

1

THE PREPARATION OF SOLUTIONS IN BIOSCIENCE RESEARCH

1.1 Introduction

To help understand the many complex processes and interactions that occur within a cell (*in vivo*) it is often necessary to simulate cellular events in the laboratory (*in vitro*). The many constituents of the cell vary in their concentration, size and physical characteristics and, while it is often difficult to exactly simulate the cellular environment in the laboratory, it is possible to provide an environment in which the cellular constituent under study functions. Then, by careful adjustment of the solution, an understanding of the cellular constituent's role within the cell can be ascertained. This means that the preparation of solutions is a vital step in bioscience research and a topic that all bioscience students should become familiar with.

However, to gain complete control of this topic you *must* memorize key topics, such as the common units used in bioscience research (Table 1.1). The only way to avoid this confusion is to commit to memory the various units involved, or keep a copy of Table 1.1 conveniently placed for ease of use. The units detailed in Table 1.1 are representative of the concentrations in which many metabolites are present within the cell, for example calcium ions (Ca^{2+}) can be present at mM concentrations outside the cell but only nM within the cell's cytoplasm.

Student exercise

- Memorize the units in Table 1.1
- Memorize the sub divisions to the units in Table 1.1.

Having mastered the units, you will be ready to apply the information to the preparation of solutions, although one of the major stumbling blocks you may find is overcoming an aversion to numerical topics. Many students view biosciences as descriptive subjects and try to block out the numerical aspects. If this is the case for you, then this approach will greatly limit your involvement

Table 1.1 *Common units in bioscience*

	1.0	1.0×10^{-3}	1.0×10^{-6}	1.0×10^{-9}	1.0×10^{-12}
Concentration	Molar (M)	millimolar (mM)	micromolar (μ M)	nanomolar (nM)	picomolar (pM)
Amount	mol	millimol (mmol)	micromol (μ mol)	nanomol (nmol)	picomol (pmol)
	gram (g)	milligram (mg)	microgram (μ g)	nanogram (ng)	picogram (pg)
Volume	Litre (L)	millilitre (ml)	microlitre (μ l)	nanolitre (nl)	picolitre (pl)

in the topic. In fact this fear of numeracy is usually misplaced as most of the calculations are straightforward and can become second nature with practice.

On a more practical note, from a student's point of view, numerical assessments within bioscience courses differ from descriptive topics in one notable aspect. In an assessment it is possible to get 100% of the available marks on a numerical question, whereas this is rarely true for a descriptive answer as the marker will always be reluctant to allocate 100% of the marks, signifying a perfect answer.

1.2 Concentration

The concentration of metabolites, chemicals and reagents that are used in bioscience are reported in a few different notations (e.g. molarity and percentage).

1.2.1 Molarity

Science uses molarity to ensure numerical equivalence in terms of the molecules present in a solution. In a mol there are 6.022×10^{23} molecules present (Avogadro's number). This is true for all reagents, for example a mol of sodium chloride (NaCl; Mr 54.55), a mol of glucose ($C_6H_{12}O_6$; Mr 175) or a mol of adenosine triphosphate (ATP; Mr 355) will all have the same number of molecules present. A mol of these reagents will result in different weights because different compounds will have different atoms present in their structure, in effect a different size or mass. But a mol of a reagent will *always* have the same number of molecules present.

Molarity is the most common method of describing the concentration of a reagent in solution. The sentence should be read again. . . ‘the *concentration* of a reagent *in solution*’. This describes exactly what occurs; a reagent (possibly a powder) is dissolved in a volume of liquid (usually water). Therefore, two things are required before a concentration can be ascribed; a known weight of a reagent must be physically weighed out, then transferred and dissolved in a known volume of liquid. A mol of a compound is a physical weight (Mr in grammes). You can go to the chemical shelves take the reagent required and weigh out a mol.

When a reagent is dissolved in a volume of liquid the units change from mol to molarity (unit of concentration). The shorthand version of this unit is the capital letter M (Table 1.1). This unit of concentration describes an amount of reagent dissolved in a volume of liquid.

A molar solution (M) is the molecular weight in grams (mol) dissolved in a litre (1000 ml) of water (see Table 1.1).

$$\text{A 1.0 M SOLUTION} = \frac{\text{MOLECULAR WEIGHT IN GRAMS (MOL)}}{\text{VOLUME (1 LITRE)}}$$

1.3 Using Balances to Weigh Out Reagents

1.3.1 The Use of an Electronic Balance

In practice the amount of reagent you would weigh out to prepare a solution depends on the availability of the reagent (always check and comply with the Health and Safety data sheet for the reagent before commencing any weighing out) and access to an appropriate accurate balance.

Most bioscience laboratories will have a range of balances accurate to 1, 2, 3 or 4 decimal places. Large amounts of inexpensive reagents are routinely weighed out on ‘top pan’ balances (see Figure 1.1), whereas small amounts of expensive reagents should be weighed out using an analytical balance. If a hazardous reagent is being weighed out, use a balance contained within a designated safety area with a positive air pressure to ensure that no particles of the reagent can enter the laboratory air space.

Always check to see that the balance is clean. If you need to clean the balance take the appropriate health and safety precautions and dispose of the debris in a prescribed container.

1.3.2 The Use of ‘top pan’ Balances:

- Place a suitable container (e.g. beaker, plastic weighing boat or aluminium foil) gently onto the top pan balance (see Figure 1.1a) and wait for the

(a)



(b)



Figure 1.1 Examples of typical balances (a) top pan (b) analytical.

electronic readout to stabilize. Press the zero button (or TARE) on the balance to bring the reading to zero.

- Using an appropriate spatula (see Figure 1.2a), gently add the reagent to the container to achieve the correct weight.

Then remove the container and ensure the balance is clean for the next user.

1.3.3 The Use of Analytical Balances

Analytical balances are sensitive to surrounding vibrations and are usually placed on a vibration free bench (see Figure 1.1b).

