1

Measurements and Calculations

Learning outcomes

- To know the common measurements and their units.
- To learn the factors which affect measurements.
- To recognize the main apparatus used in volume measurements.
- To recognize the different types of pipettes and how they are used to measure specific volumes.
- To use balances to weigh out a known weight of a substance.

1.1 Units and measurements

In the study of bioscience subjects, a wide range of measurements of variables are made using various kinds of equipment. Each measurement is described by a number and a unit.

The Systeme Internationale D'Unites (SI – also known as the *metric system*) is the international system of units used to describe data. Some common measurements are shown in Table 1.1.

Some measurements, such as pH or absorbance of light, have no units.

When we are dealing with very small or large values, prefixes are used for convenience. The most common ones are shown in Table 1.2.

All measurements are affected by errors, which can be random (cannot be predicted) or systematic (biased, predictable). The measurements made can be described by:

- **accuracy** – how close the values are to the true values, i.e. the average value will be nearer the true value but individual measurements may not necessarily be close to each other (see Figure 1.1); or
- **precision** – how close together are the spread of the values, i.e. repeated measurements will be similar but not necessarily close to the true value.

Essential Laboratory Skills for Biosciences, First Edition. M.S. Meah and E. Kebede-Westhead.

Table 1.1 *Common measurements and their units and symbols*

Measurements	Units	Symbols
Length	metre, centimetre, millimetre	m, cm, mm
Mass	kilogram, gram	kg, g
Time	second, minute, hour	s, min, hr
Volume	cubic metre, litre, cubic decimetre, millilitre, cubic centimetre	m^3, l, dm^3, ml, cm^3
Concentration	molar, mole per litre, parts per million	M, mol l^{-1}, ppm
Amount of substance	mole	mol
Density	kilogram per cubic metre, gram per cubic centimetre	kg m^{-3}, g cm^{-3}
Force	Newton	N
Pressure	Newton per square metre, Pascal, millimetres of mercury	N m^{-2}, Pa, mm Hg
Energy	Joule	J

Table 1.2 *Common prefixes*

Power	Prefix	Symbol
10^{-9}	nano	n
10^{-6}	micro	μ
10^{-3}	milli	m
10^{3}	kilo	k
10^{6}	mega	M
10^{9}	giga	G

Figure 1.1 shows the results of firing a pellet gun eight times on each of four targets. This illustrates the combinations of accuracy and precision obtained by repeated firing at the targets.

Errors in the accuracy and precision can be calculated (see Appendix 5).

To reduce error, measurements are repeated (replicated). They may also be repeated at another time to see if the same results are reproducible. One important factor in ensuring accuracy of measurements is performing regular *calibration* of equipment – the process of checking the accuracy of equipment using known values and standards over the measurement range under standard conditions.

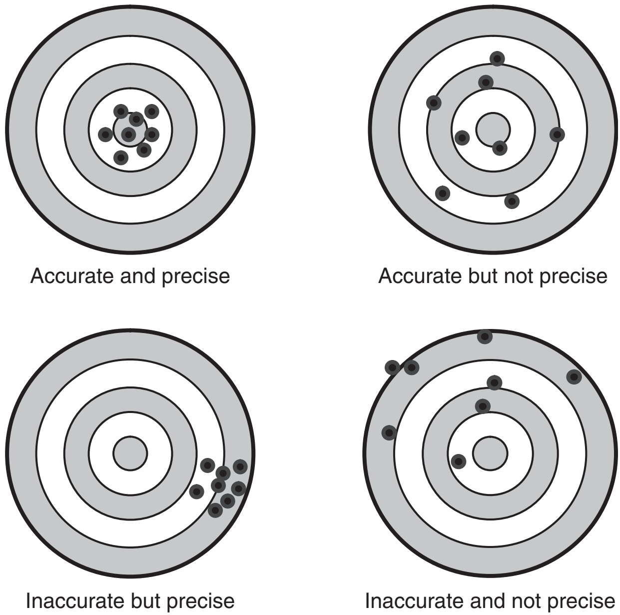

Figure 1.1 *Accuracy and precision shown on a target*

It must not be assumed that all equipment has been calibrated prior to use. It is common practice that all items of equipment are calibrated against a known standard and are checked regularly to ensure that measurements are reliable. For example, pH meters are calibrated with buffers of known pH, commonly with pH 4 and 7. Depending on the type of equipment and ease of calibration, it is possible for students to perform simple calibration in lab sessions.

