

Chapter 1

Basic principles

1.1 BIOCHEMICAL STUDIES

1.1.1 The aims of biochemical investigations

Biochemistry is concerned with the study of the chemical processes that occur in living organisms, with the ultimate aim of understanding cell function in molecular terms. Biochemists therefore undertake studies of topics such as:

- the structural, kinetic and thermodynamic characteristics of the molecules found in the whole range of living organisms;
- the function of these molecules and the mechanisms by which they recognise and interact with each other to produce ordered anabolic, catabolic, signalling, immunological and other pathways that characterise living processes;
- the pathways that operate for the synthesis and degradation of these molecules and the mechanisms responsible for errors in the pathways;
- the energetics of biological processes, including transport across cell membranes, the generation of cellular energy, energy conversion and exchange of energy with the surrounding environment;
- the storage, replication, expression, repair, recombination and control of genetic information and the development of cell specificity.

Pioneering biochemical investigations were carried out mainly on simple prokaryotic and eukaryotic organisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Neurospora crassa* and *Chlorella pyrenoidosa*. As knowledge of the nature of cellular components and control mechanisms was gained from these studies and shown to have many similarities with comparable data being gained from multicellular organisms, so the whole spectrum of biologically diverse organisms was opened up as model systems for detailed biochemical studies. Biochemists have traditionally used *in vitro* model systems rather than whole cells or organisms because of their inherently greater biochemical simplicity for experimental study and interpretation of results. Nevertheless, *in vitro* studies have the potential danger that the disruption of cells or tissues may lead to artefacts that bear little resemblance to the *in vivo* situation.

In recent years, and particularly in the past decade, the growth of biochemical knowledge relating to cellular function has grown exponentially. This has come about largely through the development of techniques for the rapid sequencing of DNA fragments released by the action of restriction enzymes, of gene cloning and site-directed mutagenesis (Chapters 5 and 6) coupled with advances in protein sequencing by mass spectrometry (Chapter 9). These developments have given rise to numerous new disciplines such as **genomics** (the study of cell genomes), **proteomics** (the study of the whole protein complement of a cell) and **molecular biology**, all of which fall within the broad discipline of biochemistry.

1.1.2 The design of biochemical investigations

Advances in biochemistry, as in all the sciences, are based on the careful design, execution and data analysis of experiments designed to address specific questions or hypotheses. Such experimental design involves a discrete number of compulsory stages:

- the identification of the subject for experimental investigation;
- the critical evaluation of the current state of knowledge (the 'literature') of the chosen subject area, noting the strengths and weaknesses of the methodologies previously applied and the new hypotheses that emerged from the studies;
- the formulation of the question or hypothesis to be addressed by the planned experiment;
- the careful selection of the biological system (species, *in vivo* or *in vitro*) to be used for the study;
- the identification of the variable that is to be studied; the consideration of the other variables that will need to be controlled so that the selected variable is the only factor that will determine the experimental outcome;
- the design of the experiment, including the statistical analysis of the results, careful evaluation of the materials and apparatus to be used and the consequential potential safety aspects of the study;
- the execution of the experiment, including appropriate calibrations and controls, with a carefully written record of the outcomes;
- the replication of the experiment as necessary for the unambiguous analysis of the outcomes;
- the analyses of the outcomes, including the use of appropriate statistical tests;
- the formulation of the main conclusions that can be drawn from the results;
- the formulation of new hypotheses and of future experiments that emerge from the study.

Biochemical experiments usually have much experimental detail in common. For example, the control and measurement of pH, temperature and oxygen tension are essential considerations for many studies. They also involve common manipulations, notably the preparation of solutions of known concentration and the dispensation of small volumes of reagents. The aim of this chapter is to address many of these common issues of experimental design and data analysis.