- Check that the balance is clean and if it requires cleaning take the appropriate health and safety precautions.
- Open the side door to the balance and place a suitable container on the balance pan (e.g. small beaker, a plastic tube, plastic weighing boat or aluminium foil).
- Close the door and allow the electronic readout to stabilize. Press the zero button (or TARE) on the balance to bring the reading to zero. Alternatively, note the weight of the beaker and add the weight of the beaker to the amount of reagent required.
- Add the reagent to the container on the pan of the analytical balance using a micro spatula (see Figure 1.2b), close the side door and allow the read out to stabilize. To weigh out accurately is a skill, but with practice it is possible to weigh out as little as 1.0 mg.
- When the desired amount has been weighed out the container should be carefully removed and the reagent dissolved.



Figure 1.2 A typical (a) spatula and (b) micro spatula.

- Remember to check that the floor and the pan of the balance are clean. Usually there are brushes and wipes available to keep these important devices clean (remember to dispose of any debris in compliance with health and safety regulations).

1.4 Practical Considerations When Making a 1.0 M Solution

No matter how careful you are in preparing a solution for use in bioscience research, there is always the possibility that bacteria or fungi may be present initially in small numbers in the final solution. This may not be a problem if the solution is to be used immediately, but it can be a problem if the solution is left in storage for a period of time.

- Storing solutions at a reduced temperature will arrest or slow down bacterial growth. A solution that is in regular use should be stored in a refrigerator (4–8 °C) and then warmed to the required operating temperature before use. Stocks of solutions can be stored frozen (–25 °C) in convenient aliquots, these can then be thawed and warmed to the required operating temperature before use.
- A concentrated stock solution (e.g. $\times 10$) may help prevent (or arrest) the growth of bacteria or fungi. Dilutions can be prepared from the stock which will take up less storage space.
- If the reagent is heat stable the prepared solution can be placed in a suitable container and heated to 120 °C under pressure in an autoclave. Allow the solution to cool before storage.
- Alternatively, before storage, the solution can be passed through a filter with a pore size (0.2 μm or 0.45 μm) which is small enough to trap bacteria.
- Another approach involves using aseptic techniques to prepare the solution in a filtered air stream (see Chapter 8 for further details).
- Different combinations of the above may be required.

Working out the amount required to make a 1.0 M solution of any reagent is relatively straight forward. You simply find the relative molecular mass (M_r) from the reagent bottle or from a reference book, accurately weigh the required amount, transfer the solid to a suitable beaker (or vessel) then add the highest quality water available¹ initially to a volume less than a litre to dissolve the reagent (see worked example 1.1).

Dissolving a reagent completely is required for the correct final concentration to be achieved. It is usual to add a magnetic stirring flea into the solution in a beaker and then place this on a metallic stirring device. Set the device to a slow stir and wait for the reagent to dissolve (see Figure 1.7). Difficult to dissolve

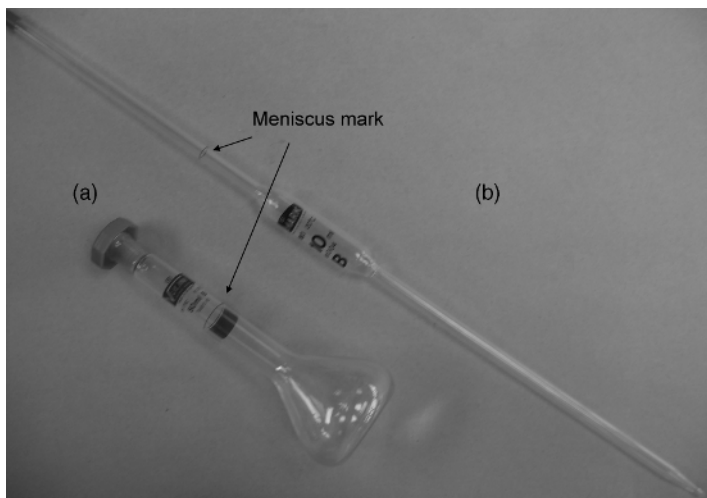


Figure 1.3 Volumetric (a) flask and (b) pipettes.

reagents may require the addition of heat and hot plate stirrers can be used in conjunction with a glass beaker (remember to use the appropriate safety precautions). Alternatively, a microwave oven can be used to slightly elevate the temperature of the liquid. In either case boiling the water should be avoided. Ultrasonic baths can be used to quickly disperse solids into a solution but remember that prolonged use of these baths will eventually elevate the temperature of the solution.

If the pH of the reagent requires (see Section 1.7.1) adjusting, then the solution should be first cooled to the temperature at which it is to be used (the reason for this is that some reagents have a different pH at different temperatures). The pH can be adjusted (see Figure 1.7) with the addition of the appropriate acid or base before the solution is added to a volumetric flask (see Figure 1.3a).

Worked Example 1.1: Prepare a 1.0 M solution of NaCl

- 1.0 M solution of NaCl will have 54.5 g of NaCl in 1000 ml.
- Weigh out 54.5 g of NaCl in a 1000 ml beaker on a top pan balance.
- Add 750 ml of distilled water to dissolve the NaCl. This can be helped by the addition of a stirring flea and then placing the beaker on a magnetic stirring device.

- When the solid has dissolved transfer the liquid to a 1000 ml volumetric flask. Volumetric flasks have a mark etched on the neck of the vessel, when the meniscus of the liquid touches this mark the correct volume is achieved.
- Add distilled water to the flask until the meniscus of the liquid is at the mark on the neck of the volumetric flask.
- Add a stopper and invert the flask 5–10 times to mix the liquid.
- Transfer the liquid in the volumetric flask to storage bottle/flask. Label this with (a) the name of the reagent, (b) the molarity, (c) the pH, (d) any specific health and safety precautions (e.g. corrosive or poisonous), (e) the date of preparation and (f) your own name and course.
- Store the liquid in the fridge or at room temperature.

1.4.1 Preparing Solutions with a Concentration Less than 1.0 M and a Volume Less than 1.0 Litre

The concentration of metabolites within the cell is rarely in the molar range, so less concentrated solutions are often required for convenience and to reduce the costs. It is these calculations that open up a variety of errors for many students, but if you break the calculation down to its constituent parts and practice, the calculation will become second nature.

