapman model, where it defines the potential right at a charged surface. Equation 3.62 works well in solutions of high ionic strength, where the double-layer thickness \( k^{-1} \), defined as the Debye screening length in Equation (3.24), is much less than the particle radius \( a \) (i.e. \( ak \gg 1 \)). This is the case for most aqueous electrolytes because the effective Debye screening length is at most a few nanometers (see Figure 3.5). However, for nano-sized colloidal particles and very low ionic strength solutions, as depicted in Figure 3.32, the Debye length can exceed the particle radius. The retardation force caused by the atmosphere of counterions thus acts much further from the particle surface and is reduced. For the case \( ak < 1 \), an improvement to the theory introduced by Hückel in 1924 predicts the following relationship for the electrophoretic mobility of charged nanoparticles in very dilute electrolytes:

![Figure 3.32](image_url)

**Figure 3.32** Equation 3.62 provides a good description of the electrophoretic mobility of a charged particle in an electrolyte of high ionic strength, where the thickness (Debye length \( 1/\kappa \)) of the electrical double layer is much smaller than the particle radius \( a \). Nano-sized particles in an electrolyte of low ionic strength can have a Debye length much larger than the particle radius. In this situation the retarding force transmitted to the particle is weakened and Equation (3.63) more accurately describes the electrophoretic mobility.
An important biological application of electrophoresis is in the separation of a mixture of proteins according to their size (molecular weight) and electrophoretic mobility. If an electric field is applied to an aqueous solution containing free proteins, convection streams caused by local heating effects at the electrodes or within the solution can occur. These convection fluid currents will disturb the electrophoretic mobility of the protein molecules. This is avoided by mixing the proteins into a pH buffered stabilising medium such as agar or gels composed of silica, agarose or acrylamide, for example. The electrophoretic separation of protein molecules through a gel will then be based on both molecular sieving, related to the pore size of the gel, as well as the electrophoretic mobility of the molecules. The smaller proteins (those of lower molecular weight) will migrate more rapidly through the gel than those of larger molecular weight. Sections of the gel containing the isolated bands of a stained protein mixture can be removed for further analysis. The molecular weight of an unknown protein can be determined by comparing its migration through the gel against the migrations of protein molecular weight standards. This is shown schematically in Figure 3.33.

\[
\mu_e = \frac{2e_r e_0 \xi}{3\eta}.
\]  

3.16.1.1 Isoelectric Focusing of Proteins

As described in Chapter 2, protein molecules carry charged amino groups on their surface. Table 2.4 of Chapter 2 lists the \( pK \) values for these functional groups. The \( pK \) corresponds to the pH at which half of the members of that group are protonated. As the pH changes, the net charge on a protein’s surface will change. At high pH, most proteins will have many deprotonated surface groups, and will carry a net negative charge. At low pH, with many protons added to the surface, most proteins have a net positive charge. At some intermediate pH, different for every protein type, the net charge on the protein will be zero. The protein is not without charged groups, it carries equal numbers of positive and negative charges. The pH at which a protein has a net charge of zero is designated its isoelectric point (pI).

A protein dissolved in buffer at its pI has no net charge and thus no net electrophoretic mobility. In isoelectric focusing (IEF) a pH gradient is established along the length of a gel,
as shown in Figure 3.34. Proteins migrate through this gradient until they reach their pI. As shown in Figure 3.34, the gradient is set up so that negatively charged molecules migrate into a decreasing pH region. If a protein is in a region where the pH is above its pI, it has a negative charge and moves to a lower pH. If it is in a pH below its pI, it has a positive charge which moves it into higher pH regions. This gives rise to the self-focusing aspect of IEF, as proteins are continually swept back into tight bands centred on the appropriate pI. IEF is thus an equilibrium electrophoresis system, run until protein movement ceases.

3.16.1.2 Ampholytes are Used to Set Up the pH Gradient

The pH gradient in the isoelectric focusing gel shown in Figure 3.34 is generated by the inclusion of ampholytes, which are low molecular weight amphoteric molecules. Amphoteric molecules can react as either an acid or a base. A mixture of ampholytes is used, each having a different pI. Like protein molecules, the ampholytes migrate through the gel until they reach a region where the pH is equal to their pI. Unlike the proteins, the ampholytes are present in high enough concentration to change their local pH. The gel is set up with a uniform mixture of ampholytes throughout, and its anodic and cathodic ends are immersed in dilute acid and base respectively. Ampholytes near the ends of the gels will be positively charged near the positive electrode, and negatively charged near the negative electrode. They therefore begin to migrate into the gel, with the most charged (i.e. the ones furthest from their pI) moving the fastest. Over time they separate into zones of defined pH. If the ampholyte system is well designed, a smooth gradient of pH is created.

Various mixtures of amphoteric substances have been used as ampholytes, namely amino acids, proteins, and synthetic poly acidic, poly basic, molecules. Proteins can be good ampholytes, but they interfere with analyses of protein samples by introducing new proteins into the mixture. Polycarboxylic acid polyamines are the most commonly used ampholytes. These molecules have excellent buffering capacity across a broad pH range, and are usually provided in a molecular weight range of 300–500 daltons. Their sole disadvantage is that they may bind tightly to the sample proteins, due to ionic interactions, and can be very difficult to remove.

Figure 3.34 Isoelectric focusing is used to determine the pI of a protein, or to separate proteins based on their pI values. (a) An amphoteric fluid is mixed into a gel and a stable pH gradient established by applying an electric field. (b) A protein solution is added and the electric field reapplied. (c) Stained proteins distributed along pH gradient according to their pI values.
By combining the gel electrophoresis and isoelectric focusing techniques shown in Figures 3.33 and 3.34, two-dimensional gel electrophoresis can be performed. This technique, given the acronym 2D-PAGE, is depicted in Figure 3.35. Thousands of different proteins can be resolved from a single sample using 2-D gel electrophoresis. By combining mass spectrometric surface analysis of the gels, over 2500 proteins that comprise the *E.coli* proteome is capable of being detected from a single *E.coli* sample [18].

