2

Partition coefficient and biopharmacy

When a substance (or *solute*) is added to a pair of immiscible solvents, it distributes itself between the two solvents according to its affinity for each phase. A polar compound (e.g. a sugar, amino acid or ionised drug) will tend to favour the aqueous or polar phase, whereas a non-polar compound (e.g. an unionised drug) will favour the non-aqueous or organic phase. The added substance distributes itself between the two immiscible solvents according to the partition law, which states that 'a given substance, at a given temperature, will partition itself between two immiscible solvents in a constant ratio of concentrations'. This constant ratio is called the *partition coefficient* of the substance, and may be expressed mathematically as

$$P = \frac{[\text{organic}]}{[\text{aqueous}]} \tag{2.1}$$

where P is the partition coefficient of the substance; [organic] is the concentration of substance in the organic, or oil phase; and [aqueous] is the concentration of substance in the water phase.

As an example, consider the distribution of 100 mg of a drug between 50 mL of an organic solvent (e.g. ether, chloroform or octanol) and 50 mL of water. The drug is added to the two immiscible solvents in a separating funnel and allowed to equilibrate. When the organic layer is analysed, it is found to contain 66.7 mg of compound. From these data the partition coefficient and the percentage of the drug extracted into the organic layer can be calculated (see Figure 2.1).

The mass of drug in the water phase = 100 − 66.7 mg = 33.3 mg; the concentration of drug in the organic phase = 66.7/50 = 1.33 mg mL^{-1}, and the concentration of drug in the water phase = 33.3/50 =0.67 mg mL^{-1}. Therefore, the partition coefficient is given by

$$\frac{[\text{organic}]}{[\text{aqueous}]} = \frac{1.33 \text{ mg mL}^{-1}}{0.67 \text{ mg mL}^{-1}} = 2$$

The partition coefficient is a ratio of concentrations, so the units cancel and P has no units.

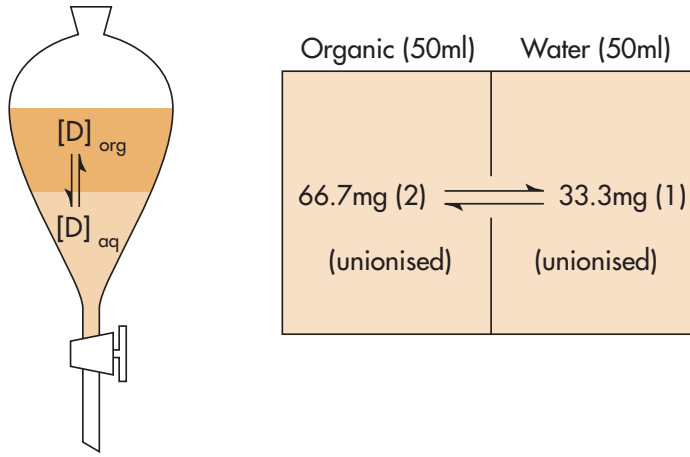

Figure 2.1 Simple partition law.

The percentage of drug extracted in the above example is simply given by the mass of drug in the organic phase divided by the total mass of drug, i.e. 66.7/100 = 66.7%.

The partition coefficient is an important piece of information as it can be used to predict the absorption, distribution and elimination of drugs within the body. Knowledge of the value of *P* can be used to predict the onset of action of drugs or the duration of action of drugs, or to tell whether a drug will be active at all. Part of medicinal chemistry, the science of rational drug design, involves structure–activity relationships, where the partition coefficient is used in mathematical equations that try to relate the biological activity of a drug to its physical and chemical characteristics.

In case this sounds too much like an advert for the partition coefficient, in reality the simple relationship above only applies if the solute in question does not ionise at the pH of measurement. If the solute is a weak acid or weak base (and a huge number of drugs are), then ionisation to form an anion or a cation will considerably alter the solubility profile of the drug. A fully ionised species will be much more soluble in water than the unionised acid or base, and so the above ratio will vary depending on the pH at which the measurement was carried out.

There are two ways round this problem: either the experimental conditions are adjusted to ensure that the measured *P* is the partition coefficient of the unionised molecule (this means that the *P* value for acids is measured at low pH when the acid is unionised and, similarly, the partition coefficient of a base is measured at high pH to prevent ionisation); or,

better, the ratio above is redefined as the *apparent partition coefficient*, to differentiate it from the partition coefficient of the unionised species, which is now termed the *true partition coefficient*.

The apparent partition coefficient (P_{app}) is dependent on the proportion of substance present in solution, which in turn depends upon the pH of the solution, or

$$P_{app} = P \times f_{unionised} \tag{2.2}$$

where $f_{unionised}$ equals the fraction of the total amount of drug unionised at that pH. It follows that if $f_{unionised} = 1$ then $P_{app} = P_{true}$ and the compound is unionised.

To illustrate the effect of ionisation, consider again the drug in the example above. If the pH of the aqueous phase is adjusted so that the drug becomes 66.7% ionised, only 40 mg of the drug partitions into the organic phase (since the ionised drug will be less soluble in the organic solvent), and the partition coefficient can be recalculated (see Figure 2.2).

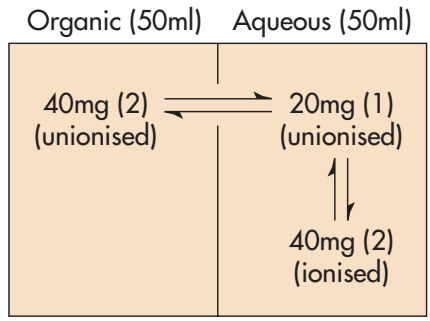

Figure 2.2 The partition of ionised drug.

The mass of drug in the water phase = 100 − 40 = 60 mg.

The mass of ionised drug in the water phase = total mass × fraction ionised, which is 60 × 0.666 = 40 mg.

The mass of unionised drug in the water phase = 60 × 0.333 = 20 mg.

The concentration of drug in the organic phase = 40/50 = 0.8 mg mL^{-1}.

The concentration of unionised drug in the water phase = 20/50 = 0.4 mg mL^{-1}.

The concentration of total drug in the water phase = 60/50 = 1.2 mg mL^{-1}.

The percentage of drug extracted into the organic phase = (40 mg/100 mg) × 100 = 40%.

The partition coefficient of the unionised drug (the true partition coefficient) should remain constant and is given by

$$P = \frac{\text{[drug] in organic phase}}{\text{[unionised drug] in water}}$$

$$P = \frac{0.8 \text{ mg L}^{-1}}{0.4 \text{ mg mL}^{-1}} = 2$$

the same answer as obtained above.