Worked Example 1.2: Prepare 25 ml solution of adenosine triphosphate (ATP) at a final concentration of 50 mM

- *Write down what weight of ATP would be required for a 1.0 M solution* 507.2 g of ATP would need to be weighed out and dissolved in 1.0 L (1000 ml) to make a 1.0 M solution. Clearly with expensive reagents this is a prohibitive amount.

- *Adjust for the volume required.*

We don't want 1000 ml (1.0 litre), we only require 25 ml (1.0 ml is 10^{-3} of 1.0 litre). So divide the Mr of ATP by 1000 to get to 1.0 ml and then multiply by 25 to get to the required volume. This is now the weight in grams of ATP required to make 25 ml of a 1.0 M solution.

$$\frac{507.2 \times 25}{1000} = 12.68 \text{ g} \dots (\text{A})$$

We are preparing a solution which is in a smaller volume than a litre so always check that the weight calculated is less than the Mr of the required reagent.

- *Adjust for the concentration.*

We don't want 25 ml of a 1.0 M solution, we require a final concentration of 50 mM (1.0 mM is 10^{-3} of 1.0 M). So divide the weight of ATP obtained

above (A) by 1000 to get to 1.0 mM and then multiply by 50 to get to the required molarity.

$$\frac{12.68 \times 50}{1000} = 0.63 \text{ g} \dots (\text{B})$$

This is the weight (0.63 g) of ATP required to make 25 ml of a 50 mM solution.

This may seem a basic piece of advice for graduate students, but everyone is capable of making a mistake in these calculations and if you stick to this method you should avoid many errors.

There are sometimes requirements for less concentrated solutions. The series of calculations outlined above can be used for these less concentrated solutions as well.

Worked Example 1.3: : Prepare 50 ml solution of ATP at a final concentration of 750 μM

- *Write down what weight of ATP would be required for a 1.0 M solution*
507.2 g of ATP would need to be weighed out and dissolved in 1.0 L (1000 ml) to make a 1.0 M solution. Clearly with expensive reagents this is a prohibitive amount.

- *Adjust for the volume required.*

We don't want 1000 ml (1.0 litre) we require 50 ml (1.0 ml is 10^{-3} of 1.0 litre). So divide the Mr of ATP by 1000 to get to 1.0 ml and then multiply by 50 to get to the required volume. This is now the weight in grams of ATP required to make 50 ml of a 1.0 M solution.

$$\frac{507.2 \times 50}{1000} = 25.36 \text{ g} \dots (\text{A})$$

We are preparing a solution which is in a smaller volume than a litre so always check that the weight calculated is less than the Mr of the required reagent.

- *Adjust for the concentration.*

We don't want 50 ml of a 1.0 M solution, we require a final concentration of 750 μM (1.0 μM is 10^{-6} of 1.0 M). So divide the weight of ATP obtained above (A) by 1 000 000 to get to 1.0 μM and then multiply by 750 to get to the required molarity.

$$\frac{25.36 \times 750}{1000 \times 1000} = 0.019 \text{ g} \dots (\text{B})$$

This is the weight (0.019 g) of ATP required to make 50 ml of a 750 μM solution.

Using this method it is possible to correctly calculate the amount in grams of any reagent required to make up any concentration or volume.

1.4.2 Preparing Percentage Solutions

In some circumstances percentage solutions are used in bioscience research and these solutions have different units.

A percentage solution means that an amount of reagent in grams is dissolved for every 100 ml of liquid. When the percentage unit is (w/v) it is a weight of a reagent in a 100 ml volume (w/v) (e.g. 0.9% w/v NaCl).

This means that if 100 ml of this solution is required, it is prepared by weighing out 0.9 g of NaCl into a suitable vessel. A volume of water less than 100 ml is added and the NaCl is dissolved. When the NaCl has dissolved the solution can then be made up to 100 ml to give the final percentage solution of 0.9% (w/v) NaCl.

If a different final volume is required then both the amount of reagent and volume should be adjusted accordingly.

Worked Example 1.4: : The preparation of % solutions

- *Prepare 25 ml of 0.9% (w/v) NaCl*

Adjust for the volume by dividing by 100 to get the amount required for 1.0 ml and then multiply by 25 to get the amount required for 25 ml.

$$\begin{aligned}
 &= \frac{0.9 \times 25}{100} \text{ g} \\
 &= 0.225 \text{ g}
 \end{aligned}$$

Dissolve this amount of reagent in a volume less than 25.0 ml; when this has dissolved, make the volume up to 25.0 ml.

- *Prepare 375 ml of 0.9% (w/v) NaCl*

Adjust for the volume by dividing by 100 to get the amount required for 1.0 ml and then multiply by 375 to get the amount required for 375 ml.

$$\begin{aligned}
 &= \frac{0.9 \times 375}{100} \text{ g} \\
 &= 3.375 \text{ g}
 \end{aligned}$$

Dissolve this amount of reagent in a volume less than 375.0 ml; when the reagent has dissolved make the volume up to 375.0 ml.

Liquids can also be weighed out to give a weight in a volume solution for example 2% (w/v) glycerol. If 100 ml of 2% (w/v) glycerol was required, 2.0 g of the liquid glycerol would be weighed into a beaker and water added to a volume less than 100 ml. When the glycerol had dissolved the volume could then be made up to 100 ml.

However, liquids are frequently prepared as a volume within a volume concentration (v/v) (e.g. 5% v/v methanol). If 100 ml of 10% (v/v) methanol solution is required, 10 ml of methanol is measured out and made up to 100 ml with distilled water. Smaller volumes of the same percentage can be prepared by adjusting the volumes of both liquids proportionally.

1.4.2.1 *Weight in a Volume (w/v) Solutions*

Some common materials encountered in bioscience, for example proteins and nucleic acids are commonly dissolved as a weight in a declared volume for example mg ml^{-1} (this can also be written as mg/ml). The reason for this is that solutions of proteins can be a heterogeneous population of different molecules, which makes constructing molar concentrations meaningless. The concentrations of proteins from cellular extracts are commonly estimated using reagents which interact with proteins to produce a coloured product (see Chapter 3.8).