Table 1.1 SI units – basic and derived units

Quantity	SI unit	Symbol (basic SI unit)	Definition of SI unit	Equivalent in SI units
Basic units				
Length	metre	m		
Mass	kilogram	kg		
Time	second	s		
Electric current	ampere	A		
Temperature	kelvin	K		
Luminous intensity	candela	cd		
Amount of substance	mole	mol		
Derived units				
Force	newton	N	kg m s^{-2}	J m^{-1}
Energy, work, heat	joule	J	$\text{kg m}^2 \text{s}^{-2}$	N m
Power, radiant flux	watt	W	$\text{kg m}^2 \text{s}^{-3}$	J s^{-1}
Electric charge, quantity	coulomb	C	A s	J V^{-1}
Electric potential difference	volt	V	$\text{kg m}^2 \text{s}^{-3} \text{A}^{-1}$	J C^{-1}
Electric resistance	ohm	Ω	$\text{kg m}^2 \text{s}^{-3} \text{A}^{-2}$	V A^{-1}
Pressure	pascal	Pa	$\text{kg m}^{-1} \text{s}^{-2}$	N m^{-2}
Frequency	hertz	Hz	s^{-1}	
Magnetic flux density	tesla	T	$\text{kg s}^{-2} \text{A}^{-1}$	V s m^{-2}
Other units based on SI				
Area	square metre	m^2		
Volume	cubic metre	m^3		
Density	kilogram per cubic metre	kg m^{-3}		
Concentration	mole per cubic metre	mol m^{-3}		

1.2 UNITS OF MEASUREMENTS

1.2.1 SI units

The French [Système International d’Unités](#) (the SI system) is the accepted convention for all units of measurement. Table 1.1 lists basic and derived SI units. Table 1.2 lists numerical values for some physical constants in SI units. Table 1.3 lists the commonly used prefixes associated with quantitative terms. Table 1.4 gives the interconversion of non-SI units of volume.

1.2.2 Solutions – the expression of concentration

A solution is a homogeneous mixture of one or more substances ([solute\(s\)](#)) in a major liquid component ([solvent](#)). The concentration of the solutes in the solution expresses the amount of each solute in a given amount (weight or volume) of the solvent. The simplest expression of concentration is in terms of

Table 1.2 SI units – conversion factors for non-SI units

Unit	Symbol	SI equivalent
Avogadro constant	L or N _A	6.022 × 10 ²³ mol ⁻¹
Faraday constant	<i>F</i>	9.648 × 10 ⁴ C mol ⁻¹
Planck constant	<i>h</i>	6.626 × 10 ⁻³⁴ J s
Universal or molar gas constant	<i>R</i>	8.314 J K ⁻¹ mol ⁻¹
Molar volume of an ideal gas at s.t.p.		22.41 dm ³ mol ⁻¹
Velocity of light in a vacuum	<i>c</i>	2.997 × 10 ⁸ m s ⁻¹
Energy		
Calorie	cal	4.184 J
Erg	erg	10 ⁻⁷ J
Electron volt	eV	1.602 × 10 ⁻¹⁹ J
Pressure		
Atmosphere	atm	101 325 Pa
Bar	bar	10 ⁵ Pa
Millimetres of Hg	mmHg	133.322 Pa
Temperature		
Centigrade	°C	(<i>t</i> °C + 273.15) K
Fahrenheit	°F	(<i>t</i> °F – 32)5/9 + 273.15 K
Length		
Ångström	Å	10 ⁻¹⁰ m
Inch	in.	0.0254 m
Mass		
Pound	lb	0.4536 kg

s.t.p., standard temperature and pressure.

Table 1.3 Common unit prefixes associated with quantitative terms

Multiple	Prefix	Symbol	Multiple	Prefix	Symbol
10 ²⁴	yotta	Y	10 ⁻¹	deci	d
10 ²¹	zetta	Z	10 ⁻²	centi	c
10 ¹⁸	exa	E	10 ⁻³	milli	m
10 ¹⁵	peta	P	10 ⁻⁶	micro	μ
10 ¹²	tera	T	10 ⁻⁹	nano	n
10 ⁹	giga	G	10 ⁻¹²	pico	p
10 ⁶	mega	M	10 ⁻¹⁵	femto	f
10 ³	kilo	k	10 ⁻¹⁸	atto	a
10 ²	hecto	h	10 ⁻²¹	zepto	z
10 ¹	deca	da	10 ⁻²⁴	yocto	y

weight per unit volume (w/v). Alternatives are v/v and w/w. Such expressions may also be in the form of a percentage in which case the w/v, w/w or v/v is multiplied by 100. Thus a 1% (w/v) sodium chloride solution contains 1 g NaCl in 100 cm³ water. Less commonly, solutions may be expressed as parts per million (p.p.m.) or parts per billion (p.p.b.) of total solution. Such units can mean grams per million (or billion) grams or cm³ in a million (or billion) grams or cm³. Thus