Using the total concentration of drug in the aqueous phase allows the apparent partition coefficient to be calculated:

$$P_{app} = \frac{\text{[drug] in organic phase}}{\text{total [drug] in aqueous phase}}$$

$$P_{app} = \frac{0.8 \text{ mg mL}^{-1}}{1.2 \text{ mg mL}^{-1}}$$

$$P_{app} = 0.67$$

The answer for P_{app} can be checked by use of equation (2.2):

$$P_{app} = P \times f_{unionised}$$

$$0.67 = 2 \times 0.33$$

The range of possible values of P found in drug molecules is huge, from small fractions through to values of several thousand. For this reason, it is common to quote the logarithm (to the base 10) of the partition coefficient, or log P. This is particularly true in *quantitative structure–activity relationships* (QSAR), where the physicochemical properties of a drug (such as hydrophobicity, steric interactions or electronic effects) are quantified and an equation is derived that can be used to predict the biological activity of other, similar drugs. The technique of QSAR became popular with the advent of powerful computers able to handle the multiple regression analysis necessary to obtain the quite complex equations required. A detailed study of QSAR is beyond the scope of this book, but more advanced textbooks of medicinal chemistry contain many examples of the ability of QSAR equations to predict biological activity.

Experimental measurement of the partition coefficient

There are three convenient ways in which *P* can be determined in the chemistry laboratory. These are the original *shake flask method*, the use of *thin-layer chromatography* or the use of *reversed-phase*, *high-performance liquid chromatography*.

Shake flask method

In the shake flask method, the drug whose *P* is to be determined is traditionally added to a separating funnel containing the two immiscible phases, although it works just as well to use a centrifuge tube (and requires less sample). The two immiscible phases chosen are usually 1-octanol and pH 7.4 buffer. Octanol is used in partition coefficient work because the answers obtained from octanol seem to correlate best with biological data obtained *in vivo*. This may be because the eight carbon atoms are essentially *hydrophobic* (or water-hating) and the one hydroxyl group is *hydrophilic* (water-loving) and together they give the closest balance to that found in human cell membranes. The aqueous buffer at pH 7.4 represents aqueous compartments within the body, e.g. blood plasma.

The two phases are thoroughly mixed to give buffer-saturated octanol in the top phase and octanol-saturated buffer in the bottom. Once the two phases have separated (this can take a while), the drug is added and the whole flask is shaken mechanically for at least an hour. The two phases are allowed to separate (or centrifuged, if you are in a hurry) and the concentration of drug in the aqueous phase is then determined. This may be done by titration if the drug is sufficiently acidic or basic or, more usually, spectrophotometrically. The concentration in the octanol phase is found by subtraction and the value of *P* is calculated. This method works perfectly well if there is sufficient sample and the drug possesses a chromophore to allow spectroscopic assay of the aqueous phase.

What is important in liquid–liquid extractions of this type is not the volume of the organic phase but rather the number of times the extraction is carried out. Five extractions of 10 mL organic phase will remove more compound than one extraction of 50 mL, even though the total volume of organic solvent used is the same. Similarly, ten extractions of 5 mL will be more efficient still, and so on. This effect (which is general to all extractions) is obvious when thought about. Each time one phase is removed and replaced by fresh solvent, the equilibrium for the partitioning process must re-establish according to the partition coefficient ratio and drug must leave the aqueous phase to enter the organic phase and restore the equilibrium ratio.

An equation can be derived to calculate the increase in efficiency of multiple extractions versus one single extraction:

$$W_n = W\left(\frac{A}{PS + A}\right)^n \tag{2.3}$$

where W_n is the mass of drug remaining in the aqueous phase after n extractions, W is the initial mass of drug in the aqueous phase, A is the volume of the aqueous phase, S is the volume of solvent (or organic) phase, P is the partition coefficient and n is the number of extractions.

Equation (2.3) is derived as follows:

$$P = \frac{[\text{organic}]}{[\text{aqueous}]}$$

or, using the terms defined above,

$$P = \frac{(W - W_1)/S}{(W_1/A)}$$

Therefore,

$$P = \frac{(W - W_1)}{W_1} \times \frac{A}{S}$$

or

$$\frac{PS}{A} = \frac{(W - W_1)}{W_1} = \frac{W}{W_1} - 1$$

Hence,

$$\frac{W}{W_1} = \frac{PS}{A} + 1 = \frac{PS}{A} + \frac{A}{A} = \frac{(PS + A)}{A}$$

Therefore, the fraction of drug remaining in the aqueous phase is

$$\frac{W_1}{W} = \frac{A}{(PS + A)} \tag{2.4}$$

This expression is valid for one extraction; it follows that if the extraction is repeated n times, the overall expression is simply given by equation (2.4) repeated n times, which, with subscript 1 replaced by n, is equation (2.3).

Thin-layer chromatography (TLC)

In this technique, the R_f value of the drug is related mathematically to the partition coefficient. A thin-layer plate, or a paper sheet, is pre-coated with organic phase (usually paraffin or octanol) and allowed to dry. Sample is applied to the origin and the plate is allowed to develop. The mobile phase used is either water or a mixture of water and a miscible organic solvent (such as acetone) to improve the solubility of the drug.

Once the plate has developed, the spots are visualised (using an ultraviolet lamp if the drug possesses a chromophore, or iodine vapour if it does not) and the R_f for each spot is determined. The R_f is the distance moved by the spot divided by the distance moved by the solvent front, and is expressed as a decimal. The R_f can be related to the partition coefficient by equations of the type

$$P = \frac{k}{(1/R_f) - 1} \tag{2.5}$$

where k is a constant for the given system, which is determined by running a number of standard compounds of known P in the system and calculating k.

The TLC method of determining P works best for compounds of similar structure and physical properties. The advantages of using this technique to determine P are that many compounds can be run simultaneously on one plate, and very little sample is required. On the other hand, finding suitable standards can be difficult, and mobile phases containing a large amount of aqueous solvent may take many hours to run up a large TLC plate.

High-performance liquid chromatography (HPLC)

This method of analysis relies on the same chemical principles as the determination by TLC, except that the efficiency (and the cost) of the technique has increased greatly. Instead of the R_f value, the retention time of the drug is measured and related to P by equations similar to equation (2.5) for TLC. The retention time, as its name suggests, is the time taken for the sample to elute from the HPLC column. The major drawback with using this technique to determine P is detecting the drug if it does not possess a chromophore, when a UV detector cannot be used. In cases like this, use must be made of an HPLC system connected to a refractive index (RI) detector or an electrochemical detector (ECD).

A RI detector relies on changes in the refractive index of the mobile phase as a solute elutes to detect a signal, while an ECD functions like a little electrode to oxidise or reduce the analyte as it elutes. In either case, before the determination of *P* is carried out, you should seriously consider measuring *P* for another drug! My PhD supervisor had a saying: 'Never make a compound you cannot name'; to that can be added the advice 'Never make a compound that cannot be detected by a UV detector'. Many entertaining hours can be spent optimising HPLC systems with RI or ECD, but if you want to finish before your children grow up, these methods of detection are best avoided. There are some advantages to the HPLC method of determining *P*, namely that HPLC does not require much sample and that the sample does not have to be 100% pure. Also, once the complete system has been obtained, the cost of the determination is limited to the purchase of HPLC-grade solvents and electricity.