Example 1.1

Check a weighing balance by using known measurements of volume.

Weigh a beaker repeatedly by adding a series of 1 ml of water. Assuming the density of water is about $1.00\,g\,ml^{-1}$, the weight should increase by 1 g with every addition of 1 ml.

Example 1.2

Check a pipette by weighing dispensed volume.

Using a 1 ml pipette, dispense aliquots of 1 ml of water into a small beaker and weigh the volume repeatedly after each addition. As the volume increases by 1 ml, the weight should increase by 1 g. You may need to pipette 5–10 ml, as the measurement may not be sensitive with small volumes if you are not using a sufficiently sensitive balance.

Calibration is also performed in another context, namely to determine unknown concentrations using standard solutions (see Chapter 4).

1.2 Measuring the volumes of liquids

The first step in measuring the volumes of liquids is to identify the glassware or equipment that would be most appropriate to use. This will depend on the volume to be measured and the accuracy required in the measurement. The smaller the amount you need to measure, the more sensitive the equipment will need to be. The figures below (Figures 1.2a–2f) illustrate the various types of volumetric labware that you will commonly use in the lab.

Beaker

- Graduated
- Rough measure
- Used for routine dispensing, mixing solutions, not for accurate measurements
- Volume labels are approximate, accurate to within 5%

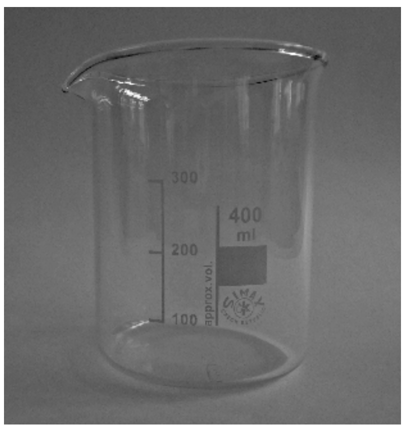

Figure 1.2a Beaker

Conical (Erlenmeyer) flask

- Graduated
- Rough measure
- Used for routine dispensing, mixing solutions, not for accurate measurements
- Volume labels are approximate, accurate to within 5%

Figure 1.2b *Conical (Erlenmeyer) flask*

Measuring Cylinder

- Also known as graduated cylinder
- For general purpose use in measuring liquid volumes
- More accurate than beakers or flasks
- Accurate to within 1%

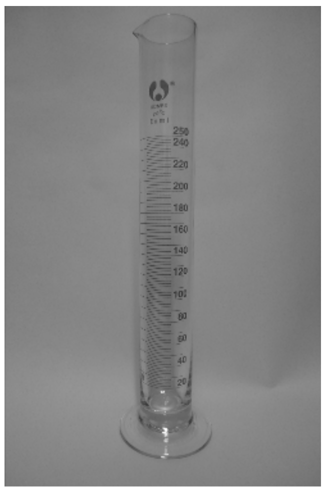

Figure 1.2c *Measuring cylinder*

Burette

- Graduated, with a stopcock at the bottom to dispense liquid precisely
- Used mainly for titration to deliver volumes accurately
- Funnel is used to fill the burette, and readings are taken at the bottom of the meniscus, starting at 0 from the top
- A stopcock is used to deliver the solution

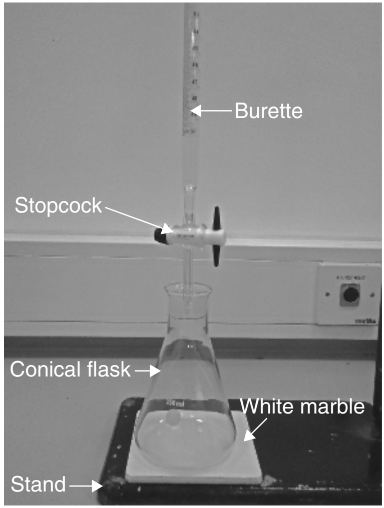

Figure 1.2d Burette

Volumetric Flask

- Calibrated to deliver only one specific volume; e.g. 25, 50, 1000 ml
- Used to prepare stock and standard solutions very accurately
- Narrow neck with etched ring which indicates the volume, e.g. 500 ml ± 0.2 ml.