### 3.16.2 Electro-Osmosis

We have seen that electrophoresis is the motion of a charged molecule or particle in a fluid that is induced by an electric field. Electro-osmosis, on the other hand, is a phenomenon where liquid is induced to flow through a narrow channel or capillary by an applied electric field. For this effect to occur, immobilised electric charges must be present on the inner surface of a channel wall in contact with the liquid. This surface charge can arise from the adsorption of charged species in the liquid, or to have ionisable groups as part of the wall structure. A way to ensure that the walls are charged is to fabricate them from glass or fused silica, where deprotonation of Si-OH silanol groups occurs above pH 3 to form negatively charged silanoate (Si–O−) groups. This surface charge induces the formation of an electric double layer by attracting ions of opposite charge from the buffer solution, as shown in Figure 3.36.

If an electrical field is applied along the axis of the channel, a volume Coulombic force \((\rho E)\) will be exerted on the buffer solution. However, the net charge density \(\rho\) in the buffer is significantly different from zero only in a thin annular region, within the Debye length.

![Figure 3.36](image_url) The formation of an electrical double layer at the surface of internal charged channel or capillary wall. The net charge density \(\rho\) in the buffer solution is significantly different from zero only in a thin annular region, within the Debye length region of thickness \(1/\kappa\).
region close to the channel wall, as shown in Figure 3.36. Therefore, only the counterions in the fluid close to the wall will experience a Coulombic accelerating force and induce fluid movement – in a direction that depends on the field direction and polarity of the counterions. Due to the radial velocity gradient that is formed, the adjacent fluid annuli will be accelerated by the momentum transfer caused by viscous forces until the velocity gradient approaches zero across the whole radius of the capillary. This evolution of the electro-osmosis flow profile is shown in Figure 3.37.

As depicted in Figure 3.37, the charged fluid layer ‘drags’ the adjacent fluid layer along, until finally the entire channel moves at a uniform velocity. The ‘stationary-plate/moving-plate’ scheme outlined in Figure 9.6 of Chapter 9, to describe the viscosity of a Newtonian fluid, has in effect been created. Numerical simulations by Dose and Guiochon [19] demonstrated that this process develops on a timescale between $100\,\mu s$ and $1\,ms$. After that time, the whole fluid inside the channel moves at a constant velocity, with the resulting flow profile across the capillary being of a rectangular ‘plug’ shape as shown in Figure 3.38 [20]. This uniform velocity profile occurs if the channel characteristic length (diameter) is at least 7-times that of the electric double layer thickness (Debye length), and if other sources of fluid acceleration such as convection due to Joule heating are absent. This velocity profile is very different from that of pressure-driven flow, which has the parabolic profile shown in Figure 3.38, and is described further in Chapter 9. As a special characteristic of electro-osmotically pumped systems, fluid zones can be transported without significant hydrodynamic dispersion. This is of particular interest in capillary electrophoresis and other elements of microfluidic devices.

\[ v = \mu_{eo} E \]

\[ v(x) = 6\nu f[(x/X) - (x/X)^2] \]

**Figure 3.37** (a) On application of an electric field the counterions in the electrical double layers at the channel wall are accelerated and induce local fluid flow. (b) Shear forces accelerate neighbouring fluid lamina, until a steady-state is reached where all fluid lamina move at a uniform velocity given by Equation (3.64).

**Figure 3.38** The rectangular ‘plug’ flow profile of electro-osmosis differs from the laminar (parabolic) flow profile induced by pressure-driven flow (see Figure 9.15 and Equation (9.22) in Chapter 9).
Under the assumption that the buffer viscosity $\eta$ and the permittivity $\varepsilon_0\varepsilon_r$ are the same in the double layer as in free solution, the Smoluchowski theory described in Section 3.16.1 is valid, and the velocity $v_{eo}$ of a fluid undergoing electro-osmosis with an applied electric field $E$ is given by:

$$v_{eo} = \frac{\varepsilon_0\varepsilon_r\zeta E}{\eta}$$  \hspace{1cm} (3.64)

As for electrophoresis of a charged particle, the zeta potential $\zeta$ in this equation is the potential difference at the interface between the tightly held counterions close to the charged surface (the channel wall) and the bulk solution. This interface is called the slip plane or the surface of shear. The electro-osmotic velocity therefore depends on the characteristics (viscosity $\eta$ and permittivity $\varepsilon_0\varepsilon_r$) of the fluid undergoing transport, as well as the channel wall material that determines the surface charge and the value of $\zeta$. Fluid properties such as pH, ionic strength or composition, can have an enhanced or opposing effect. For example, in a bare glass capillary, the surface charge increases with pH. If the pH is increased through addition of high concentrations of a metallic salt, the ionic strength will increase and reduce the double layer thickness, which in turn will reduce electro-osmosis.

Microfluidic devices made from glass tend to have a well-characterised surface charge that varies predictably as a function of fluid pH and composition. Under physiological conditions, glass and silica have a negative zeta potential. With reduction of the local pH, the silanol groups on the glass surface become protonated and the zeta potential falls in magnitude. Many different surface modification techniques have been developed for glass, which allow the user to change the surface charge or to alter its biocompatibility (e.g. cell adhesion or nonfouling coatings). However, with an increasing use of polymeric components (silicone, Mylar, Teflon) electro-osmotic behaviour is less predictable. Biological fluids, in particular, can lead to problems such as protein adsorption on polymeric surfaces.