Drug absorption, distribution and bioavailability

The study of the fate of a drug administered to an organism is called *pharmacokinetics*. This discipline involves measuring or predicting the ***a**bsorption, **d**istribution, **m**etabolism and **e**xcretion* (usually known by the acronym ADME) of the drug in the body. Pharmacokinetics has been described as 'what the body does to the drug' as opposed to *pharmacodynamics*, which is the study of mechanisms of drug action and the biochemical changes brought about by treatment with the drug or 'what the drug does to the body.' Some older textbooks use the expression 'molecular pharmacology' instead of pharmacodynamics.

Bioavailability (symbol *F*) is a measure of the extent to which a drug reaches the bloodstream and is available at its site of action. The bioavailability of a drug administered by intra-venous (i.v.) injection is defined as 1 (since the entire dose is available in the systemic circulation). Problems start to appear, however, if the drug is administered by a non-parenteral route (e.g. oral, rectal, topical). In these cases, the bioavailability of a drug is often considerably less than 1 due to a number of factors, such as poor absorption from the gut (in the case of an oral medicine), extensive binding to circulating plasma proteins, or rapid 'first pass' metabolism in the liver.

The most popular method of administering drugs and medicines, at least in the UK, is the oral route. Tablets, capsules or oral liquids are swallowed and, once in the stomach, the tablet or capsule disintegrates to release the active drug molecule. Interestingly, a drug is not considered to be *in* the body until it has been absorbed across the gut wall and into the bloodstream. The gut can be thought of as a hollow tube running through the

body, open at both ends (hopefully not at the same time) and, as such, the gut contents are considered *outside* the body. Passage into the body must be achieved by absorption across a biological membrane; for the oral route of drug administration, this is the cell membrane of cells lining the wall of the stomach and the intestine. Once the drug has passed through the gut membrane into the bloodstream, it then has to travel to its site of action and diffuse out of the bloodstream to the receptor on some, perhaps distant, cell membrane. In the case of drugs acting on the brain or spinal cord (the central nervous system, or CNS) the drug must partition across the *blood–brain barrier* to gain access to the CNS. The blood–brain barrier is, in reality, the cell membranes of glial cells (or *astrocytes*) lining the blood vessels within the brain. These cells fuse together very closely to form a tight, high-resistance 'lipid barrier' that restricts the passage of many drug molecules, especially if the drug molecules are polar. It has been estimated that the blood–brain barrier prevents the brain uptake of >98% of all potential neurotherapeutics. This barrier is designed to protect the delicate structures of the brain from damage by harmful compounds that may gain access to the bloodstream, but it can be a problem for drug administration. Some infectious diseases, such as malaria, can spread to the brain and, once established, can be very difficult to treat, since drugs used to eradicate the infection in other parts of the body cannot cross the blood–brain barrier to get at the infection in the CNS. This creates a 'reservoir of infection' which can re-infect the rest of the body after treatment.

A similarly depressing picture exists with tumours in the brain. Conventional anticancer chemotherapy often cannot penetrate the barrier to attack the tumour. The CNS, however, does require low-molecular-weight molecules to grow and function and these small polar molecules (e.g. amino acids, sugars) have their own transport proteins located at the blood–brain barrier that act to transfer the essential compound through the barrier in a process called carrier-mediated transport.

Biological membranes vary in structure and function throughout the body, but there are some common structural features and properties (see Figure 2.3). A cell membrane is composed of a bilayer of fatty molecules known as phospholipids. These compounds are amphoteric in nature, possessing a non-polar region of hydrocarbon chains that are buried inside the cell membrane, and a polar region comprising negatively charged phosphoric acid head groups. These ionised groups are exposed to the aqueous surroundings of the extracellular and intracellular fluids of the cell. The cell membrane has to be fatty and non-polar in nature to allow it to successfully separate the aqueous compartments of the body. Buried within this lipid bilayer are large globular protein molecules. These macromolecules

function as ion channels (e.g. the Na^+ channel of nerve membranes), transmembrane receptors (like the β adrenoceptor) or transport proteins (as in the electron transport chain of mitochondria). Human cell membranes also contain high concentrations of the steroid cholesterol, particularly in nerve tissue. Chemically, cholesterol is a cyclopentanoperhydrophenanthrene derivative, but it is much simpler to use the trivial name and call this important group of compounds 'steroids.' The structure of cholesterol and a general structure of membrane phospholipids are shown in Figure 2.4.

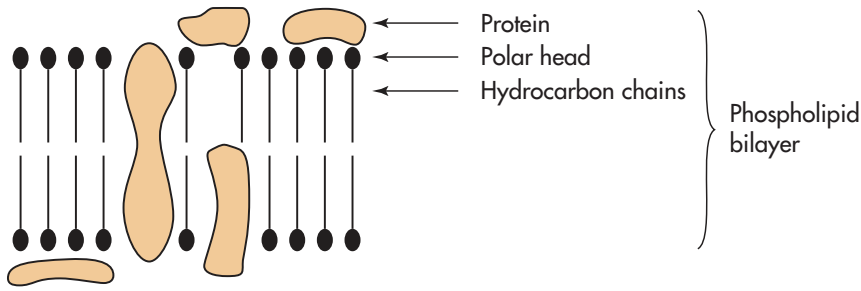

Figure 2.3 A fluid mosaic model of a cell membrane.

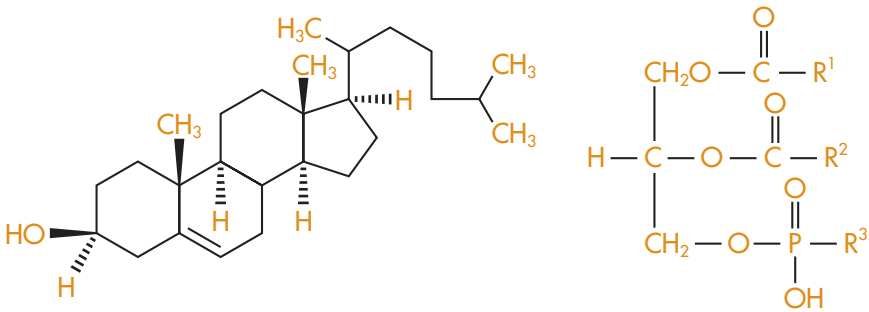

Figure 2.4 The structures of cholesterol and phospholipids. R^1 and R^2 = palmityl, stearyl or oleyl. R^3 = ethanolamine, choline, serine, inositol or glycerol.

Cholesterol gets a bad press nowadays. Tabloid newspapers and television programmes seem to have latched onto cholesterol as the villain of a healthy lifestyle. It is true that high levels of cholesterol in the diet, coupled with high salt intake and lack of exercise, are blamed for causing coronary heart disease and strokes. In cell membranes, however, cholesterol increases membrane rigidity and is essential for maintaining the integrity of the

membrane – without cholesterol your cells would leak. Other important steroids include the male sex hormone, testosterone, and the female sex hormones oestrogen and progestogen in addition to drugs such as digoxin and beclometasone.

The lipid bilayer of the cell membrane presents a significant barrier to drug transport and for a small drug molecule to travel across membranes, one of two things must happen: either the drug must cross the membrane by passive diffusion down its concentration gradient or the drug has to be transported across the membrane, against the concentration gradient, with the expenditure of energy, a process called active transport.