Table 1.4 Interconversion of non-SI and SI units of volume

Non-SI unit	Non-SI subunit	SI subunit	SI unit
1 litre (l)	10 ³ ml	= 1 dm ³	= 10 ⁻³ m ³
1 millilitre (ml)	1 ml	= 1 cm ³	= 10 ⁻⁶ m ³
1 microlitre (μl)	10 ⁻³ ml	= 1 mm ³	= 10 ⁻⁹ m ³
1 nanolitre (nl)	10 ⁻⁶ ml	= 1 nm ³	= 10 ⁻¹² m ³

air contains approximately 8 p.p.m. carbon monoxide, where the units would be in volume. If the p.p.m relates to a solution in water, the approximation can be made that 1 g water is equivalent to 1 cm³. Hence 8 p.p.m. would be equivalent to 8 g in 1000 dm³ or 8 mg in 1 dm³ or 8 μg in 1 cm³ or 8 ng in 1 mm³ (see Table 1.4).

Molarity

The SI unit of the amount of any substance is the **mole**, defined as the amount that contains Avogadro’s number (N_A) of molecules (6.022 × 10²³). It can also be defined as the amount of a substance in which the number of elementary entities is equal to Avogadro’s constant. It is therefore possible to have a mole of molecules, of atoms, of ions or even of electrons. In practical terms, one mole of a substance is equal to its **molecular mass** expressed in grams, where the molecular mass is the sum of the atomic masses of the constituent atoms. Note that the term molecular mass is preferred to the older term molecular weight. The SI unit of concentration is expressed in terms of moles per cubic metre (mol m⁻³) (see Table 1.1). In practice this is far too large for normal laboratory purposes and a unit based on a cubic decimetre (dm³, 10⁻³ m) is preferred. However, some textbooks and journals, especially those of North American origin, tend to use the older unit of volume, namely the litre and its subunits (see Table 1.4) rather than cubic decimetres. In this book, volumes will be expressed in cubic decimetres or its smaller counterparts (Table 1.4). The **molarity** of a solution of a substance expresses the number of moles of the substance in 1 dm³ of solution. Molarity is expressed by the symbol M, but, since this has another use in the SI system (mega = M), mol dm⁻³ is recommended instead. Nevertheless, molarity continues to be expressed as M in many textbooks and international journals as well as in conversation and will be used in this book.

It should be noted that atomic and molecular masses are both expressed in **daltons** (Da) or **kilodaltons** (kDa), where one dalton is an atomic mass unit equal to one-twelfth of the mass of one atom of the ¹²C isotope. However, biochemists prefer to use the term **relative molecular mass** (M_r). This is defined as the molecular mass of a substance relative to one-twelfth of the atomic mass of the ¹²C isotope. M_r therefore has no units. Thus the relative molecular mass of sodium chloride is 23 (Na) plus 35.5 (Cl), i.e. 58.5, so that one mole is 58.5 g. If this was dissolved in water and adjusted to a total volume of 1 dm³, the solution would be one molar (1 M).

Table 1.5 Interconversion of mol, mmol and μmol in different volumes to give different concentrations

Molar (M)	Millimolar (mM)	Micromolar (μM)
1 mol dm^{-3}	1 mmol dm^{-3}	$1\text{ }\mu\text{mol dm}^{-3}$
1 mmol cm^{-3}	$1\text{ }\mu\text{mol cm}^{-3}$	1 nmol cm^{-3}
$1\text{ }\mu\text{mol mm}^{-3}$	1 nmol mm^{-3}	1 pmol mm^{-3}

Biological substances are most frequently found at relatively low concentrations and in *in vitro* model systems the volumes of stock solutions regularly used for experimental purposes are also small. The consequence is that experimental solutions are usually in the mmol dm^{-3} , $\mu\text{mol dm}^{-3}$ and nmol dm^{-3} range rather than molar. Table 1.5 shows the interconversion of these units.