Some of the advantages and disadvantages of employing electro-osmosis to drive fluid flow in microchannels or capillaries can be summarised as follows:

**Advantages:**

- Uniform flow profile.
  This results in uniform retention times for all particles in a given section of a device, which can greatly simplify calculations and analysis. Because fluids move as a bolus, the leading and trailing edges of materials are minimised. This reduces the time and material required to change solutions in a device.
- No moving-part pumps are required.
- A simple fluidic interface.
  The interface between the source of pumping (electrodes) can be as simple as two wires placed into holes in the device. Unlike pressure-driven flow, we do not require a leak-tight interface between the source of the hydraulic force and the fluid being driven.

**Disadvantages:**

- Strong dependence on the electrochemical properties of channel wall and fluid.
  If a device is expected to process a variety of fluids or a fluid of unknown pH and ionic strength, the electro-osmotic velocity will be unpredictable.
Often requires high voltages (typically in the kV to MV range). This requires isolation of the electrodes from the sample fluid to avoid the products of electrolysis (bubbles, acid or base production) from entering the sample fluid, whilst at the same time retaining electrical connectivity.

Heat produced by the electric field may have to be dissipated.

A summary of the various ways to control electro-osmotic induced fluid flow in microfluidic devices is given in Table 3.7.

### 3.16.3 Capillary Electrophoresis

Capillary electrophoresis, also known as capillary zone electrophoresis, can be used to separate ionic species by their charge and frictional forces and mass. As discussed in Section 3.16.1, in conventional electrophoresis electrically charged molecules or particles move in a liquid under the influence of an electric field. The technique of capillary electrophoresis was designed in the 1960s to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte. This technique provides an alternative approach to gel electrophoresis to alleviate thermal convection problems in free solution electrophoresis.
The essentials of capillary electrophoresis are shown in Figure 3.39. A narrow-bore capillary, typically of length 25~75 cm, outside diameter 300~400 μm, inside diameter 25~75 μm, is filled with an electrolyte buffer to maintain a fixed pH of the solution. Due to its narrow bore, heat is dissipated efficiently by the capillary and allows high electric fields to be used. A liquid sample is introduced into the capillary via capillary action, pressure, or siphoning. The migration of the sample along the capillary is then initiated by an electric field, applied between the inlet and outlet reservoirs using electrodes energised by a high-voltage. All molecular components of the sample, whether or not they are positively or negatively charged, will be pulled through the capillary in the same direction (towards the cathode) by electro-osmosis. This is because the electro-osmotic flow of the buffer solution will be greater than the electrophoretic movement of the analytes. Even small, triply charged, anions will be directed to the cathode by the relatively powerful electro-osmosis of the buffer solution. Negatively charged components of the sample are thus retained longer in the capillary due to their conflicting electrophoretic mobilities. Experimentally, the electro-osmotic mobility can be determined by measuring the retention time of an electrically uncharged analyte. The velocity ($u$) of an analyte in an electric field can then be defined in terms of the sum of its electrophoretic and electro-osmotic mobility:

$$u = u_e + u_{eo} = (\mu_e + \mu_{eo})E.$$

The order of migration of species along the capillary is shown in Figure 3.40. Neutral species travel at the same velocity as the fluid towards the cathode (assuming that the capillary walls are negatively charged). Small multiply charged cations migrate quickly, whereas small multiply charged anions are strongly retarded. Thus, the chemical components of an injected sample separate as they migrate along the capillary, according to their characteristic electrophoretic mobilities. They are detected near the outlet reservoir in the form of an electropherogram, which displays the detected ‘peaks’ as a function of time, with the various ‘peaks’ spaced apart according to the different retention times of the analyte components. An example is given in Figure 3.41 of the separation at pH 4.5 of a set of proteins having pI values of 7.0 or higher (see Table 3.8). The proteins shown in Figure 3.41 each have a pI higher than 4.5 and so will have protonated amino acid side chains (see Table 2.4 of
Chapter 2) and carry a net positive charge. From Tables 2.5 and 3.8 we find that cytochrome-
c and lysozyme have similar molecular weights and pI values, and so it is not surprising to
see that their electro-osmotic migration times through a capillary are quite similar. Chymo-
trypsinogen, with a molecular weight of 25.7 kDa, is about twice the size of cytochrome-c,
and this is reflected in its much longer migration time.

A common form of detection is by optical absorbance at visible and UV wavelengths. A
section of the capillary is used as an optical
cell, and this ‘on-tube detection’ enables

Figure 3.40 A schematic to show the order of migration of charged species of different size and
charge, carried by electro-osmotic flow, during capillary electrophoresis. The chemical components of
an injected sample will separate as they migrate along the capillary to an extent determined by their
characteristic electrophoretic mobilities.

Figure 3.41 The migration times of four basic proteins (pI ≥ 7) obtained at pH 4.5 by capillary elec-
trophoresis (based on data presented by [20]).

Chapter 2) and carry a net positive charge. From Tables 2.5 and 3.8 we find that cytochrome-
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A common form of detection is by optical absorbance at visible and UV wavelengths. A
section of the capillary is used as an optical cell, and this ‘on-tube detection’ enables

Table 3.8 Isoelectric points (pI) of some proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>pI</th>
<th>Protein</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>&lt;1.0</td>
<td>Haemoglobin</td>
<td>6.8</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>4.6</td>
<td>Myoglobin</td>
<td>7.0</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>4.9</td>
<td>Chymotrypsinogen</td>
<td>9.5</td>
</tr>
<tr>
<td>Urease</td>
<td>5.0</td>
<td>Cytochrome-c</td>
<td>10.7</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>5.2</td>
<td>Lysozyme</td>
<td>11.0</td>
</tr>
</tbody>
</table>
detection of separated analytes with no loss of resolution. In general, capillaries used in capillary electrophoresis are coated with a polymer for increased stability, but the portion of the capillary used for optical detection must be optically transparent. Bare capillaries can break relatively easily and, as a result, capillaries with transparent coatings are available to increase the stability of the cell window. The path length of the detection cell in capillary electrophoresis (∼50 μm) is far less than that (∼1 cm) used in normal spectrometers. According to the Beer Lambert Law, described in Section 4.4 of Chapter 4, the sensitivity of the detector is proportional to the path length of the cell. To improve the sensitivity the path length can be increased. The capillary tube itself can be expanded at the detection point, creating a ‘bubble cell’ with a longer path length or additional tubing can be added at the detection point. Both of these methods, however, will decrease resolution of the separated analytes.