Passive diffusion

Passive diffusion is probably the most important mechanism by which small drug molecules gain access to the body. The drug molecule must be in solution and it partitions into the lipophilic cell membrane, diffuses across the cell and then partitions out of the cell and into the aqueous compartment on the other side. Drugs that are very lipid soluble (such as the antifungal agent griseofulvin) are so water insoluble that they partition into the cell membrane but then stick in the lipid membrane and do not partition out of the membrane and into the aqueous compartments inside the cell. Similarly, drugs that are very water soluble will not partition well into a non-polar lipid membrane and will tend to stay in the aqueous contents of the gut, or if they do manage to cross the gut membrane will stick in the aqueous intracellular solution. Clearly, for a drug to be successfully absorbed from the gut it must possess an intermediate level of water solubility and lipid solubility: a sort of 'Goldilocks effect' whereby the drug is not too hydrophobic, not too hydrophilic, but possesses just the right degree of solubility to partition through biological membranes. In general, drugs that are strongly acidic with a $pK_a < 2$ or strongly basic ($pK_a > 10$) will not cross membranes very well since they will be >99.99% ionised at the pH values found in the gut.

Transfer across membranes occurs down a concentration gradient (i.e. from regions of high drug concentration to regions where the concentration is lower). The process of diffusion can be described by Fick's law (named after the German physiologist, Adolf Fick), which states

$$\frac{dm}{dt} = \frac{PDA(C_2 - C_1)}{d}$$

where dm/dt is the rate of appearance of drug within the cell (or rate of transfer), P is the partition coefficient of the drug, D is a diffusion coefficient

for the membrane, A is the surface area of membrane available for absorption, C_2 and C_1 are the concentrations of drug on the external and internal surfaces, respectively, and d is the thickness of the cell membrane. Rate of diffusion is favoured by high values of P, high membrane surface area and a steep concentration gradient across a thin membrane.

Passive diffusion can only occur with small molecules (e.g. drugs with relative molecular masses of approximately 1000 or less). This excludes large macromolecules such as proteins, which are polyelectrolytes and do not partition well across lipid membranes. This can be important for drugs that are extensively bound to proteins in the bloodstream. These drugs are effectively trapped in the blood plasma and cannot easily gain access into and through cells. The effect is most noticeable for drugs that do not distribute widely around the body and are highly bound to plasma proteins (>90% of the given dose). Examples of these include the anticoagulant warfarin, the antibacterial sulfonamides and oral hypoglycaemic drugs such as tolbutamide.

The potential for serious drug interactions occurs with drugs bound to plasma proteins. The binding sites on the protein molecules are relatively non-specific, and a bound drug can easily be displaced by another drug with affinity for the protein. The well-documented interaction between the anticoagulant warfarin and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indometacin and phenylbutazone arises in this way. When warfarin is administered, >90% of the dose can circulate in the blood bound to plasma proteins; this means that the patient is effectively stabilised on the remaining 10% of the dose of drug. If aspirin is co-administered with warfarin, the aspirin can displace warfarin from binding sites on the protein and increase the 'effective' concentration of warfarin in the body, leading to increases in clotting time and haemorrhage. This serious effect is potentiated because NSAIDs can inhibit the metabolism of warfarin. Treatment of warfarin overdose is by prompt intravenous administration of vitamin K and clotting factors II, VII, IX and X.

The pH partition hypothesis

Biological membranes are, essentially, non-polar or hydrophobic, due to the long hydrocarbon chains of the phospholipid molecules. For a drug to cross a membrane of this type, the drug must pass from the aqueous solution of the extracellular fluid, through the lipid membrane to the aqueous solution of the intracellular fluid (see Figure 2.5), i.e. the drug must be sufficiently soluble in both the aqueous and the lipid phases to succeed.

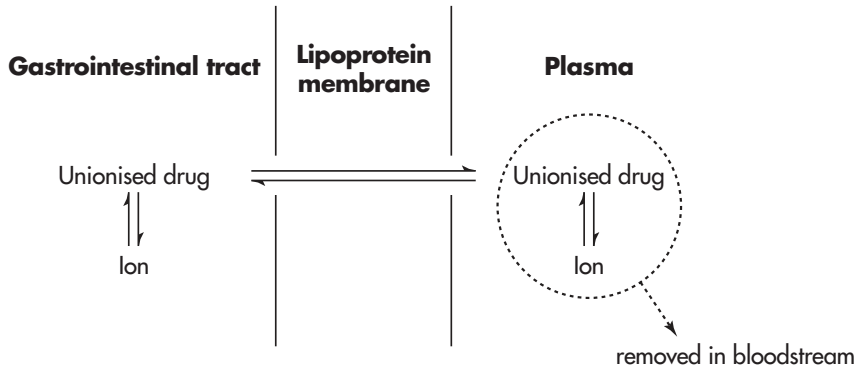

Figure 2.5 A partition diagram of a cell membrane.

For any given drug (or, for that matter, for any biological membrane) there must exist an optimal value of partition coefficient for transport of drug across the membrane. This value is called P_0.

The situation becomes (even) more complicated if the drug ionises at the pH of the body compartment. For weak acids and weak bases, the aqueous and lipid solubility of the compound will depend on the extent to which the drug is ionised, which in turn will depend on the pK_a of the acidic and basic groups involved and the pH of the surroundings.

For weak acids that ionise as

$$HA \rightleftharpoons H^+ + A^-$$

the unionised species, HA, will be much more lipid soluble, and will therefore cross biological membranes much more rapidly than will the anion A^-. This suggests that weak acids will be absorbed more efficiently across a membrane when the pH of the surrounding solution is low and the weak acid will be predominantly unionised. Such a situation is found in the gastric juice of the stomach, which, due to the high concentration of hydrochloric acid present, is at a pH of 1–2 (this is why gastric ulcers are so painful: the hole in the stomach lining allows the acid to burn the underlying muscle layer). This theory is called the *pH partition hypothesis*, and predicts that weakly acidic drugs such as aspirin, barbiturates, phenytoin, etc. will be absorbed preferentially from the stomach rather than from the more alkaline small intestine. In a region of high pH, the acidic drug will ionise to give A^-, which, since it is charged, will not diffuse well through a hydrophobic lipid membrane.

For weak bases that ionise as

$$B + H_2O \rightleftharpoons BH^+ + OH^-$$

the more lipid-soluble species is the unionised free base, B, which will be present to the greatest extent in solutions of high pH, such as are found in the small intestine (pH range 6–8). The pH partition hypothesis predicts that basic drugs (such as morphine, codeine, antihistamines, etc.) will be absorbed into the body better from the small intestine than from the acidic stomach, since the base will be predominantly unionised in the intestine, rather than existing as the ionised conjugate acid in the stomach. This is important for the patient, since, if a drug can only be absorbed from the small intestine, there will inevitably be a delay in onset of action if the drug is taken orally. The drug has to be swallowed and pass through the stomach (where if it is basic it will exist predominantly in the ionised form) before the stomach empties and the drug enters the small intestine and begins the process of absorption. If, for example, a patient takes a basic drug such as an antihistamine for travel sickness, they should be advised to swallow their medication at least an hour before they set off on their journey to allow time for the drug to reach the site of absorption and partition into the bloodstream. An estimate of the extent to which a weak acid or base will be ionised at any given pH can be made using the approximate 'rule of thumb' introduced in Chapter 1 if the pK_a of the acid or base is known.