Example 1 **CALCULATION OF MOLARITY**

Question 1

How would you prepare 250 cm^3 of 0.1 M glucose?

Answer

The molecular formula of glucose is $\text{C}_6\text{H}_{12}\text{O}_6$ so its molecular mass is $(6 \times 12) + (12 \times 1) + (6 \times 16)$, i.e. 180 daltons. Hence, 180 g dissolved in 1 dm^3 would give a 1 M solution, so that 18 g dissolved in 1 dm^3 would give a 0.1 M solution. Hence to prepare 250 cm^3 of 0.1 M solution, 4.5 g of glucose would be dissolved in water and the total volume adjusted to 250 cm^3 in a volumetric flask.

Question 2

How would you prepare 10 cm^3 of 0.01 M glucose from a 0.1 M stock solution?

Answer

Applying the dilution formula, $M_1 V_1 = M_2 V_2$, $M_1 = 0.1$, V_1 is unknown, $M_2 = 0.01$, $V_2 = 10\text{ cm}^3$. Hence $0.1 \times V_1 = 0.01 \times 10$, so V_1 must be 1.0 cm^3 . Hence 1.0 cm^3 stock solution (dispensed via an accurate automatic pipette) would be diluted to 10 cm^3 in a volumetric flask.

Question 3

What is the approximate molarity of a solution of glucose that contains 20 p.p.m.?

Answer

A 20 p.p.m. solution contains 20 g in one million grams or 20 mg in one kilogram. Assuming that the density of the solution is 1 g cm^{-3} , this is equivalent to 20 mg dm^{-3} . Hence the molarity of the solution is $20 \times 10^{-3} / 180\text{ M}$, i.e. $0.11 \times 10^{-3}\text{ M}$ or 0.11 M .

Question 4

What is the molarity of pure water?

Answer

Water has a molecular mass of $2 + 16 = 18$ daltons. The molarity of 1 dm^3 of water (equivalent to 1000 g if the density is assumed to be 1 g cm^{-3}) is therefore equal to $1000/18$, i.e. 55.6 M.

Dilution

In the preparation of experimental solutions it is common practice to prepare dilute solutions from more concentrated stock solutions. This dilution is easily achieved using the formula $M_1 V_1 = M_2 V_2$, where M_1 and M_2 represent the initial and final molarities and V_1 and V_2 represent the initial and final volumes. For the preparation of a given dilution, three of the variables will be known and the fourth can be calculated.

1.2.3 Concentration or activity?**Ionisation and ionic strength**

A solution of sodium chloride in water does not contain molecules of NaCl but rather individual sodium (Na^+) and chloride (Cl^-) ions due to the process of **ionisation**. Ionisation is possible in this case because sodium chloride forms a crystal lattice in which the sodium and chloride ions are held together by purely ionic attraction, i.e. there is no covalent bond formation. Sodium chloride is typical of the majority of inorganic salts, all of which ionise more or less completely in solution. The process of ionisation in such cases is therefore shown as being irreversible. Collectively these salts are said to be **strong electrolytes** and contrast with many other compounds, mainly organic acids and bases, which are only partially ionised in solution and are therefore said to be **weak electrolytes**. The process of ionisation of weak electrolytes is shown as being reversible. Ionisation of weak electrolytes, such as the carboxylic acids, is possible because, although there is a covalent bond between the oxygen and hydrogen atoms in the carboxyl group, the bond is highly polarised so that there pre-exists a partial positive charge on the hydrogen atom. Ionisation to release a proton places a negative charge on the oxygen atom that can be delocalised over the other oxygen atom in the carboxyl group. This stabilises the carboxyl anion (COO^-) relative to the carboxyl group and encourages ionisation. The relative ease with which the ionisation of weak electrolytes occurs is discussed in Section 1.3. Yet other organic compounds, for example alcohols including simple sugars such as glucose, do not ionise at all in solution and are therefore said to be **non-electrolytes**.