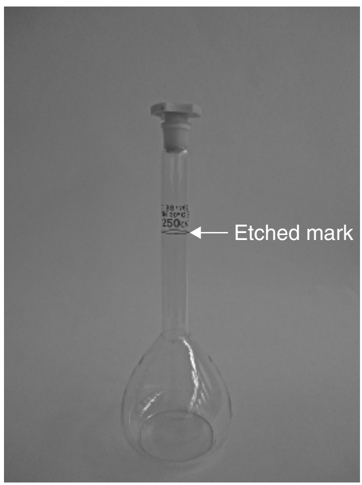

Figure 1.2e Volumetric flask

Pipette

- Used to measure and transfer small amounts of solution accurately
- Device with a mechanism for drawing solution into the pipette and for dispensing (e.g. bulb, plunger)
- Several design variations
- Common lab use: Gilson-type pipettes - various size ranges; different volumes shown here are P20, P200, P1000, P5000.

Figure 1.2f *Pipette*

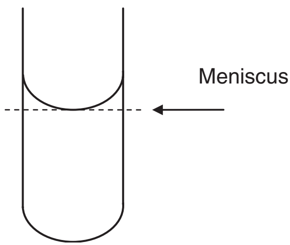

Figure 1.3 *Location of the meniscus of a liquid*

1.2.1 Reading at the meniscus

The surface of a liquid is curved rather than flat, and it is called the *meniscus* (see Figure 1.3). This curvature is due to the relatively strong attractive force between the glass and water molecules (surface tension); it is more obvious in narrow containers.

When reading a volume, precise reading should be taken at the lowest point of the meniscus, which is determined by looking at the liquid surface at eye level. It should not be read from above or below, as this would lead to an error called parallax. If the eyes are below the meniscus level, then the value will be lower than the correct volume; conversely, if the eyes are above the meniscus, then the volume will seem to be higher.

Table 1.3 Common measurement units of volume

	Large	→	Small
Units	litre (l)	millilitre (ml)	microlitre (μl)
Equivalents	1l	1000 ml	1 000 000 μl
		10^3 ml	10^6μl

1.2.2 Common units of volume measurement

The standard unit of volume used in the metric system is the litre (l) and its variations, the millilitre (ml) and microlitre (μl), etc.

To convert from a larger to a smaller measurement unit (Table 1.3), we would multiply (e.g. by 1000 from l to ml, by 1000 from ml to μl).

To convert from a smaller to larger measurement unit, we would divide (e.g. by 1000 from μl to ml, and by 1000 from ml to l).

Example 1.3

a) Express 0.85 l in ml → $0.85 \times 10^3 = 850$ ml
b) Express 850 μl in ml → $850 \div 10^3 = 0.850$ ml
c) Express 1.5 ml in μl → $1.5 \times 10^3 = 1500$ μl

1.3 Pipetting

Pipettes are used to measure and dispense accurate volumes of solutions. There are various different types of pipettes in use in laboratories. Although most of these are similar, their settings for adjusting the volume differ. Three popular types of pipettes which are commonly used in the laboratory are described below.

1.3.1 Gilson Pipetman

These are adjustable mechanical pipettes that come in various sizes (Table 1.4 and Figure 1.5), and the most frequently used sizes lie in the range between 0.2 μl to 10 ml. They are autopipettors which work by air displacement. They consist of the following parts (see Figure 1.4):

(i) a push button plunger (to uptake and dispense liquid)
(ii) adjustment ring and volume scale (to set the correct volume)
(iii) a barrel to which a disposable tip is attached
(iv) tip ejector button and tip ejector (to expel the tip after dispensing liquid).