Limitations of the pH partition hypothesis

The pH partition hypothesis is very useful as a model to explain the extent of drug absorption in the body, but it must be borne in mind that the model has some limitations. As usual, the real-life situation is more complex than this simple model suggests. The theory predicts that weak acids will preferentially be absorbed from the stomach, and weak bases from the small intestine but, in reality, *the vast majority of drugs are absorbed from the small intestine irrespective of their degree of ionisation*. This is because the small intestine has evolved as the organ where absorption of food (and drugs) takes place. The small intestine has three sections, the *duodenum*, a short curved section attached to the back wall of the abdomen, and the *jejunum* and *ileum*, two larger coiled segments that can move about within the abdominal cavity. The existence of these three sections means that the small intestine is long (about 6.5 metres in an adult) and has much higher surface area (estimated as 100 m^2) than the stomach. The large intestine, which frames the coils of the small intestine, follows on from the small intestine and is itself about 1.5 metres long. This means the total length of the gut is over 8 metres, or approximately five to six times an individual's height. The high surface area of the small intestine is achieved due to its convoluted folded structure (see Figure 2.6), which is increased further by

the presence of *microvilli*, small tube-like structures, like tiny hairs, which extend into the gut lumen. The small intestine is also supplied with a rich blood supply, which means that food or drug molecules, once they cross the gut membrane, are carried away in the bloodstream, initially to the liver, and from there are distributed around the body.

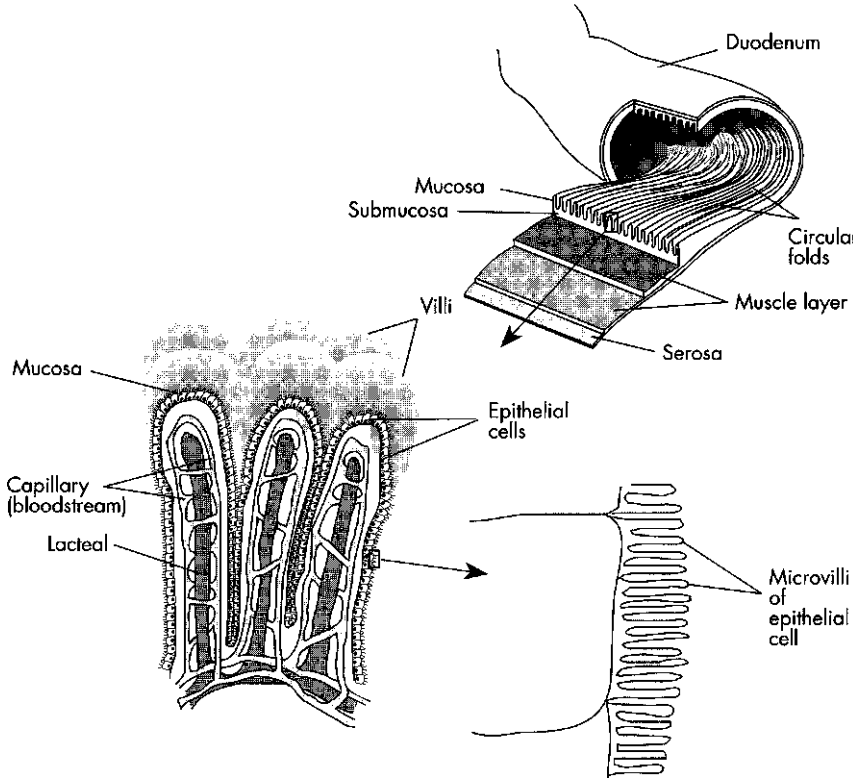

Figure 2.6 A diagram of the small intestine, showing the convoluted surface made up of villi, which are lined with microvilli.

What all this means in practice is that drugs are absorbed quite effectively from the small intestine even if they exist in a predominantly ionised form. The absorption process obeys the *law of mass action*, which was introduced in Chapter 1. This law is fundamentally an equilibrium process, and, as with any equilibrium, rapid removal of the 'products' or compounds on the right-hand side of the equilibrium arrow will shift the equilibrium in that direction. This is exactly what happens in drug absorption across the gut membrane: a small amount of unionised drug is absorbed by passive diffusion and whisked away by the rich blood supply of

the gut. This allows the equilibrium to re-establish, and more drug is absorbed. In this way, acidic or basic drugs, which may be >99% ionised at the pH of the gut, may be absorbed quite effectively into the body.

Some ionised drug molecules can traverse the lipophilic gut membrane by combining with an ion of opposite charge (a *counter ion*) to form an ion pair. The ion pair, although composed of two ionic species, behaves as a neutral molecule with a high partition coefficient and can cross biomembranes effectively. Quaternary ammonium compounds, which are charged at all values of pH, may be absorbed into the body in this way:

$$N^+R_4 + A^- \rightleftharpoons [N^+R_4A^-] \qquad \text{easily absorbed ion pair}$$

Active transport mechanisms

Occasionally, a chemical is so essential to the functioning of the body that special mechanisms are established to allow the essential molecule or ion to cross cell membranes. Glucose and ions such as sodium and chloride must cross membranes efficiently, but they are too polar to diffuse across a phospholipid bilayer passively. Their transport is 'facilitated' by proteins that span the membrane and allow these chemicals to enter cells. If the transport occurs down a concentration gradient, the process is described as *facilitated diffusion* and does not usually require expenditure of energy in the form of hydrolysis of ATP (adenosine triphosphate). The protein merely aids the uptake process by allowing an alternative route of access (this mechanism includes gated hydrophilic pores, such as ion channels, which are discussed below). In the alternative process where the transport occurs against an existing concentration gradient, the process is defined as *active transport* and does require metabolic energy to be expended in the form of hydrolysis of ATP to ADP (adenosine diphosphate). A good example of this type of active transport occurs with amino acids. As discussed in Chapter 1, amino acids are the monomers from which proteins are made and exist predominantly as the zwitterion at neutral pH. This 'internal salt' is far too hydrophilic to partition through a lipophilic lipid membrane by passive diffusion, so energy generated from the oxidation of food must be used in order to ensure that these essential molecules are absorbed from the diet. The active transport mechanism usually involves a carrier molecule, which 'recognises' the desired compound and forms a complex with it at the cell surface. These carrier molecules are proteins and are specific for the molecule in question. The protein complex diffuses across the cell membrane and, once on the other side, dissociates to release the compound. The carrier protein is then free to return to the outside of the cell membrane

to pick up another molecule to transport. A drug that is similar in structure to an essential natural compound can, in some cases, fool the transport mechanism and be absorbed actively into the body. The anticancer drug *melphalan* was synthesised in order to make use of the existing active transport pathway for the amino acid phenylalanine (see Figure 2.7). The phenylalanine part of the molecule takes no part in the anticancer action; it is merely there to improve the molecule's chances of being absorbed across biomembranes. Interestingly, only the natural L-phenylalanine analogue of melphalan is absorbed actively; the opposite D form is only absorbed slowly by passive diffusion. This fact neatly illustrates that active transport, like most of the body's biochemical mechanisms, is chiral in nature, and can easily discriminate between enantiomers.