For some biochemical studies involving the use of both strong and weak electrolytes it is more important to measure the amount of individual ions present in solution than to know the concentration of the compound from which they arise. **Ionic strength** (μ) is a measure of the total ion charge in solution and is determined by both the concentration of all the individual ions present and their charge. Its value is calculated by use of equation 1.1:

$$\begin{aligned}\mu &= \frac{1}{2} (c_1 z_1^2 + c_2 z_2^2 \cdots c_n z_n^2) \\ &= \frac{1}{2} \sum c z^2\end{aligned}\tag{1.1}$$

where Σ indicates the sum of all the terms of the following type, $c_1, c_2 \dots c_n$ is the concentration of each individual ion in molarity, and $z_1, z_2 \dots z_n$ is the charge on the individual ions (thus for Na^+ and K^+ $z = +1$, for Cl^- and NO_3^- $z = -1$, for Ca^{2+} $z = +2$, and for SO_4^{2-} $z = -2$).

Whilst salts such as NaCl and KNO_3 , which consist of monovalent ions, ionise almost completely in aqueous solution, those that consist of divalent ions, such as MgSO_4 , ionise to a smaller extent owing to the process of **ion-pairing**. In this process, ions of opposite charge are attracted to each other in aqueous solution to form a tightly bound **ion-pair** that behaves as a single particle. Thus a 0.25 M solu-

Example 2 **CALCULATION OF IONIC STRENGTHS**

Question

Calculate the ionic strength of (i) 0.1 M NaCl , (ii) 0.1 M NaCl + 0.05 M KNO_3 + 0.01 M Na_2SO_4 .

Answer

Ionic strength can be calculated using the equation $\mu = \frac{1}{2} \sum cz^2$.

(i) Calculating cz^2 for each ion:

$\text{Na}^+ = 0.1 \times (+1)^2 = 0.1 \text{ M}$

$\text{Cl}^- = 0.1 \times (-1)^2 = 0.1 \text{ M}$

Hence

$\frac{1}{2} \sum cz^2 = 0.2/2 = 0.1 \text{ M}$

(ii)

$\text{Na}^+ = 0.1 \times (+1)^2 + 0.02 \times (+1)^2 = 0.12 \text{ M}$

$\text{Cl}^- = 0.1 \times (-1)^2 = 0.10 \text{ M}$

$\text{K}^+ = 0.05 \times (+1)^2 = 0.05 \text{ M}$

$\text{NO}_3^- = 0.05 \times (-1)^2 = 0.05 \text{ M}$

$\text{SO}_4^{2-} = 0.01 \times (-2)^2 = 0.04 \text{ M}$

Hence

$\frac{1}{2} \sum cz^2 = \frac{1}{2} (0.36) = 0.18 \text{ M}$

Note 1: the unit of ionic strength is M.

Note 2: that for Na_2SO_4 $c = 0.02$, since there are 2Na^+ per mole.

Note 3: that for a 1 M 1 : 1 electrolyte such as NaCl , the ionic strength is 1 M; for a 1 M 2 : 1 electrolyte such as MgCl_2 , the ionic strength is 3 M, for a 1 M 2 : 2 electrolyte such as MgSO_4 , the ionic strength is 4 M and for a 3 : 1 electrolyte such as FeCl_3 , the ionic strength is 6 M.

Note 4: As the concentration and ionic strength increase, this type of calculation becomes progressively inaccurate owing to the importance of activity coefficients.

tion of MgSO_4 is 65% ionised (i.e. 65% consists of individual magnesium and sulphate ions in solution), the remaining 35% existing as ion-pairs. As a consequence of ion-pairing it is more difficult to calculate the ionic strength of solutions of salts of this type.

In aqueous solution, anions and cations are surrounded by an **ionic atmosphere** or **shell** owing to the attraction by the ion of oppositely charged species, including water molecules in which the O—H bond is polarised to give a δ^+ on each of the hydrogen atoms and δ^- on the oxygen atom. This ionic atmosphere has a net charge that is opposite to, but smaller than, that of the ion it surrounds. Its presence results in a reduction of the effective charge of the central ion and hence its attraction for oppositely charged ions. This effect is enhanced by an increase in the ionic strength of the solution. This is the basis of the salting out of proteins (Section 8.3.4).