The resolution $R_s$ of capillary electrophoresis is often given [22] as:

$$R_s = 0.177(\mu_1 - \mu_2) \sqrt{\frac{V}{D_{av}(\mu_{av} + \mu_{eo})}},$$

where $\mu_1$ and $\mu_2$ are the effective mobilities of the more mobile and less mobile analyte, respectively, $\mu_{av}$ is their average mobility, $D_{av}$ is their average diffusion coefficient, $\mu_{eo}$ is the electro-osmotic mobility, and $V$ is the applied voltage. This expression has been greatly refined in subsequent work by Rawjee and Vigh [23]. Maximum resolution is attained when the electrophoretic and electro-osmotic mobilities are similar in magnitude and opposite in sign. In addition, high resolution requires low diffusion coefficients, and a low electro-osmotic velocity with a correspondingly increased analysis time.

Fluorescence detection can also be used in capillary electrophoresis for samples that naturally fluoresce or are chemically modified to contain fluorescent tags (see Section 4.2.4 in Chapter 4). Ethidium bromide, fluorescein and green fluorescent protein are common fluorophore tags. This mode of detection offers high sensitivity and improved selectivity for these samples, but cannot be utilised for samples that do not fluoresce. Laser-induced fluorescence has been used with a detection limit as low as $10^{-18}$ to $10^{-21}$ mol. This sensitivity arises from the high intensity of the incident light and the ability to accurately focus the laser on the capillary. In order to obtain the identity of sample components, capillary electrophoresis can be directly coupled with mass spectrometers or the surface enhanced Raman spectroscopy (SERS) technique described in Chapter 4. In most systems, the capillary outlet is introduced into an ion source that utilises electrospray ionisation. The resulting ions are then analysed by the mass spectrometer. This requires volatile buffer solutions, which will affect the range of separation modes that can be employed and the degree of resolution that can be achieved. For SERS, capillary electrophoresis eluants can be deposited onto a SERS-active substrate. Analyte retention times can be translated into spatial distance by moving the SERS-active substrate at a constant rate during capillary electrophoresis. This allows the subsequent spectroscopic technique to be applied to specific eluants for identification with high sensitivity. As shown by Lin et al. [24] SERS-active substrates can be chosen that do not interfere with the spectrum of the analytes.

Capillary electrophoresis can be incorporated into lab-on-chip devices (see Figure 3.42) and the electrical control of fluid flow in networks of channels and capillaries, without the
use of valves, is also possible. Early demonstrations of such lab-on-chip techniques were described by Seiler et al. [25].

The various advantages of capillary electrophoresis can be summarised as follows:

- small sample sizes (1–10 μl) can be used, and high sensitivity obtained (see Figure 3.43). Femtomole ($10^{-15}$ M) and zeptomole ($10^{-21}$ M) detection levels can be achieved using UV and fluorescence detectors, respectively;
- relatively fast separation times (1 ~ 45 min) can be achieved;
- easy technique to use with predictable selectivity;
- automation is possible;
- extremely high separation efficiencies are attainable;
- reproducible results can be obtained;
- Can be coupled to a wide range of detectors, such as UV/Visible absorption spectroscopy, mass spectrometry, electrochemical, conductivity, with laser-induced fluorescence being the most sensitive option.

Examples of the various ways in which capillary electrophoresis can be employed for the study and characterisation of biological molecules are given in Table 3.9.

\[ V = -3 \text{ kV} \]

Figure 3.42  Electro-osmosis can be used with capillary electrophoresis in lab-on-chip devices to both control fluid flow, fluidic mixing, and in this simple example to analyse the components of a sample using capillary electrophoresis. The direction and rate of fluid flow in the various arms of this fluidic T-junction can be controlled by the magnitude and polarity of the applied voltages, without the use of valves.
Dielectrophoresis (DEP) is defined as the translational motion of electrically neutral matter in a nonuniform electric field. The field can be either AC or DC. DEP is capable of selectively isolating, concentrating, or purifying target bioparticles when present in complex mixtures. Examples include the isolation of stem cells, cancer cells and bacteria from blood for therapy or further analysis. DEP also lends itself readily to miniaturisation and automation, either as standalone microdevices or as the means for rapid and efficient sample collection and preparation [26].

The DEP collection of particles at electrode edges is shown in Figure 3.44. An effect not possible by magnetophoretic manipulation of magnetic particles, namely the attraction to as well as the repulsion from a magnetic pole of the same particles under essentially the same conditions, is possible by DEP and is also shown in Figure 3.44. The ability to attract or repel particles from electrodes is an important aspect of DEP. The translational forces producing

<table>
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<th>Table 3.9  Summary of main of applications of capillary electrophoresis for the study and characterisation of biological molecules</th>
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<td><strong>Proteins, peptides</strong></td>
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<td>- Environment</td>
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<tr>
<td>- Foods, additives</td>
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<tr>
<td>- Ions (organic and inorganic)</td>
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3.16.4 Dielectrophoresis (DEP)

Dielectrophoresis (DEP) is defined as the translational motion of electrically neutral matter in a nonuniform electric field. The field can be either AC or DC. DEP is capable of selectively isolating, concentrating, or purifying target bioparticles when present in complex mixtures. Examples include the isolation of stem cells, cancer cells and bacteria from blood for therapy or further analysis. DEP also lends itself readily to miniaturisation and automation, either as standalone microdevices or as the means for rapid and efficient sample collection and preparation [26].