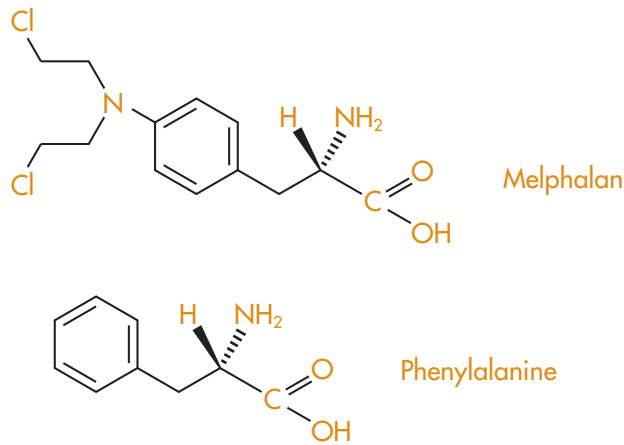

Figure 2.7 The structures of melphalan and phenylalanine.

The action of local anaesthetics

The physicochemical properties of drugs that underlie their absorption within the body can be complex, and the pH partition hypothesis is not sufficiently comprehensive to explain all the processes that occur *in vivo*; it is, however, a good place to start. Perhaps surprisingly for such a simple theory, the pH partition hypothesis can explain quite complicated pharmacological observations. The processes that occur when a patient swallows a tablet are so complicated that the most powerful computers known to science cannot adequately model the process. It is astonishing, therefore, that a few physicochemical constants (pK_a and partition coefficient, for example) can provide useful information and, when used properly, predict

the possibility and extent of drug absorption. An example of drug action that can be adequately explained by the pH partition hypothesis, is the mechanism(s) of action of local anaesthetic drugs. Local anaesthetics are drugs that are used to induce a state of temporary analgesia, or freedom from pain. They achieve this by blocking the conduction of impulses along nerve fibres responsible for the transmission of painful stimuli from a site of an injury to the brain and CNS.

Local anaesthetics are basic drugs, all derived originally from cocaine (see Figure 2.8), an alkaloid obtained from the leaves of *Erythroxylum coca*, a small shrub which grows wild in the Andes region of South America. Cocaine is a very effective local anaesthetic, but due to a profound stimulant action on the CNS it has been replaced in most routine procedures with synthetic, non-addictive, analogues such as lidocaine (lignocaine), prilocaine, procaine, etc. These drugs are aliphatic amines, with pK_a values for their conjugate acids of approximately 8–9. Applying the 'rule of thumb' shows that local anaesthetics will exist approximately 99% ionised at blood pH (7.4).

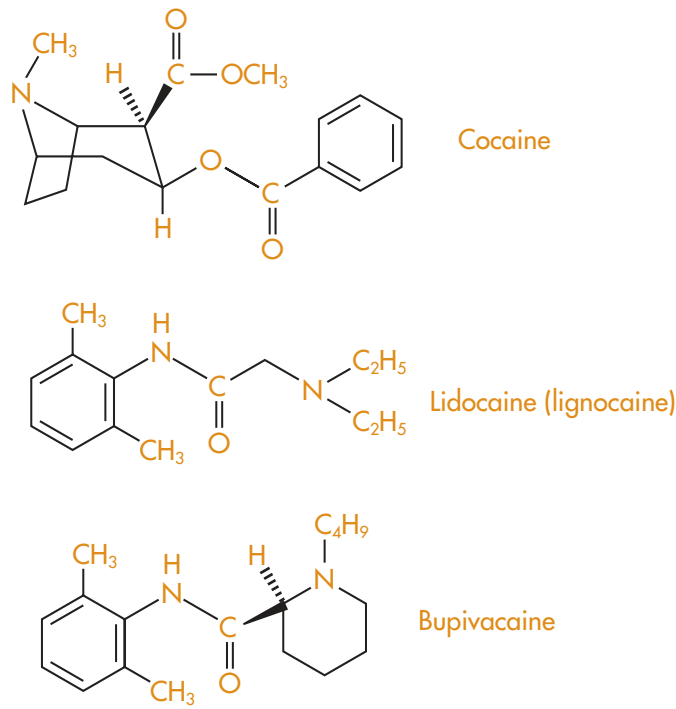

Figure 2.8 The structures of cocaine, lidocaine (lignocaine) and bupivacaine.

The site of action of most local anaesthetics is a Na^+ ion channel found in the cell membrane of nerve cells (or *neurons*). This sodium channel, as its name suggests, allows Na^+ ions to travel through the cell membrane to depolarise the resting membrane potential and allow the nerve cell to fire. Local anaesthetics block nerve conduction by attaching to the protein of the sodium channel and disrupting the flow of Na^+ ions. Recent research using radiolabelled local anaesthetics has shown that the local anaesthetic attaches to a structure at the *intracellular* opening of the sodium channel, and that the form of the drug active at the receptor is the positively charged *conjugate acid*, which prompts the question 'How does an ionised drug get to the internal opening of the ion channel?' The apparently obvious answer is that the cationic form of the local anaesthetic gains entry to the nerve cell by the same route as the Na^+ ions, i.e. down the open sodium channel. Although this may explain part of local anaesthetic action, it cannot be the full story, since most local anaesthetics are too large to pass through the channel. The answer lies within the properties of equilibria and can be predicted from the pH partition hypothesis (see Figure 2.9).

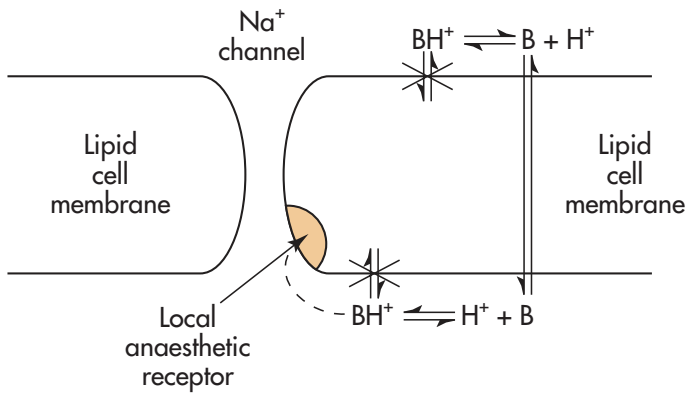

Figure 2.9 A diagram of local anaesthetic equilibrium.