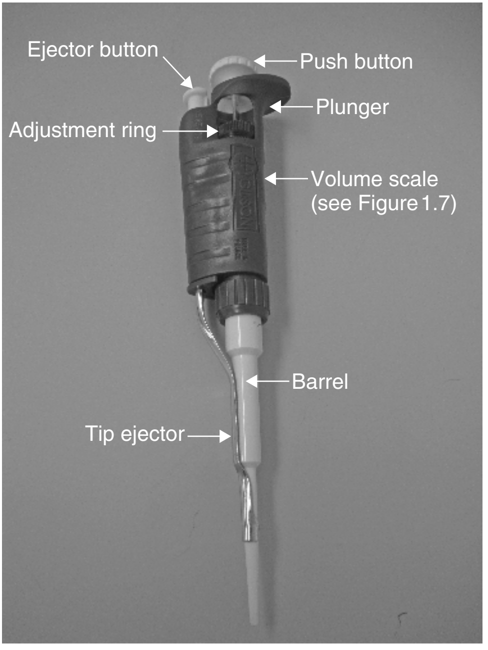

Figure 1.4 *Parts of a Gilson pipette*

Figure 1.5 *Circle of pipettes*

Figure 1.6 *Tips used with pipettes*

Note that there are also other types of pipettes very similar to the Gilson Pipetman (e.g. Eppendorf), but with slight variations in volume adjustments, such as on the adjustment rings or dials.

The P20 and P200 use yellow pipette tips, and the P1000 uses blue tips (Figure 1.6). The volume of each of the Gilson pipettes is set by turning the adjustment knob to get the desired volume in the window.

The reading window for the volume shows three levels (Figure 1.7), each level denoting a difference of one order of magnitude. For example, with a P1000 pipette, a dial reading 1–0–0 is set to pipette 1000 μl, while a dial reading 0–1–0 is set to pipette 100 μl.

Choosing the right pipette is important if the required volume is to be measured accurately. For example, although a 10 ml pipette has a range of 1–10 ml, a 5 ml pipette should be used to pipette accurately 5 ml or

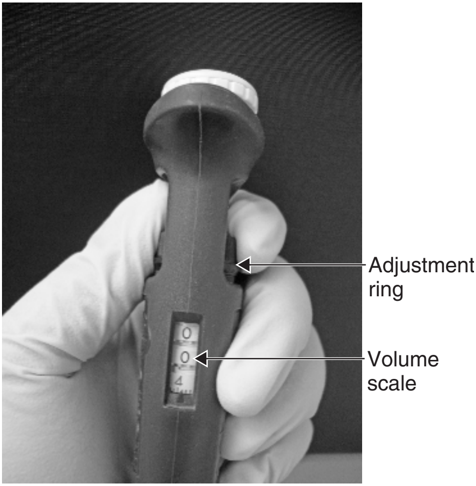

Figure 1.7 *Setting the volume on a Gilson pipette*

less. Similarly, a P200 with the range of 50–200 µl can deliver 100 µl, but it is more accurate to use a P100.

Using the wrong pipette is frequently a source of common error in practical work involving accurate volume measurements, such as preparing standard solutions. First note the volume required, then choose the pipette which has the closest range to this (see Table 1.4).

Table 1.4 *Volume ranges for Gilson pipettes*

Pipette	Minimum recommended volume	Maximum volume
P2	0.5 µl	2 µl
P10	2	10
P20	5	20
P100	20	100
P200	50	200
P1000	200	1000 µl = 1 ml
P5000	1 ml	5 ml
P10 000	1 ml	10 ml

1.3.2 How to pipette

Step 1: Choose the right pipette for the volume range required and attach the right coloured tip (Figure 1.8).