1.5 Dilutions and the Use of Pipettes

It was proposed (Section 1.4) that a convenient method to prepare solutions of different concentrations is to initially prepare a concentrated stock solution and then dilute down from this concentrated solution to the concentration and volume required. The ability to prepare accurate dilutions is an important skill in bioscience research (also see Chapters 2 and 8).

Preparing large volume dilutions can be achieved using a measuring cylinder (see Figure 1.4a) but accurate dilutions requires the use of a pipette. Volumetric glass (see Figure 1.3b) or cylindrical graduated glass/plastic pipettes (see Figure 1.4b) are still in use but these have been superseded by the use of mechanical (or electronic) micropipettes (see Figure 1.5), primarily on health and safety issues, accuracy and ease of use. All pipettes work by creating a vacuum above the liquid that requires dispensing in a liquid holding barrel. The liquid is dispensed by releasing the vacuum with care.

KEY POINTS TO REMEMBER 1.1.

- A dilution implies that the concentration of the final working solution is *less* than the concentration of the stock solution. If your calculation produces a more concentrated solution than the stock you must start the

calculation again. It is also important to ensure that the units of volume and concentration (see Table 1.1) are the same on both sides of the equation.

- A 1 in 10 dilution is 1 part in a final volume of 10. (*Not* one part added to ten parts).

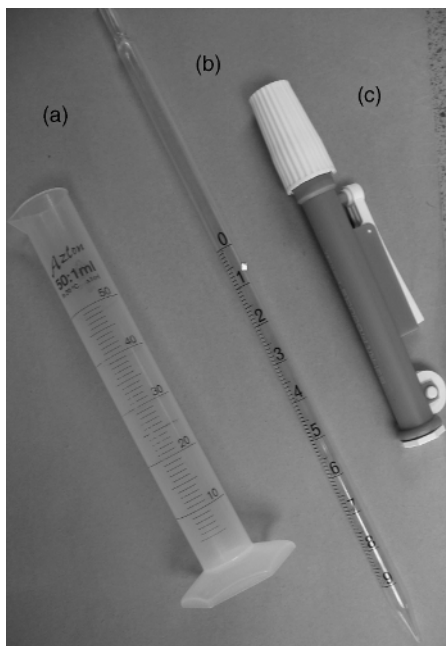


Figure 1.4 An example of (a) a measuring cylinder, (b) cylindrical pipette and (c) pipette filler.

1.5.1 The Use of Graduated Cylindrical Glass or Plastic Pipettes

If a graduated cylindrical glass or plastic pipette is to be used to prepare a dilution from a stock solution:

- Never use your mouth to draw liquid into the pipette!!
- Ensure that the pipette is clean and has no flaws at either end.

- *Hold the pipette at the top* and insert it into the nozzle of a pipette filler (see Figure 1.4c or Figure 8.1) using a firm hold. Do not try and force the pipette into the filler; if there is an obviously poor grip between the filler and the pipette then exchange the pipette filler for a new one.
- Draw liquid into the cylindrical barrel to the level required as recommended by the manufacturer. Hold the pipette above the stock liquid and check to see if the pipette drips liquid. If the pipette drips liquid replace the pipette (or the pipette filler/or both).
- Dispense the stock solution into a suitable vessel.
- Place the used pipette in a suitable tray for cleaning.

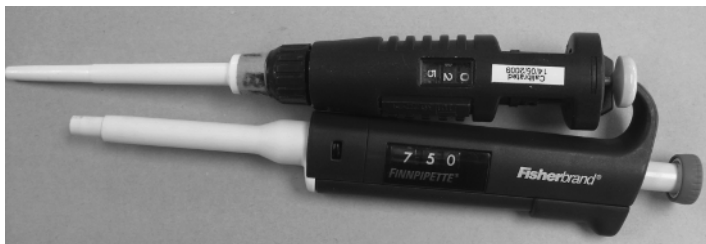


Figure 1.5 *Example of two common laboratory pipettes.*

1.5.2 The Use of Hand Held Micropipettes

Micropipettes are routinely used in bioscience laboratories and, although there are many different manufacturers of hand held micropipettes, the design is inherently similar (see Figure 1.5).

- Micropipettes are accurate only between the range of volumes designated by the manufacturer (e.g. 100–1000 μl). This range is usually on the side and top of the pipette, so check to see if the pipette covers the range required. If it doesn't, then change the pipette.
- Adjust the volume on the pipette to the volume required (remember that 1.0 ml is 1000 μl – see Table 1.1). Do not adjust the pipette beyond its designated range.
- Place a suitable tip on the end of the pipette (pipettes with different volume ranges require different tips. Please check if you are unsure).
- Before drawing up the liquid, hold the pipette in your hand and depress the plunger using the thumb. Notice that there are two stops to which the plunger can be depressed; the first stop on the pipette will allow the required volume of

liquid to be taken up into the pipette tip and the second stop allows any trace liquid to be expelled from the pipette tip.

- With the pipette tip on the end of the micropipette depress the plunger to the first stop; place the tip into the liquid, slowly allowing the plunger to return to the starting position. Check to see that no air bubbles have been drawn into the tip.
- Move the pipette to the dispensing container and depress the plunger initially to the first stop, this is followed by moving the plunger to the second stop.
- The pipette tip can then be removed (most micropipettes have a mechanical device to remove the tip) and disposed of according to health and safety regulations.
- Your ability to pipette accurately can be practiced using an empty beaker on a balance and some distilled water. Set the pipette to a volume and dispense water into the beaker on the balance. Note the weight at each addition and after ten additions work out the average weight and the standard deviation. The average weight should correspond to the setting on the pipette which will indicate the accuracy of the pipette and the standard deviation will show you how adept you are at dispensing the same volume.

KEY POINTS TO REMEMBER 1.2

- 0.1 ml of water weighs 0.1 g (100 mg)
- 1.0 ml of water weighs 1.0 g
- 1000 ml weighs 1.0 kg.