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![Figure 3.44](image-url)  
(a) Cells collecting at electrode edges under the influence of positive dielectrophoresis.  
(b) Cells repelled from electrode edges into a field ‘cage’ under the action of negative dielectrophoresis. The only difference in experimental conditions is the frequency of electrical excitation of the electrodes.
these effects arise from the interaction of the particle’s dipole moment $m$ with the non-uniform electric field.

### 3.16.4.1 Force imposed on an Infinitesimal Dipole in an Electric Field Gradient

A useful starting point in formulating the DEP behaviour of particles is to estimate the net force exerted on a small physical dipole when placed in a nonuniform electric field. In Section 3.2.4 a dipole is described as consisting of equal and opposite charges $+\delta q$ and $-\delta q$ located a vector distance $d$ apart. We define the dipole moment as $m = \delta q d$. From Equation (3.41) the electric potential $\phi$ at a distant point (but not at the dipole origin) from the dipole is given by:

$$
\phi = \frac{|m| \cos \theta}{4\pi \varepsilon_0 \varepsilon_r r^2},
$$

where $\theta$ and $r$ are, respectively, the polar angle and radial position (measured from the centre of the dipole) in spherical coordinates. This result is obtained on condensing the dipole to the limit of a double-point singularity in a manner, where as $d$ tends to zero, $q$ is increased in magnitude so that the dipole moment $m$ remains constant. As in our earlier treatment of electrostatic interactions, the quantity $\varepsilon_0 \varepsilon_r$ is the absolute permittivity of the medium surrounding the dipole. A plot of the equipotentials around a dipole is shown in Figure 3.45. In this figure the potential at any location is given by the sum of the individual potentials generated by the two point charges at that location. This follows from the Principle of Superposition, and is shown more clearly in the 3-D plot shown in Figure 3.45b. The electric potential $\phi$ (Volts) is a scalar quantity. The electric field $E$ generated around the dipole at any point is a vector quantity $E = -\nabla \phi$, where $\nabla$ is the del (gradient) vector operator. The total electric field at any point is the vector sum of the electric fields due to each charge. The field of a dipole is cylindrically symmetrical about the dipole axis, so that from Equation (3.65) the radial and transverse components of the field intensity in any meridian plane are given by:

$$
E_r = -\frac{\partial \phi}{\partial r} = \frac{1}{2\pi \varepsilon_0 \varepsilon_r} \frac{m \cos \theta}{r^3}; \quad E_\theta = -\frac{1}{r} \frac{\partial \phi}{\partial \theta} = \frac{1}{4\pi \varepsilon_0 \varepsilon_r} \frac{m \sin \theta}{r^3}.
$$

In Figure 3.46 the dipole is located in a nonuniform electric field $E$, with the negative charge $-q$ having a vector position $r$. The dipole contribution to the total electric field cannot exert a force on itself, and so is not included in the field $E$ of Figure 3.46. (If this were to be

![Figure 3.45](image)

(a) A two-dimensional plot of the equipotentials around an electric dipole. (b) A three-dimensional plot of the equipotentials shown in (a).
the case, it would be rather like the dipole ‘pulling itself up by its own bootlaces’!\) The total Coulombic force $F$ acting on the dipole is given by:

$$F = qE(r + d) - qE(r).$$  \hfill (3.67)

We can simplify this equation using a vector Taylor series expansion,\(^1\) and expand the electric field about position $r$ as:

$$E(r + d) = E(r) + d \cdot \nabla E(r) + \frac{d^2}{2!} \nabla^2 E(r) + \text{higher order terms}$$  \hfill (3.68)

so that $\nabla E$ (pronounced grad $E$) is the field gradient.\(^2\) If the dipole particle size is small compared to the length scale of the field nonuniformity, we can ignore the terms containing $d^2$ and higher in Equation (3.68). Substituting Equation (3.67) into (3.68) and condensing the dipole (i.e. taking the limit $d \to 0$) in such a way that the dipole moment $m$ (given by $m = qd$) remains finite, we obtain the force on an infinitesimal dipole as:

$$F = q(E + d \cdot \nabla E) - qE = qd \cdot \nabla E = m \cdot \nabla E.$$  \hfill (3.69)

This force is known as the \textit{Dielectrophoresis} (DEP) force and can be interpreted as the energy which must be expended to withdraw the dipole particle from the local field $E$ into a region where there is no field.

### 3.16.4.2 Dielectrophoretic Forces on Particles

We can make the conceptual transition from dealing with hypothetical point dipoles to real particles using the fact that materials are polarised when subjected to an electrical field. This polarisation produces surface charges on the material, creating in effect a macroscopic

---

\(^1\)Taylor’s series: $f(x + h) = f(x) + h \cdot f'(x) + \frac{h^2}{2} f''(x) + \frac{h^3}{6} f'''(x) + \ldots$ (where $f', f''$, $f'''$ are the 1st, 2nd, 3rd differentials, and so on).

\(^2\) $\nabla = i \frac{\partial}{\partial x} + j \frac{\partial}{\partial y} + k \frac{\partial}{\partial z}$ and $E = -\nabla V$ (written as $E = -\nabla V$) where $V$ is the electric potential. $E$ thus belongs to a special class of vector fields that can be expressed as the gradient of a scalar field (or scalar point function) and is said to be an \textit{irrotational field}. 