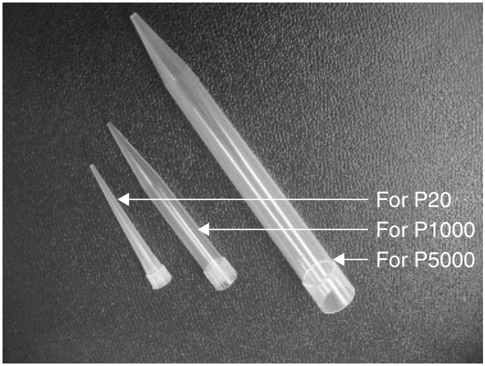

Figure 1.8 *Types of pipette tips*

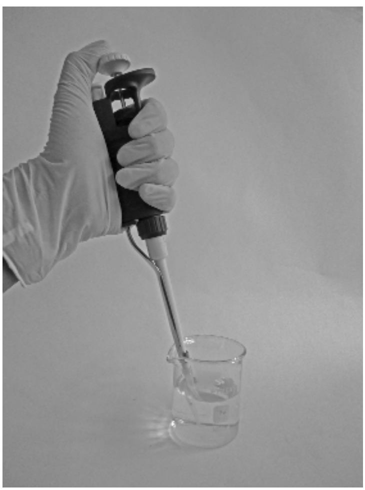

Figure 1.9 *Pipette inserted into beaker*

Step 2: Set the volume to be measured by turning the dial. Note there are only three spaces for setting the volume in the window. For example, for the P20, in order to set 5, 10, 15 and 20 µl, you would set the dial (from the top) to 0-5-0, 1-0-0, 1-5-0 and 2-0-0 respectively.

Step 3: Practise pushing the plunger to find the two 'stop' positions (opposition or resistance to pushing plunger).

Step 4: Push the plunger to the first 'stop', then place the tip of the pipette into the solution and draw the solution into the pipette tip (Figure 1.9).

Step 5: Place the pipette into the receiving tube or container and push the plunger all the way to the second 'stop' position to ensure that all the solution is pushed out of the pipette tip.

Step 6: Eject the tip into waste using the ejector behind the plunger (Figure 1.10).

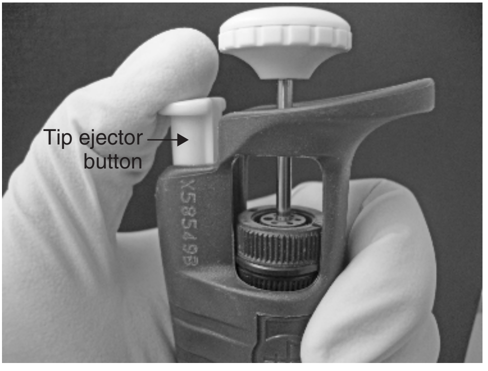

Figure 1.10 *Ejecting tip using tip ejector button*

Precautions in handling pipettes

- Don't forget to use the pipette tip and make sure it fits correctly.
- Don't force the plunger.
- Always put in a pipette holder when not in use (look at Figure 1.5).
- Don't walk around with a pipette.

- Hold the pipette upright, and do not leave it on bench at an angle as its contents may flow backwards, losing liquid and blocking the pipette.
- Inspect the tip for blockages or dirt.
- Never use 5 ml or 19 ml pipette without a filter.

1.3.3 Multichannel pipettes

For repetitive tasks (for example, filling a 96-well tray), a multichannel pipette is used. These draw and expel the same volume in each of the individual tubes making up the multichannel pipette (see Figure 1.11).

1.3.4 Vacuum pipettes

Different from the air displacement pipettes described above (1.3.1–1.3.3), in these pipettes where liquid is drawn up a cylinder by filling up a vacuum which is created by an attached pipette filler, pipette pumps or bulbs. The pipettes are typically made of borosilicate glass or polystyrene, and can be autoclavable or disposable for use in sterile work. Volumetric pipettes dispensing single volumes (e.g. 10, 25, 50 ml) are commonly used in preparing large quantities or stock solutions. Variable quantities of liquids can be dispensed with pipettes that are graduated along the length of the cylinders.

Figure 1.11 *Multichannel pipette*

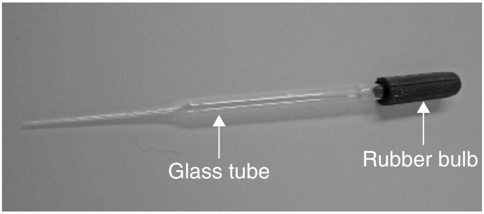

Figure 1.12 *Pasteur pipette*

Pasteur pipette (Figure 1.12) is another type of pipette commonly used in the lab for transferring or dispensing small amounts of liquid. These are small glass tubes with an attached rubber bulb which can be squeezed to create a vacuum and liquid is sucked up when released, but it is not quantitative.