1.5.3 Diluting Down from a Stock Solution

A convenient formula can be used to calculate dilutions from a stock solution

$$C_1 V_1 = C_2 V_2$$

Initial concentration (C_1) x initial volume (V_1) = final concentration (C_2) x final volume (V_2)

Worked Example 1.5:

- *You are supplied with 1.0 M stock solution of pyruvate.*

What volume of the 1.0 M pyruvate stock is required if you need to prepare 5 ml of a 25 mM solution?

$$C_1 V_1 = C_2 V_2$$

$$1000 \times V_1 = 5 \times 25 \text{ (note } C_1 \text{ is 1000 mM – see Table 1.1)}$$

$$V_1 = \frac{5 \times 25}{1000} \text{ ml}$$

$$= 0.125 \text{ ml (note this is also } 125 \mu\text{l – see Table 1.1)}$$

Take 0.125 ml of the 1.0 M stock solution and make this up to 5.0 ml to arrive at a 25 mM solution.

- *You are supplied with a 25 mM stock solution of L-lysine.*

What volume of the 25 mM L-lysine stock is required if you need to prepare 0.75 ml (750 μ l) of a 500 μ M solution?

$$C_1 V_1 = C_2 V_2$$

Convert all the units to the 1×10^{-3} level (milli) (see Table 1.1)

$$25 \times V_1 = 0.75 \times 0.5 \text{ (note } C_1 \text{ is 0.75 mM – see Table 1.1)}$$

$$V_1 = \frac{0.75 \times 0.5 \text{ ml}}{25}$$

$$= 0.015 \text{ ml (note this is also } 15 \mu\text{l – see Table 1.1)}$$

Answer: Take 0.015 ml (15 μ l) of the 25 mM stock solution and make this up to 0.75 ml to arrive at a 500 μ M solution.

Alternatively:

- *Convert all the units to 1×10^{-6} level (micro) (see Table 1.1)*

$$25,000 \times V_1 = 750 \times 500 \text{ (note } C_1 \text{ is 25 mM or } 25000 \mu\text{M – see Table 1.1)}$$

$$V_1 = \frac{500 \times 750}{25,000} \mu\text{L}$$

$$= 15 \mu\text{l (note this is also } 0.015 \text{ ml see Table 1.1)}$$

Take 15 μ l of the 25 mM stock solution and make this up to 750 μ l (0.75 ml) to arrive at a 500 μ M solution.

1.6 Water, Acids and Bases

Most living matter is composed of approximately 70% water and many of its properties, including the formation of H-bonds and the ability to ionize, are fundamental to the correct structure and properties of many biological compounds. Understanding the properties of water and how it interacts with other molecules is fundamental to understanding many topics within biosciences.

The structure of water means that there are electron rich areas (enriched negative charge) within the molecule and electron depleted areas (enriched positive charge) called a charge dipole. This dipole of charge contributes many of the properties of water because the dipole on one molecule of water can interact with the dipole on other water molecules (see Figure 1.6a), thus establishing a network of interactions described as hydrogen bonds (H-bonds). In water most of the molecules are engaged in hydrogen bonding with their nearest neighbours and each bond lasts between 1–20 pico seconds (1 picosecond = 10^{-12} seconds) before the bond is reformed or a new bond is formed with a neighbouring water molecule. Hydrogen bonds are relatively weak bonds (the *bond dissociation energy* is low 23 kJ mol^{-1}) if this is compared to some covalent bonds (470 kJ mol^{-1} to break the O=H covalent bond or 348 kJ mol^{-1} to break a C=C covalent bond). This is also reflected in the greater bond distance of the hydrogen bond compared to a covalent bond (see Figure 1.6a). This means that, as the temperature rises (hence an increase in available energy), the amount of H-bonding decreases. In ice, H_2O molecules form four hydrogen bonds with other H_2O molecules to form a low temperature crystal lattice. The elevated temperature of water results in an increase in the random movement of the H_2O molecules and each molecule hydrogen bonds with only 3.4 water molecules.

KEY POINTS TO REMEMBER 1.3

- Hydrogen bonds are important weak bonds that hold the structures of proteins and nucleic acids together. In most cases elevated temperatures (e.g. $>37^\circ\text{C}$) will alter the structure of biological macromolecules.
- Hydrogen bonding can be formed between molecules other than water in biological systems. The dipole on electron rich atoms such as oxygen and nitrogen (relatively negative and thus a hydrogen bond acceptor) and the dipole on the hydrogen attached to oxygen or nitrogen (relatively positive and thus a hydrogen bond donor) may interact if they are within the correct bond forming distance.

1.6.1 Water as a Solvent

Water is a polar solvent because the dipole on water molecules enables water to interact with itself and other charged (*polar*) molecules, allowing them to dissolve (*hydrophilic*) (see Figure 1.6b) compounds. In contrast, non polar (*hydrophobic*) molecules cannot interact with water, and in the presence of water non polar molecules tend to group together. The water molecules surrounding the non polar molecules form caged (*clathrate*) structures (see Figure 1.6c).