The important thing to remember in this situation is that although 99 out of every 100 local anaesthetic molecules are ionised, there exists an equilibrium between the cation and the unionised free base. This unionised free base (B) can diffuse easily through the cell membrane, where it will become instantly ionised due to the H^+ ions present within the cell. Once ionised to the cation (BH^+), the local anaesthetic cannot easily diffuse back outside the cell, but it can approach the receptor situated at the internal opening of the sodium channel. Once the 1% of free base has diffused into

the cell, *the equilibrium must re-establish to give a further 1% free base*. This unionised free base can diffuse into the cell easily, the equilibrium re-establishes, and so on. These so called 'sink conditions' mean that a substantial portion of a drug dose can reach the site of action even though, at first glance, there appears to be insufficient unionised drug to partition across the membrane. This system is an example of a *dynamic equilibrium* and should be studied carefully. Dynamic equilibria occur in many sites in the body and are responsible for a significant amount of drug absorption.

Excretion and reabsorption of drugs

Previously in this chapter, the pH partition hypothesis was applied to the absorption of drugs across biological membranes following administration by the oral route. The same types of physicochemical processes occur when drugs are reabsorbed into the bloodstream following excretion by the kidneys.

The two kidneys are situated at the back of the abdomen on either side of the vertebral column. They carry out many functions in the body, the most important of which is the production of urine and the excretion from the body of low-molecular-weight (relative molecular mass less than 68 000 daltons) water-soluble compounds, including many drugs. Each kidney contains approximately one million urine-producing structures called nephrons. The nephron in turn consists of a bundle of blood capillaries termed a *glomerulus*, which functions as a very efficient filter to remove waste products and impurities from the blood, and a long tube-like structure called a *tubule* (see Figure 2.10).

The kidneys receive a large blood flow (approximately a quarter of the total cardiac output of 5 litres per minute) and from this volume of blood approximately 170 litres of filtrate are produced every day. Clearly, the body would quickly become dehydrated if this volume of fluid were lost to the sewage system, so most of it is reabsorbed from the kidney tubule and returned to the bloodstream. Small molecules that are dissolved in the glomerular filtrate are also reabsorbed back into the bloodstream, either by passive diffusion (which obeys Fick's law) or by the utilisation of energy in an active transport process similar to the mechanisms for gut absorption discussed previously. It should be realised that reabsorption from the glomerular filtrate and return to the bloodstream are involved in the duration of action of many drugs, and a drug molecule may be filtered and reabsorbed many times before it is finally excreted from the body.

In cases of drug overdose it is desirable to eliminate the toxic drug from the body as quickly as possible and techniques have been devised to

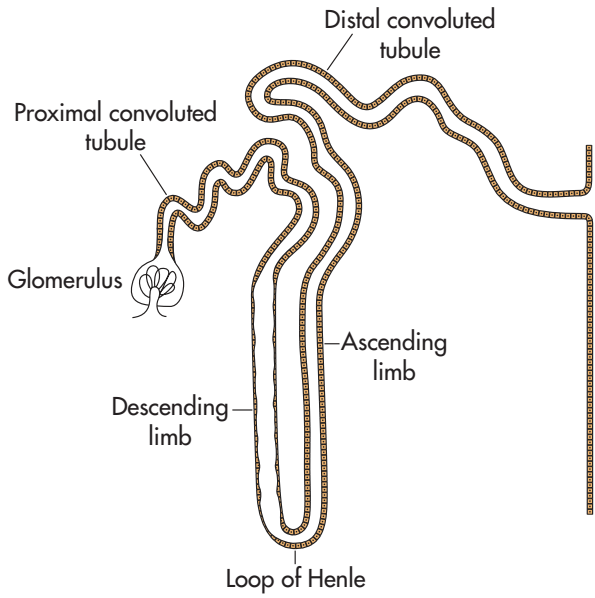

Figure 2.10 A diagram of a nephron.

minimise the process of reabsorption of drug from the kidney tubule and so expedite excretion. If a drug is to be reabsorbed by passive diffusion through the tubule cell membrane, then it must exist predominantly in the *unionised* form. This is entirely in keeping with the process of absorption into the body following oral administration discussed above. If the pH of the urine is adjusted to increase the proportion of the drug that is ionised, then reabsorption will be decreased (since the unionised species crosses the membrane more easily by passive diffusion). If the drug taken in overdose is a weak acid (e.g. a barbiturate, phenytoin, or most NSAIDs), then excretion should be favoured, and reabsorption minimised, by addition of an agent that will raise the pH of the urine. This technique is called *forced alkaline diuresis* and should result in more rapid clearance of an acidic drug. An example of an agent used to raise the pH of urine is sodium bicarbonate, $Na^+HCO_3^-$ (the salt of a strong base and a weak acid, which will be basic by partial hydrolysis), usually administered as an 8.4% w/v infusion.

If the drug taken in overdose is a base, for example, a benzodiazepine tranquilliser or an antihistamine, excretion should be favoured by acidification of the urine. Agents that may be used to achieve this include ammonium chloride, $NH_4^+Cl^-$ (an acidic salt by partial hydrolysis) and ascorbic acid (vitamin C). If the pH of the urine is artificially lowered, the technique is called *forced acid diuresis*.

Food and drink

Solubility and partition effects do not only occur with drug molecules. In everyday life, the effects of water solubility, or lack of it, can be observed. This can be neatly illustrated using two consumables familiar to generations of students, namely alcohol and curry!

In France, it is the custom before a meal to partake of an aperitif, usually an aniseed-flavoured spirit called *pastis*. Pastis (e.g. 'Ricard', 'Pernod') when it comes out of the bottle is a clear, light brown coloured solution of volatile oils from the seeds of the anise plant (*Pimpinella anisum*), which impart the characteristic aniseed flavour to the drink, dissolved in approximately 40% v/v ethanol. When a pastis is drunk, it is mixed with water and ice, whereupon the liquid becomes cloudy. This happens because the anise oils are hydrophobic, non-polar liquids and not very water-soluble. They are only held in solution by the high alcohol content of the drink. When the alcohol is diluted with water, the oils come out of solution and form an emulsion of oil droplets in the aqueous phase. This is what gives the drink its cloudy appearance. Oral solutions of anise oils have been used pharmaceutically for their carminative action and as an aid to digestion for many years, although it seems to this author preferable to consume anise oils in the form of a pastis, rather than in the form of a bottle of medicine.

Spicy foods such as curries and chillies and flavourings such as tabasco and paprika derive their hot pungent taste from the compound *capsaicin* (Figure 2.11). Capsaicin is found in the fruits of various species of *Capsicum* and is a powerful irritant causing intense pain if administered in a pure form. As can be seen from Figure 2.11, capsaicin is a non-polar compound possessing few polar groups to hydrogen-bond to water. This means that capsaicin is virtually insoluble in water. This is important information for people who eat spicy food. If a curry or chilli is too hot, there is little point in trying to counteract the burning in your mouth by consuming water (or beer!) as capsaicin is not soluble in aqueous solution. A far better strategy to put out the fire is to consume a non-aqueous liquid such as milk (an oil-in-water emulsion) in which the capsaicin can dissolve. Alternatively, eating fatty food such as bread with butter can help the capsaicin partition into the fat on the bread rather than the lipid of your epithelium. Incidentally, the temperature of the mouth does not increase while eating a hot, spicy meal, even though you may feel warm as a result. Capsaicin is a chemical irritant and does not raise the temperature of the mouth at all.