1.4 Weighing

You will be frequently asked to weigh small amounts of chemicals to make up solutions. This will involve using balances (Figure 1.13), which will give the mass to either two or three decimal places in grams. A common

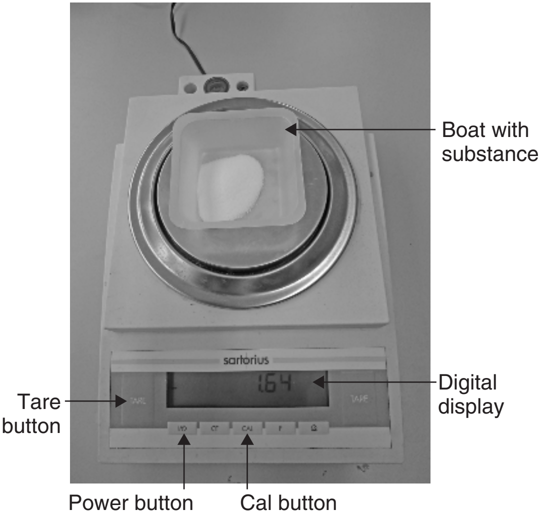

Figure 1.13 *Portable digital top-loading balance*

procedure in the use of these balances is *taring*, which means to remove the weight of the container holding the chemical by setting the scale to zero while the container is on the balance. Increases in the reading on the balance would then be accounted to the mass of the substance added.

1.4.1 Top-loading balance

A typical balance used to measure mass will consist of a zero button or knob, a measuring pan, movable masses for an analogue balance or a power button for a digital balance. A portable top-loading balance (Figure 1.13) is most frequently used for routine laboratory procedures such as making up solutions. It can show accuracy to three or four decimal places.

Measurement range is usually 0.01–0.1 g or 1–50 g.

How to weigh using a top-loading balance

Step 1: Place the balance on a clean, level bench away from draughts and vibrations.
Step 2: Check that the balance pan is clean.
Step 3: Check the balance is level – the spirit level should be in the centre. If not, use the adjustable feet to make it level.
Step 4: Put an empty beaker, a boat (Figure 1.14) or foil on the pan.
Step 5: Press the Tare button. Always press this before any new measurement.

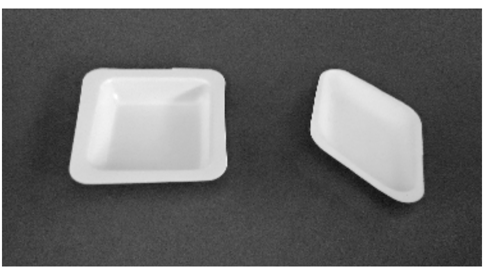

Figure 1.14 *Plastic boat*

Step 6: Check that the scale displays zero and the correct number of decimal places, depending on the balance used (e.g. 0.000).
Step 7: Using a clean spatula (Figure 1.15), add the substance to the centre of the container.

Step 8: Keep adding the substance, reducing the amount added when approaching close to the target mass.

Figure 1.15 Spatula

Step 9: If you go over the target mass, carefully remove small amounts and discard safely. Do not return the substance back into the container.

Step 10: Record the mass when the display shows a constant reading.

Precautions

Any spills should be cleaned with a brush and disposed carefully, but make sure the balance is switched off before cleaning.

1.4.2 Analytical balance

A more sensitive balance used for weighing very low masses (0.0001 g) is the analytical balance (see Figure 1.16). This is a high-precision beam balance composed of a level with two equal arms and a pan suspended from each arm. It is enclosed by glass, with two sliding sides. Measurement range is typically 0.01–0.10 mg.

How to weigh using an analytical balance

Step 1: Switch the balance on and the display should read zero (0.0000).

Step 2: Place a small weighing boat on the weighing pan.

Step 3: Close the glass doors and wait a few seconds.
Step 4: Press the control button so that the display again reads 0.0000 (taring).
Step 5: Open the doors gently and add the substance carefully until the required mass is reached.
Step 6: Again close the doors and wait until the reading is stable, then record the final mass.

Precautions

- An analytical balance is a sensitive instrument, so make sure you do not make any sudden movements that will shift the balance.
- Any spill should be cleaned carefully before proceeding with more measurements, as it could be a danger to the next person who works in this area, who will not know what the chemical left behind is – an unknown white powder could be just salt or it might be cyanide!