Activities and activity coefficients

Ionic strength influences the effective concentration of a compound that can ionise in solution such that the effective concentration, referred to as the *activity* (A), is related to the nominal concentration by a factor known as *the activity coefficient* (γ) as shown in equation 1.2:

$$A_x = [X] \gamma_x \quad (1.2)$$

where A_x is the activity of species X, $[X]$ is the concentration of X, and γ_x is the activity coefficient of X. An activity coefficient is a measure of the deviation of the behaviour of a species from the expected. As the ionic strength increases, the activity coefficient decreases, reducing the activity relative to the concentration. Thus the activity coefficient for Na^+ is 0.964 at 0.001 M and 0.79 at 0.1 M. The reverse of this, namely that, as the ionic strength decreases, the activity coefficient approaches unity, is important, since it means that under these circumstances the activity and concentration of the ion converge. The implications of this will be discussed later in the context of pH (Section 1.3.2). If the ionisable species gives rise to multiply charged ions, the activity coefficient decreases, irrespective of the sign, + or −, of the ions. Thus the activity coefficient for Mg^{2+} and Fe^{3+} , each at 0.001 M, is 0.872 and 0.738, respectively, at 25 °C.

Practical biochemical studies quite commonly include the use of reactants that are subject to discrepancies between concentration and activity. The design of such experiments has to include an assessment of the importance of this effect. Clearly, the impact will be greatest in those cases where the effect of a particular ion on a process or response is being studied. It is also important to realise that electrodes, such as the pH electrode and ion-selective electrodes commonly used in biochemical work, respond to the activities rather than concentration of the ion being measured. In the majority of other types of study it is generally acceptable to assume that activity and concentration are interchangeable, bearing in mind that in most biochemical studies the concentrations of reagents are generally low. When needed, the values of activity coefficients of organic and inorganic ions can be found in tables of physical constants.

1.3 WEAK ELECTROLYTES

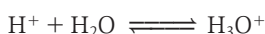
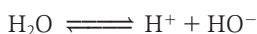
1.3.1 The biochemical importance of weak electrolytes

Many molecules of biochemical importance are weak electrolytes in that they are acids or bases that are only partially ionised in aqueous solution. Examples include the amino acids, peptides, proteins, nucleosides, nucleotides and nucleic acids. The biochemical function of many of these molecules is dependent upon their precise state of ionisation at the prevailing cellular or extracellular pH. The catalytic sites of enzymes, for example, contain functional carboxyl and amino groups, from the side-chains of constituent amino acids in the protein chain, that need to be in a specific ionised state to enable the catalytic function of the enzyme to be realised. Before the ionisation of these compounds is discussed in detail, it is necessary to appreciate the importance of the ionisation of water.

1.3.2 Ionisation of weak acids and bases

Ionisation of water

One of the most important weak electrolytes is water, since it ionises to a small extent to give hydrogen ions and hydroxyl ions. In fact there is no such species as a free hydrogen ion in aqueous solution, as it reacts with water to give a **hydronium ion** (H_3O^+):



Even though free hydrogen ions do not exist, it is conventional to refer to them rather than hydronium ions. The equilibrium constant for the ionisation of water has a value of 1.8×10^{-16} at 24°C . Since the ionic strength of water is very low, the activity coefficients for the hydrogen ions and hydroxyl ions will both effectively be unity so that the activity of each of these two ions is equal to their concentration. As calculated previously, the molarity of pure water is 55.6. This can be incorporated into a new constant, K_w . Thus, effectively, the activity of water is set at unity. It follows that:

$$1.8 \times 10^{-16} \times 55.6 = [\text{H}^+][\text{HO}^-] = 1.0 \times 10^{-14} = K_w \quad (1.3)$$

K_w is known as the **autoprotolysis constant** of water and does not include an expression for the concentration of water. Its numerical value of exactly 10^{-14} relates specifically to 24°C . At 0°C K_w has a value of 1.14×10^{-15} and at 100°C a value of 5.45×10^{-13} . The stoichiometry in equation 1.3 shows that hydrogen ions and hydroxyl ions are produced in a 1 : 1 ratio; hence both of them must be present at a concentration of $1.0 \times 10^{-7}\text{M}$. Since the Sørensen definition of pH is that it is equal to the negative logarithm of the hydrogen ion activity, it follows that the pH of pure water is 7.0. This is the definition of neutrality.

This theoretical background to the pH of water is well known, but what is not so well appreciated is the influence on the pH of water of adding a salt such as NaCl.