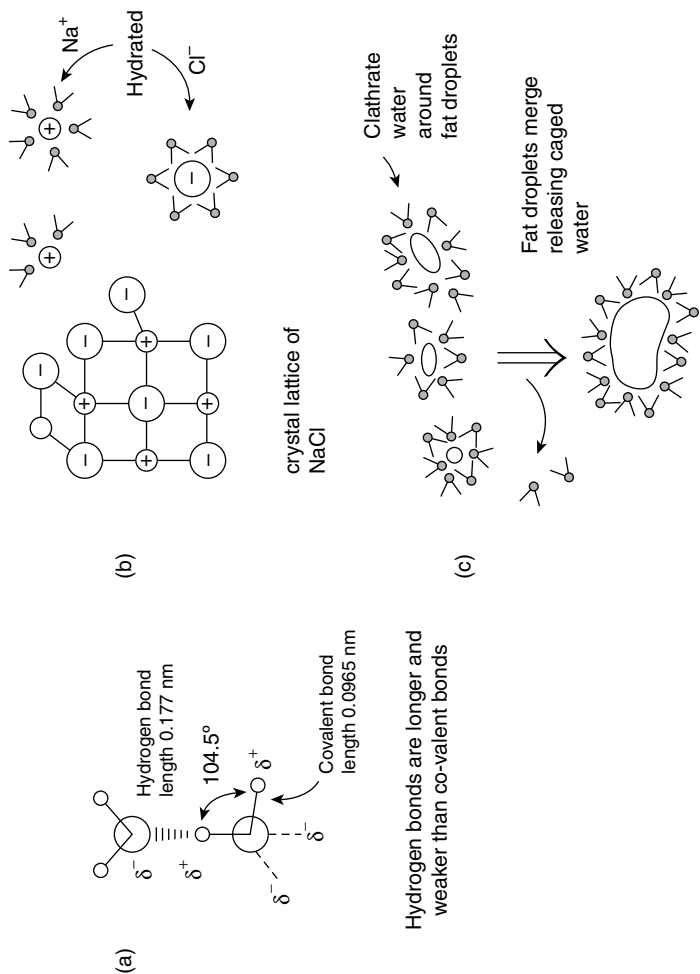


Figure 1.6 The structure of water (a) contributes to its ability to solubilise ionic compounds (b) but not non ionic compounds (c).

1.6.2 The ionization of water

For every billion water molecules (10^9) there are approximately two molecules that ionize at room temperature. This of course is a very low degree of ionization but this still represents another important property of water.

Water ionizes into a positively charged hydrogen ion $[H^+]^+$ and a negatively charged hydroxyl ion $[OH^-]^{*-}$



All reactions can reach equilibrium where the rate of the dissociation is equalled by the rate of association. The equilibrium constant for water K_{eq} at $25^\circ C$ is given by the equation.

$$K_{eq} \rightleftharpoons \frac{[H^+][OH^-]}{[H_2O]} \quad (1.1)$$

In pure water the molar concentration of water is 55.5 M (Divide the weight of 1.0 litre of water [i.e. 1000 g] by the relative molecular mass of water; $M_r = 18$) and the K_{eq} for water has been determined experimentally to be 1.8×10^{-16} .

Substitute these values into Equation (1.1).

$$1.8 \times 10^{-16} \rightleftharpoons \frac{[H^+][OH^-]}{[55.5]}$$

The ion product of water (K_w) at $25^\circ C$ is

$$K_w = [1.8 \times 10^{-16}] [55.5] = [H^+][OH^-]$$

$$K_w = 1 \times 10^{-14} \text{ M}$$

The ion product of water (K_w) always equals $1 \times 10^{-14} \text{ M}$. When the concentration of $[H^+]$ is equal to the concentration of $[OH^-]$ the solution is at the neutral pH value (7.0).²

This value can be calculated when the concentration of $[H^+]$ is equal to the concentration of $[OH^-]$.

$$K_w = [H^+][OH^-] = [H^+]^2$$

Solving this for $[H^+]$

$$\begin{aligned} [H^+] &= \sqrt{K_w} = \sqrt{1 \times 10^{-14}} \\ &= 1 \times 10^{-7} \end{aligned}$$

The ion product of water is constant which means that when the concentration of $[H^+]$ is high, there is a corresponding decrease in the concentration of $[OH^-]$.

Table 1.2 *The pH scale*

$[\text{H}^+]$ (M)	pH	$[\text{OH}^-]$ (M)
10^0 (1.0)	0.0	10^{-14} (0.000000000000001)
10^{-1} (0.1)	1.0	10^{-13} (0.00000000000001)
10^{-2} (0.01)	2.0	10^{-12} (0.0000000000001)
10^{-3} (0.001)	3.0	10^{-11} (0.000000000001)
10^{-4} (0.0001)	4.0	10^{-10} (0.00000000001)
10^{-5} (0.00001)	5.0	10^{-9} (0.000000001)
10^{-6} (0.000001)	6.0	10^{-8} (0.00000001)
10^{-7} (0.0000001)	7.0	10^{-7} (0.0000001)
10^{-8} (0.00000001)	8.0	10^{-6} (0.000001)
10^{-9} (0.000000001)	9.0	10^{-5} (0.00001)
10^{-10} (0.0000000001)	10.0	10^{-4} (0.0001)
10^{-11} (0.00000000001)	11.0	10^{-3} (0.001)
10^{-12} (0.000000000001)	12.0	10^{-2} (0.01)
10^{-13} (0.0000000000001)	13.0	10^{-1} (0.1)
10^{-14} (0.00000000000001)	14.0	10^0 (1.0)

The ion product of water (K_w) is the basis of the pH scale, which denotes the molar concentration of $[\text{H}^+]$ in solution.

$$\text{pH} = -\log_{10}[\text{H}^+]$$

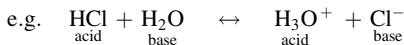
By taking the negative logarithm the exponential values (1×10^{-x}) values seen above are converted into linear arithmetic numbers (e.g. at neutral pH the $[\text{H}^+] = 1 \times 10^{-7} \text{ M}$ which is converted to pH 7.0 (see Table 1.2).

- The pH scale is the negative logarithm of the hydrogen ion concentration $[\text{H}^+]$. This means that the values for the molar concentration of $[\text{H}^+]$ decrease by a factor of 10 with each pH number not by a factor of 1.

1.6.3 Acids and Bases

The pH scale outlined in the previous section is a measure of the acidity of a solution describing the molar concentration of $[\text{H}^+]$ in solution. The compounds which can release H^+ ions into solution are known as acids. In 1923 two scientists Johannes Nicolaus Brønsted (Denmark) and Thomas Martin Lowry

(England) independently proposed essentially the same theory of acids and bases. This is now known as the Brønsted-Lowry theory of acids and bases, which proposes that an acid is a proton (H^+) donor and a base is a proton (H^+) acceptor. In addition, every acid has a conjugate base and every base a conjugate acid.