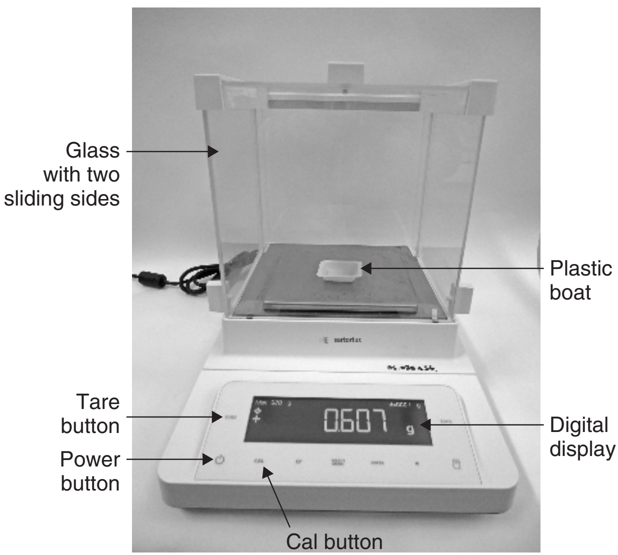

Figure 1.16 *A typical analytical balance*

Conversions between units of volume and mass

Volume

$$1\,\text{l} = 1000\,\text{ml}$$
$$1\,\text{ml} = 1000\,\mu\text{l}$$

Mass

$$1\,\text{kg} = 1000\,\text{g}$$
$$1\,\text{g} = 1000\,\text{mg}$$
$$1\,\text{mg} = 1000\,\mu\text{g}$$
$$1\,\mu\text{g} = 1000\,\text{ng}$$
$$1\,\text{ng} = 1000\,\text{pg}$$

1.5 Calculations

All quantities of measurements need to be reported by a *number* and the *unit* of measurement, except in cases such as absorbance readings on a spectrophotometer (in which case the wavelength at which absorbance was read should be included).

When using units, the numbers should be separated from the unit by a space. The forward slash means ‘per’ or ‘divide by’ – for example, 5 mg/l is read as ‘five milligrams per litre’. The slash is replaced by a superscripted $^{-1}$ after the unit in scientific writings and publications; so mg/l should correctly be written as $\text{mg}\,\text{l}^{-1}$.

1.5.1 Significant figures

Significant figures provide a way to express the degree of accuracy in experimental data which should be recorded with as many digits as can be accurately measured. The number of significant figures is the number of non-zero values in a measurement. Where there are many calculations on the same data, then the final value would be rounded up (i.e. if >5 then increase by one; if <5 then leave as it is).

Example 1.4

a) 7849 to 2 significant figures = 7800
b) 0.0457 to 2 significant figures = 0.046
c) 0.0457 to 2 decimal places = 0.05

1.5.2 Powers

In the decimal counting system that we use, the values of numbers are represented by columns which represent multiples or divisions of 10. For example, the number 1451.25 represents 1 thousand, 4 hundreds, 5 tens, 1 unit, 2 tenths and 5 hundredths. The value of these numbers are so because the columns to the left of the decimal point are represented by multiples of 10, i.e. units (10^0), tens (10^1), hundreds (10^2), thousands (10^3) and so on, while the columns to the right of the decimal point are represented by divisions of 10, i.e. tenths (10^{-1}), hundredths (10^{-2}), thousandths (10^{-3}), etc. This factor of 10 by which the number is increasing or decreasing is called the *base*, and the superscript is the *power* or *exponent*. For example, 10^{-2} has the base as 10 and the power as $^{-2}$.

In general, when a number is multiplied by itself a number of times or divided by itself a number of times, the number of times is called the *power* and the number is called the *base*. For example, $10 \times 10 \times 10$, can also be written as 10^3, where 10 is the base and the superscripted 3 is the power.

Similarly, three divisions of 10 ($10 \div 10 \div 10$) can be expressed as 10^{-1}, where 10 is the base and -1 is the power; alternatively, 10^{-1} multiplied 3 times ($1/10 \times 1/10 \times 1/10$) can be written as 10^{-3}, where 10 is the base and -3 is the power. Other bases can also be used, the best known being the binary system used in computers, which has base 2 – i.e. 2^0, 2^1, etc.