---

**Figure 3.46** (a) A dipole located in an electric field gradient, with the negative charge $-q$ having a vector position $r$. (b) Because the two dipole charges each experience different values of the electric field, they experience different Coulombic forces. This gives rise to a dielectrophoretic force acting on the dipole.
The induced surface charge density $\Delta \rho$ gives a measure of the polarisability of the dielectric material. We can relate this to a molecular property of the dielectric by defining a polarisability parameter $p$ as the average induced dipole moment per unit volume.

This relationship between induced surface charge on a polarised particle and an effective dipole moment is shown in Figure 3.47. Our task is to formulate an expression for this effective dipole moment and to make sure it generates the same potential contours around itself as the point dipole moment shown in Figure 3.45.

The effective dipole moment $m_{\text{eff}}$ of a particle of radius $R$, suspended in a medium of permittivity $\varepsilon_m$ and polarised in a field $E$, to replace the dipole moment $m$ in Equation (3.69) is:

$$m_{\text{eff}} = pvE = 4\pi\varepsilon_0\varepsilon_m R^3 \frac{f(\varepsilon)}{\varepsilon}E,$$

(3.70)

where $f(\varepsilon)$ is the effective polarisability (per unit volume) of the particle – generally referred to as the Clausius-Mossotti factor (named after a German and Italian scientist who made early contributions in the 1800’s to an understanding of dielectric properties). From Equations 3.69 and (3.70):

$$F_{\text{DEP}} = 4\pi\varepsilon_0\varepsilon_m R^3 \frac{f(\varepsilon)\langle E, \nabla \rangle E}{\varepsilon} = 2\pi\varepsilon_0\varepsilon_m R^3 \frac{f(\varepsilon)\nabla E^2}{\varepsilon}.$$  

(3.71)

Equation 3.71 teaches several important facts, namely that the DEP force $F_{\text{DEP}}$ is zero if the field is uniform (i.e. $\nabla E = 0$), and that the force depends on:

- the square of the applied electric field magnitude;
- the polarisability $p$ of the particle;
- the effective volume $v$ of the particle;
- the geometry of the electrodes producing the nonuniform field.

The product $(E, \nabla)E$ in Equation (3.71) has dimensions of $V^2/m^3$. The voltage-squared dependence indicates that the direction of the DEP force is insensitive to the polarity of the applied field – either DC or AC voltages can be used to energise the electrodes. The same DEP force can also be produced using a smaller applied voltage if the electrode dimensions are scaled down accordingly. For example, with all other factors remaining fixed, a one hundred-times smaller voltage can produce the same DEP force if the electrode dimensions are
scaled down 1000-fold. To produce a significant DEP force on a biological cell, for example, requires a value for \((E.\nabla)E\) of at least \(10^{12} \text{ V}^2/\text{m}^3\). With suitably scaled electrodes this can be achieved using an applied voltages of the order 1 V.

Biological particles such as bacteria and cells exhibit a conductivity associated with mobile ions in their structures, and the medium in which they are suspended is usually a conducting electrolyte. When A.C. fields are applied, these conduction losses can be described in the form of either a complex permittivity \(\varepsilon_p^s\):

\[
\varepsilon_p^s = \varepsilon_0 \varepsilon_p - \frac{j\sigma_p}{\omega}
\]

or a complex conductivity \(\sigma_p^s\):

\[
\sigma_p^s = \sigma_p + j\omega\varepsilon_0 \varepsilon_p,
\]

where \(j\) is the imaginary vector \((j = \sqrt{-1})\) and \(\omega\) is the angular frequency \((\omega = 2\pi f)\) of the applied A.C. field. The Clausius-Mossotti factor \(f(\varepsilon)\) in Equation (3.70) can be expressed as a complex function using either of the two equivalent forms:

\[
f(\varepsilon) = \left( \frac{\varepsilon_p^s - \varepsilon_m^s}{\varepsilon_p^s + 2\varepsilon_m^s} \right) \quad \text{or} \quad f(\varepsilon) = \left( \frac{\sigma_p^s - \sigma_m^s}{\sigma_p^s + 2\sigma_m^s} \right).
\] (3.72)

The total current in the particle can be considered to comprise two elements – one associated with field-induced movement of free charges such as ions, and the other arising from the field-induced perturbation of bound charges known as a displacement current. At low frequencies, as \(\omega \to 0\), the current is dominated by the conduction of free charges and there is essentially no phase difference between the field and the current. At high frequencies, \(\omega \to \infty\), the dielectric displacement current dominates and the particle acts like a capacitor with the current leading the applied field by a phase angle close to \(\pi/2\) radians. Depending on the relative values of the permittivity \(\varepsilon_p\) of the particle and that of the surrounding medium \(\varepsilon_m\), \(f(\varepsilon)\) will have a value bounded by the limits \(-0.5 \leq f(\varepsilon) \leq 1.0\). A positive value for \(f(\varepsilon)\) corresponds to the effective moment \(m_{\text{eff}}\) being colinear with the applied field \(E\), whilst \(m_{\text{eff}}\) acts against \(E\) when \(f(\varepsilon)\) is negative, which in turn corresponds to either a positive or negative DEP force, respectively. Examples of these two types of DEP response are shown in Figure 3.44. From Equation (3.72) it is clear that \(f(\varepsilon)\) is a complex variable (having real and imaginary components). The translational DEP force results from the electric field interacting with the in-phase component of the induced dipole moment, and so the real component of \(f(\varepsilon^s)\) should appear in Equation (3.71) and is this is commonly signified as \(\text{Re}[f(\varepsilon^s)]\).