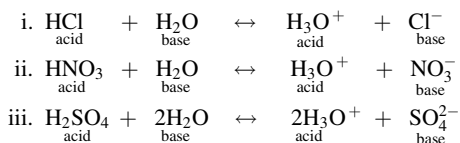


At the same time, another scientist Gilbert Newton Lewis (USA) proposed that acids can be considered as electron pair donors (Lewis acid) and bases as electron pair acceptors (Lewis base).

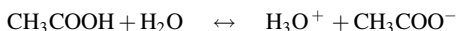
1.6.3.1 *Strong and Weak Acids*

Acids are often referred to as being ‘strong’ or ‘weak’. This does not refer to their ability to dissolve other compounds, but informs us of their ability to ionize and dissociate in solution.

A strong acid (e.g. hydrochloric acid (HCl), nitric acid (HNO_3) or sulphuric acid (H_2SO_4)) will ionize and dissociate at *all pH values* into their conjugate parts of acid and base.



In contrast, the degree of ionization and dissociation of ‘weak acids’ is dependent upon the pH of the solution in which they are dissolved. Acetic acid (CH_3COOH) is a familiar weak acid which ionizes to varying degrees across the pH scale (see Figure 1.7).



Below pH 3.0, when the $[\text{H}^+]$ concentration is high (see Table 1.2), any loss of the $[\text{H}^+]$ on the carboxyl group of acetic acid ($=\text{COOH}$) is quickly replaced. However, above pH 3.0 (i.e. when the $[\text{H}^+]$ concentration in solution decreases rapidly; see Table 1.2) the proton on the carboxyl group of acetic acid can dissociate and go into solution. This leaves a negatively charged acetic acid and a positively charged proton in solution.

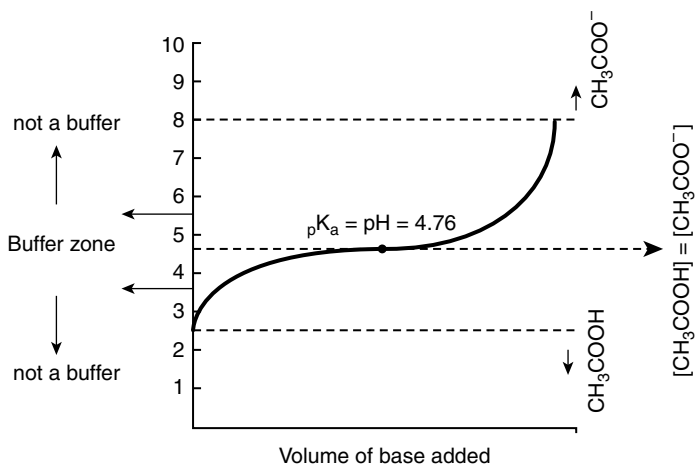


Figure 1.7 A titration curve for acetate buffer.

1.7 Buffers

Many biological compounds have weak acidic groups present within their structure. As a result, the surface charge on the molecule will vary according to the surrounding pH. Amino acids, proteins, nucleotides and nucleic acids have weak acidic groups as part of their structures and the degree of ionization of these molecules is pH dependent. As the integrity and functionality of these molecules depends on the pH, maintaining this optimum pH value is *extremely important* in biology.

1.7.1 Preparing a Buffer and Using a pH Meter

There are many different buffers used in bioscience laboratories to preserve the pH of a solution (some listed in Table 1.3). A buffer comprises of a weak acid and one of its salts (conjugate base) or a weak base and one of its salts (conjugate acid). Buffers work best when there is a balance between charged and uncharged species (pK_a) (see Figure 1.7 and section 1.8). At the pK_a buffers are able to absorb changes in the concentration of $[H^+]$ protons or hydroxyl groups $[OH^-]$ without significantly altering the overall pH. There is a limit to this buffering capacity which is approximately 1.0 pH unit either side of the buffer's pK_a . If the

Table 1.3 *A list of common buffers and their effective pH range*

Buffer	pKa	Effective buffer range
Maleate	1.97	1.2–2.6
Acetate/ CH_3COOH	4.76	3.6–5.6
MES	6.10	5.5–6.7
PIPES	6.76	6.1–7.5
$\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$	7.20	5.8–8.0
HEPES	7.48	6.8–8.2
Tricine	8.05	7.4–8.8
Tris/HCl	8.06	7.5–9.0
$\text{Na}_2\text{CO}_3/\text{NaHCO}_3$	10.33	9.0–10.7

desired pH value is at the extremes of the buffer's pKa value, the molarity of the buffer should be increased or a different buffer used.

- When the desired molarity of the buffer has been decided, the buffer is weighed out and dissolved (see Section 1.4) in a volume of water (highest purity available) less than the final volume required. Any additions to the buffer which may contribute to the pH value (e.g. EDTA) should be dissolved at this stage before the pH is finalized. When the buffer has dissolved, the pH can be adjusted using a pH meter (see Figure 1.8).
- The probe of the pH meter should be cleaned by rinsing with distilled water and the machine's response calibrated with standard buffer solutions according to the manufacturer's instructions.
- The pKa value for some buffers can vary with changes in the temperature (e.g. pKa of Tris at 4 °C is 8.8 but this will change to 8.3 at 20 °C). Most pH meters will also have a temperature adjustment switch. Consider equilibrating the buffer to the temperature it is to be used at in an experiment before adjusting the pH.
- The buffer solution can be placed on a magnetic stirring plate and a suitable magnetic stirring 'flea' should be rinsed before placing in the buffer solution. The stirring rate should not be too fast as the flea may jump and damage the pH probe (particularly important when using glass probes).
- The probe should be rinsed with distilled water prior to being placed in the buffer solution.

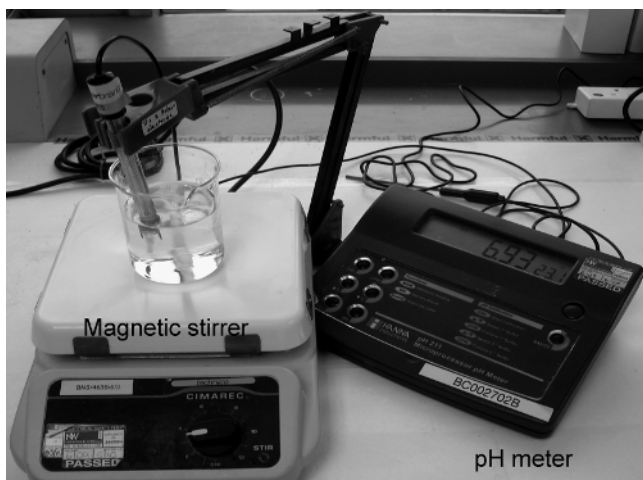


Figure 1.8 A typical set up to adjust the pH of a solution.