Powers are very useful in dealing with very large or small numbers, as in the table of prefixes elsewhere in this chapter (Table 1.2, page 2). The rules for calculations involving powers are shown in Appendix 1.

Converting between units involving powers of 10

It is very useful to be able to quickly convert between units, particularly between prefixes changing by a constant factor such as 1000, for example in volume units (l, ml and µl) and weight units (g, mg and µg).

To convert from a large unit to a small unit, multiply by 1000 for one prefix (e.g. 1 l = 1000 ml) or 1000×1000 for two prefixes (e.g. 1 l = 1000×1000 µl).

To convert from a small unit to a larger unit, divide by 1000 for one prefix (e.g. 1 µg = $\frac{1}{1000}$ mg) or divide by 1000×1000 for two prefixes (e.g. 1 µg = $\frac{1}{1000} \times \frac{1}{1000}$ g).

1.5.3 Logarithms

One application of powers is in the use of logarithms or logs, which are useful in dealing with large or small changes in values. Examples include the pH scale (Chapter 3), exponential bacterial cell growth over time, or dose response curves in pharmacology.

In such cases, it is common to use *scientific notations* – the expression of numbers using multiples (prefixes) of base 10.

e.g.

$$0.0052 = 5.2 \times 10^{-3};\ 0.003 = 3 \times 10^{-3}$$

The logarithm is *the power to which a base number must be raised to give that number*. For example, what power of 10 will give 1000? The answer is 3, since 10^3 is equal to 1000. Logs to base 10 (simply called 'logs') are important because as a number goes up by a factor of 10 (i.e., multiplied by 10), the log goes up by one.

The two most commonly used base numbers are 10, where the power is $\log_{10}$, and the number 2.714 (also known as 'e'). Logs to base 'e' are called natural logs and are written as $\log_e$ or simply 'ln'. Logarithms do not have units.

Rules for calculations involving logs are shown in Appendix 2.

Example 1.5

a) 10^1 (or 10) $\log 10^1 = 1$
b) 10^2 (or 100) $\log 10^2 = 2$
c) 10^3 (or 1000) $\log 10^3 = 3$
d) 10^{-2} (or 0.01) $\log 10^{-2} = -2$

1.5.4 Fractions, ratios and percentages

A *fraction* is the division of one number (numerator) by another (denominator), with the answer being either less than 1 (e.g. $\frac{1}{2}$) or greater than 1 (e.g., $\frac{7}{5}$). In these two examples, 1 and 7 represent the numerators and 2 and 5 represent the denominators.

A *ratio* is simply a different way of expressing a fraction. Ratios are used to express proportions or fractions, usually in integers (e.g., the fraction $\frac{1}{2}$ expressed as a ratio would be written 1:2 and expressed as 'a ratio of 1 to 2'. Ratios do not have units).

A *percentage* is a fraction in which the denominator is always 100. The symbol used for percentage is '%'. Thus 20% is equal to $\frac{20}{100}$ and is also 0.20.

Example 1.6

Find the ratio of three substances in a mixture with the quantities 20 g:15 g:30 g.

Divide by the highest common factor (HCF) which will go exactly into each of the quantities; in this case, the HCF would be 5, giving the ratio 4:3:6.

Ratios and dilutions

Ratios are used frequently in the process of making dilutions of liquids. If you are asked to do dilutions in parts or ratios, this specifies the fractions to combine (see Chapter 2). If asked to dilute to a dilution factor, you need to calculate how many times the final volume must be increased (see Chapter 5 – Cell counting).

Example 1.7

a) Perform a 1 in 10 (1:10) dilution.
   Take 1 ml of substance and add 9 ml of water or buffer.
b) Dilute 20 µl of NaCl stock solution 200-fold to a final volume of 4 ml (or 4000 µl) using distilled water.
   Multiply 200 by 20 (= 4000); but you need to subtract the volume of the stock solution that you are using from the total volume. Therefore, the volume of water to be added is

$$4000 - 20\,\mu l = 3980\,\mu l.$$