### 3.16.4.3 Dielectrophoresis of Cells

Bioparticles, such as cells and bacteria, have complicated structures and certainly cannot be modelled as homogeneous spheres. A simple method to describe many bioparticles is by means of the so-called multishell model. A simple example of this is shown in Figure 3.48a in the form of the single-shell model used to describe a red blood cell (or a liposome) of radius \(R\). The membrane (thickness \(d\)) is assigned a specific conductance \(g_m\) and specific capacitance \(C_m\), with \(g_m = \sigma_m/d\), and \(C_m = \varepsilon_m/d\). \(C_m\) values for cells are large (reflecting the
The ultra-thin nature of the membrane – typically of the order $10 \sim 20 \text{ mF/m}^2$. For viable cells the membrane acts as a barrier to passive ion flow across it, and so $g_m$ has a low value – typically $\sim 1 \text{ mS/m}^2$ or lower.

Red blood cells (erythrocytes) are normally discoid in shape, but when suspended in an electrolyte they can take the form of a spheroid ($\sim 7 \mu\text{m}$ in diameter). Human erythrocytes, unlike white blood cells (leukocytes), do not possess a nucleus and can be represented as a thin membrane (the single shell) surrounding the cytoplasm. A 3-shell model is required to represent a cell with a nucleus, comprising four separate compartments: a 1st shell to represent the cytoplasmic membrane; a 2nd shell to represent the cytoplasm; and a 3rd shell to represent the membrane surrounding the nucleus. The Clausius-Mossotti function for a multishell particle is obtained by evaluating effective values for the relative complex permittivity $\varepsilon_p^*$ or conductivity $\sigma_p^*$ of the particle. The term effective is used to signify that a heterogeneous (multishell) particle may be replaced conceptually with one having homogeneous smeared-out bulk properties, such that substitution of this homogeneous particle with the original heterogeneous one would not alter the electric field in the surrounding medium. A numerical method for achieving this has been described by Huang et al. [27]. The corresponding Clausius-Mossotti function is then obtained by substituting this effective value for $\varepsilon_p^*$ into Equations (3.71) and (3.72). The frequency variation of this function is shown in Figure 3.48b for a model of a red blood cell with an intact membrane, and for one whose membrane has been damaged and no longer acts as a high resistance to passive ionic conduction. For frequencies below $\sim 100 \text{ kHz}$ nonviable, damaged, cells experience positive DEP and the viable ones negative DEP. This effect can be used to separate viable from nonviable cells that are suspended in a fluid that flows through a DEP chamber.

In DEP studies of mammalian cells (e.g. blood cells, cancer cells, stem cells) the suspending medium commonly takes the form of a low conductivity ($10 \sim 180 \text{ mS/m}$) electrolyte containing sufficient concentrations of sugars (e.g. mannitol, sucrose, dextrose) to raise the osmolarity to the normal physiological level of around $280 \text{ mOs/kg}$. For viable cells the plasma membrane acts as an electrical insulator to passive ion conduction, and thus at low frequencies the cell will appear as an insulating object suspended in a conducting medium. This corresponds to a negative polarisability factor $f(\varepsilon)$ so that viable cells will exhibit negative DEP at low frequencies, as shown for the example of a viable red blood cells in

![Figure 3.48](image-url)
Figure 3.48b. With increasing frequency the electrical field begins to penetrate into the conductive cytoplasm. Electronic engineers will recognise this as capacitive coupling between the suspending medium and cytoplasm, where the effective capacitance of the plasma membrane shorts out the membrane resistance. The effective conductivity of the cytoplasm will be less than that ($\sim 1.4 \text{ S/m}$) of a pure physiological strength electrolyte because of the presence of insulating bodies and structures (e.g. protein cytoskeleton, lipid membranes). Values for $\sigma_p$ in the range $0.1 \sim 0.5 \text{ S/m}$ are commonly deduced for viable cells above 1 MHz, so that depending on the choice of suspending medium conductivity we can achieve the condition $\sigma_p > \sigma_m$. From this we deduce that, with increasing frequency, a transition from negative to positive DEP may occur. The frequency value, commonly referred to as the DEP crossover frequency $f_{xo}$, of such a transition occurs when $Re[f(\varepsilon')]$ is zero. For the usual experimental conditions used in DEP experiments on cells, this crossover frequency occurs at a frequency of the order 100 kHz. Theoretical modelling of the Clausius-Mossotti factor $f(\varepsilon)$ of the type shown in Figure 3.48b predicts that a second crossover of the DEP response (from positive to negative DEP) can occur at a frequency above 100 MHz for some cells when suspended in suitable media. This effect corresponds to where displacement currents begin to dominate over ionic conduction effects as the frequency increases, and where the effective permittivity of the cell interior is less than that of the surrounding medium. The first systematic experiments to measure this so-called 2nd DEP crossover frequency were those of Chung et al. [28] for myeloma cells.

A cell’s plasmic membrane acts as a capacitor because it is constructed like one – namely a thin dielectric situated between two conductors (the outer and inner electrolytes). For a cell of radius $R$ suspended in an electrolyte of conductivity $\sigma_m$ the membrane capacitance $C_{cm}$ can be determined from a measurement of the first (lower frequency) DEP crossover frequency $f_{xo1}$, using the following relationship:

$$C_{cm} = \frac{\sqrt{2}}{2\pi R f_{xo1}} \sigma_m.$$

This equation assumes that the high resistance value of the cell membrane has not been impaired due to damage or the onset of cell death, for example. For a fixed cell radius, the effective membrane capacitance of a smooth cell will be less than that for a cell having a complex cell surface topography associated with the presence of microvilli, blebs, membrane folds or ruffles, for example. This will influence the value observed for $f_{xo1}$, which has important implications for applying DEP to characterise and selectively isolate target cells from other cells [26].