- Allow a few minutes for the pH of the solution to stabilize on the pH meter's readout before starting to adjust the pH.
- Remember that buffers are made up of predetermined combinations; for example sodium acetate buffer comprises a basic component (sodium acetate) and an acidic component (acetic acid). If the desired pH value is overshoot, avoid adjusting the pH back to the correct pH with a strong acid or base, for example hydrochloric acid (HCl) or sodium hydroxide (NaOH). This will use up the buffering capacity creating a less effective buffer. Instead, adjust to the correct pH using a concentrated solution of either one of the buffer's constituents; this will alter the molarity of the buffer but retain the buffering capacity.
- When the correct pH has been attained the pH probe can be removed and rinsed in distilled water before being returned to the storage solution.
- The buffer can then be made up to volume and stored as required. If the buffer is to be used for chromatography (see Chapter 7) it is advisable to filter the buffer through a 0.2 μm filter to remove particulate matter and bacteria.
- Overnight storage in a 0.1 M HCl solution will clean and restore most dirty pH probes.

1.8 The Equilibrium/Dissociation Constant (K_a) for an Acid or Base and the Henderson –Hasselbalch Equation

A measure of the strength of an acid is the acid-dissociation equilibrium/dissociation constant (K_a) for that acid.



The dissociation constant $K_a = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]}$

- A strong acid readily dissociates at all pH values and has a high K_a value (HCl: K_a = 1 × 10³).
- A weak acid has a small K_a value (see Figure 1.7) and its dissociation is pH dependent (CH₃COOH K_a = 1.8 × 10⁻⁵).
- To convert K_a values into useable numbers, the pK_a of an acid or base is used which is the negative logarithm of the K_a. In this case a strong acid will have a low pK_a.

$$pK_a = \log \frac{1}{K_a}$$

The Henderson-Hasselbalch equation.

The quantitative aspects of buffers can be ascertained by the Henderson-Hasselbalch equation, which mathematically links pH with pK_a.

$$\text{pH} = \text{pK}_a + \log 10 \frac{[\text{A}^-]}{[\text{HA}]} \quad \text{or} \quad \text{pH} = \text{pK}_a + \log 10 \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

- Where [A⁻] is the concentration of base and [HA] is the concentration of acid. This is usually a molar concentration but because [A⁻]/[HA] is a ratio, other concentration units will also be acceptable. It is this ratio which determines the pH of a solution.
- At the mid point of the titration curve (see Figure 1.7) when the concentration of [HA] is equal to the concentration of [A⁻] the pH = pK_a and this is when buffers are most effective.

$$\begin{aligned} \text{pH} &= \text{pK}_a + \log 1 = \text{pK}_a + 0 = \text{pK}_a \\ \text{or } \text{pH} &= \text{pK}_a \end{aligned}$$

When [HA] > [A⁻] the pH of the buffer is less than the pK_a and when the [A⁻] > [HA] the pH is greater than pK_a.

- This $[A^-]/[HA]$ ratio can only be varied within certain limits usually to 1.0 pH unit either side of the pKa value. This means that there is little or no buffering at the extremes of the buffering range and it may be necessary to increase the concentration of the buffer to maintain good buffering capacity or switch to a different buffer with a different pKa.

The Henderson – Hasselbalch equation allows

- Calculation of pKa when the pH and molar concentrations of proton donor and acceptor are known.
- Calculation of the pH if the pKa and molar concentrations of proton donor and acceptor are known.
- Calculation of the molar concentrations of proton donor and acceptor if the pKa and pH are known.

Worked Example 1.6: The use of the Henderson–Hasselbalch equation

Calculate the pH of a solution containing 0.15 M acetic acid and 0.25 M sodium acetate. (For the constituents of a sodium acetate buffer see Table 1.3) .

The pKa of acetic acid is 4.76



$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA^+]}$$

or

$$pH = pK_a + \log_{10} \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

$$pH = pK_a + \log_{10} \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$pH = 4.76 + \log_{10} \frac{[0.25]}{[0.15]}$$

$$pH = 4.76 + 0.22$$

$$pH = 4.96$$

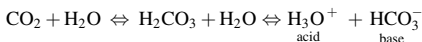
1.9 Summary

The foundation of successful experimental work is the correct preparation of solutions. If undertaking a course in the bioscience area the following skills must be developed to a satisfactory standard, particularly if you wish to pursue a career in laboratory based bioscience.

- Preparation of molar and percentage solutions.
- Preparation of dilutions prepared from a stock solution.
- The accurate use of pipettes.
- A good understanding of pH and the preparation and use of buffers.

Notes

- ¹ Water from a tap may contain impurities (such as metal ions) which may interfere with an experiment. Water obtained by distillation represents the minimum purity of water that ideally should be used to prepare solutions. Water purification machines which pump tap water through filters can be used to produce distilled and deionized water of varying degrees of purity. The highest purity of water will have a conductivity of $18.2 \Omega \text{ ms}^{-1}$ ($0.055 \mu\text{S}$).
- ² The pH of the cell is approximately 7.0. This is also called the physiological pH value. Water is rarely at pH 7.0 because carbon dioxide (CO_2) dissolves in it to produce carbonic acid, resulting in a slightly acid pH for water of approximately pH 5.5.



- * $[\text{H}^+]$ is effectively a proton and this is never available in solution. The proton joins up with another water molecule to generate a positively charged hydronium ion $[\text{H}_3\text{O}^+]$.
- ** $[\text{OH}^-]$ the square brackets indicate that a defined concentration is involved and this is usually molar concentration.