### 3.16.5 Electrowetting on Dielectric (EWOD)

In Chapter 9, surface tension is described as an inherently dominant force in the microscale. This force can be modified and controlled electrically. An early demonstration of this of relevance to lab-on-chip technologies was the demonstration by Pollack et al. [29] of rapid manipulation of discrete microdroplets along a linear array of electrodes. The direct manipulation of discrete droplets offers the means to integrate microfluidic systems without the need for conventional pumps, valves or channels. Such systems can be flexible, power efficient and capable of performing complex and highly parallel microfluidic processing tasks. To achieve this effect a voltage is applied, as shown in Figure 3.49, between a conducting liquid
droplet (e.g. an electrolyte) at rest on a dielectric layer and a counter electrode positioned below this dielectric. For maximum effect the dielectric surface should be of poor wettability – for example, a hydrophobic surface if the droplet is aqueous. The resulting charge that accumulates at the solid–liquid interface leads to a change in contact angle from $>90^\circ$ to $<90^\circ$, as shown in Figure 3.49. This is equivalent to a transition from a nonwetting to a wetting state. This effect is known as electrowetting on dielectrics (EWOD).

The liquid drop shown in Figure 3.49 is in contact with a solid insulator film of thickness $t$ and permittivity $\varepsilon_\text{o}\varepsilon_r$. A voltage $V$ is applied between the conducting liquid and a counter electrode situated beneath the dielectric film. Before the voltage is applied we assume that the solid–liquid interface is not electrically charged. When a voltage is applied the conducting liquid drop and the counter electrode form a capacitor $C$ of value proportional to the area $A_{\text{S-L}}$ formed by the solid–liquid interface at the base of the droplet. The surface capacitance $C$ (per unit area) is:

$$C = \frac{\varepsilon_\text{o}\varepsilon_r}{t}$$

The wetted dielectric surface will attain a charge of magnitude $Q = VC$, and this will decrease the surface potential energy of the water molecules at the solid-liquid surface. The water molecules will gain cohesive energy arising from their dipole attraction to these surface charges, and so reduce their potential energy. To a first order approximation the electrostatic energy stored in the capacitor ($\frac{1}{2}CV^2$) can be incorporated into an expression for the voltage-dependent solid–liquid interfacial energy $\gamma_{\text{S-L}}(V)$ to give:

$$\gamma_{\text{S-L}}(V) = \gamma_{\text{S-L}}(0) - \frac{\varepsilon_\text{o}\varepsilon_r}{2t} V^2,$$  \hspace{1cm} (3.73)

where $\gamma_{\text{S-L}}(0)$ is the interfacial energy with no voltage applied. The $V^2$ dependence indicates that either a direct current voltage, of positive or negative polarity, or an alternating current
voltage can be applied across the dielectric layer. For an ac rather than a dc voltage, $V^2$ is replaced by $V^2_{\text{peak}}$ in Equation (3.73).

The contact angle $\theta$ will be modified according to Young’s equation (see Chapter 9):

$$\gamma_{S-A} = \gamma_{S-L}(0) + \gamma_{L-A} \cos \theta(0) = \gamma_{S-L}(V) + \gamma_{L-A} \cos \theta(V),$$

where $\gamma_{S-A}$ and $\gamma_{L-A}$ are the solid-air and liquid-air surface energies, respectively.

From Equations (3.73) and (3.74) we obtain:

$$\cos \theta(V) = \cos \theta(0) + \frac{\varepsilon_a - \varepsilon_r}{2t\gamma_{L-A}} V^2.$$  \hfill (3.75)

From Equation (3.75) we can estimate that a voltage of $\sim 160$ V is required to lower $\theta$ from $110^\circ$ to $70^\circ$ for an aqueous droplet ($\gamma_{L-A} \sim 70 \times 10^{-3}$ Nm$^{-1}$) if PTFE (Teflon) or PET ($\varepsilon_r \sim 2$) of thickness $\sim 5$ $\mu$m is used as the dielectric. Equation 3.75 also indicates that the electro-wetting effect is enhanced if the dielectric thickness $t$ is reduced and $\varepsilon_r$ is increased. The field across the dielectric is $V/t$ and this should be maintained at a value well below that where charge injection and dielectric breakdown occurs. In the example considered above of 160 V applied across a dielectric thickness of 5 $\mu$m, the corresponding field is 32 MV m$^{-1}$, which is below the dielectric strength value of 60 MV m$^{-1}$ for PTFE, for example.

Some polymers can be vapour-deposited as thin hydrophobic dielectric films – a common example being various forms of poly(p-xylene) known as parylene that have values for $\varepsilon_r$ of around 3 and a dielectric strength $\sim 7$ MV m$^{-1}$. Other dielectrics being investigated for EWOD applications include the high-$\kappa$ (high dielectric constant) oxides that have replaced silicon dioxide as the gate material in the latest CMOS devices. Silicon oxynitride, for example, has a value for $\varepsilon_r$ of around 8, a dielectric strength greater than 1000 MV m$^{-1}$, and can be formed as a submicron layer on conducting silicon.

Through the suitable physical arrangement and electrical switching of electrodes, EWOD can be used to control the motion and delivery of fluid droplets in microfluidic devices. A droplet situated mainly on an electrode element, but also overlapping an adjacent electrode area, can be induced to relocate onto this neighbouring electrode by bringing the first electrode to earth potential and applying a voltage of sufficient magnitude to the neighbouring electrode (so as to change the leading dielectric-liquid contact angle from above to below $90^\circ$). A droplet can also be split into two separate portions by applying voltages to both of its adjacent electrodes. Refinements of the dielectric layers in EWOD devices, in terms of their dielectric strength, high permittivity, and surface wetting characteristics, is an active research area (e.g. [30]). EWOD can also be integrated with other technologies such as dielectrophoresis and optoelectronic tweezers to provide the means for single cell sample preparation and analysis on lab-on-chip devices (e.g. [31]).

References