The irritant properties of capsaicin are employed in pharmacological research, where it is used to stimulate sensory nerves and as an experimental

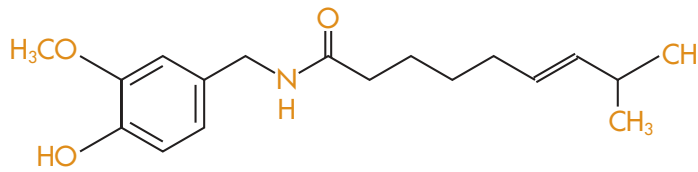

Figure 2.11 The structure of capsaicin.

treatment for chronic pain. Patients suffering intense chronic pain that can no longer be treated by analgesics may gain some relief by the use of capsaicin, which destroys the sensory nerves carrying the painful stimulus. Capsaicin is also used for more sinister purposes. A solution of capsaicin (pepper spray) is used by police forces around the world as a non-lethal weapon to temporarily blind and incapacitate criminals resisting arrest.

Tutorial examples

Q *1 A basic drug, $pK_a = 9.4$, P = 65, was administered to a patient and 5 mL of blood plasma was removed for analysis. This 5 mL of sample was extracted with 10 mL of octanol and the concentration of drug in the octanol was found to be 34 ng mL^{-1}. Calculate the following:*

(a) The apparent partition coefficient at pH 7.4

(b) The concentration of drug in plasma before extraction

(c) The percentage of drug extracted in a single extraction

A 1(a) The first step is to calculate the fraction of the drug unionised at pH 7.4. The equation for the percentage ionised for a base is

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}(\text{pH} - pK_a)}$$

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}(7.4 - 9.4)}$$

$$\% \text{ Ionised} = 99\%$$

Therefore, the percentage unionised = 1% and the fraction unionised = 1/100 = 0.01.

$$P_{app} = P \times \text{fraction unionised}$$

$$P_{app} = 65 \times 0.01$$

$$P_{app} = 0.65$$

(b) Before we can calculate the concentration of drug in the plasma *before* the extraction, we have to consider the concentration present *after* the extraction and remember that all of the drug in the octanol and the plasma started off in the plasma. Thus,

$$P_{app} = \frac{\text{concentration in octanol after extraction}}{\text{concentration in plasma after extraction}}$$

$$P_{app} = \frac{34 \text{ ng mL}^{-1}}{\text{plasma concentration}}$$

$$0.65 = \frac{34 \text{ ng mL}^{-1}}{\text{plasma concentration}}$$

Concentration in plasma after extraction = 52.3 ng mL^{-1}

To calculate the concentration of drug in the plasma *before* the extraction, we have to convert the concentrations into amounts (i.e. mass) of drug.

The amount of drug in octanol *after* extraction is volume × concentration = 10 × 34 = 340 ng

The amount of drug in plasma *after* extraction is volume × concentration = 5 × 52.3 = 261.5 ng

Total amount of drug in the initial plasma sample = 340 + 261.5 = 601.5 ng

Therefore the *initial* concentration of drug in plasma is given by

$$\frac{\text{Amount}}{\text{Volume}} = \frac{601.5}{5} = 120.3 \text{ ng mL}^{-1}$$

(c) The percentage extracted in a single extraction is easily calculated from the following.

The amount extracted into octanol = 340 ng

The total amount of drug in plasma = 601.5 ng

Therefore,

$$\% \text{ Extracted} = \left(\frac{340}{601.5} \right) \times 100 = 56.5\%$$

Q

2 5.0 mL of a plasma sample (pH 7.4) containing an acidic drug (pK_a = 6.5) was extracted with 10.0 mL of ether. The concentration of drug in both layers was determined and the results obtained were:

The total concentration (unionised + ionised) in plasma = 16 μg mL^{-1}
The concentration in ether = 7 μg mL^{-1}

From these data determine the following:
(a) The distribution of the drug between the two phases at equilibrium
(b) The apparent partition coefficient, P_{app}
(c) The partition coefficient, P
(d) The percentage extracted
(e) How the efficiency of the extraction might be improved by modification of the pH
(f) The percentage extracted under the modified conditions

A

2(a) From the pK_a value and the pH it can be seen that the acidic drug will be ionised at plasma pH.

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}(\text{pH} - pK_a)}$$

$$= \frac{100}{1 + \text{antilog}(6.5 - 7.4)}$$

$$= 88.8\%$$

Thus, the fraction ionised (f_i) = 0.888. Therefore, the fraction unionised (f_u) = 1 − 0.888 = 0.112.

Mass of drug in ether = 7 μg mL^{-1} × 10 mL = 70 μg

Mass of drug in the plasma = 16 μg mL^{-1} × 5 mL = 80 μg

Amount of drug ionised = 0.888 × 80 μg = 71 μg

Amount of drug unionised = 0.112 × 80 μg = 9 μg

Amount of drug in the total system = 70 μg + 80 μg

= 150 μg

(b) P_{app} can be calculated from the concentration data since the drug is ionised in the plasma.

$$P_{app} = \frac{\text{[drug] in ether}}{\text{total [drug] in plasma}}$$

$$= \frac{7\ \mu g\ mL^{-1}}{16\ \mu g\ mL^{-1}}$$

$$= 0.44$$

(c) The partition coefficient, P, is given by

$$P = \frac{P_{app}}{\text{fraction unionised}}$$

$$= \frac{0.44}{0.112}$$

$$= 3.93$$

It should be noted that P is greater than P_{app} since the drug will be ionised at the pH of measurement of P_{app} and hence will be less soluble in the ether phase.

(d) The percentage of drug extracted is given by

$$\frac{\text{Mass of drug in ether}}{\text{Total mass of drug in system}}$$

$$= \frac{70\ \mu g}{150\ \mu g} \times 100$$

$$= 47\%$$

(e) Since the drug is an acid, lowering of the pH of the plasma sample (e.g. by the addition of a small volume of strong acid) will suppress ionisation of the drug and allow more drug to partition into the ether phase.

(f) If the plasma sample is acidified so that $P_{app} = P = 3.93$, the mass of drug extracted when it is essentially unionised can be calculated. The mass of drug remaining in the plasma after extraction under the new conditions is x μg. Therefore, the mass extracted into the ether = $(150 - x)$ μg.

$$\text{Concentration of drug in plasma} = \frac{x}{5}\ \mu\text{g mL}^{-1}$$

$$\text{Concentration of drug in ether} = \frac{(150 - x)}{10}\ \mu\text{g mL}^{-1}$$

$$P = \frac{[\text{drug}] \text{ in ether}}{[\text{drug}] \text{ in plasma}}$$

$$= \frac{(150 - x)/10}{x/5}$$

$$= 3.9$$

When this unpleasant-looking piece of algebra is solved for x, we obtain

$$\begin{aligned}
\text{Mass of drug remaining in plasma} &= 17\ \mu\text{g} \\
\text{Mass of drug extracted into the ether} &= (150 - 17)\ \mu\text{g} \\
&= 133\ \mu\text{g} \\
\%\ \text{Extracted under new conditions} &= (133/150) \times 100 \\
&= 89\%
\end{aligned}$$

which shows, as expected, that acidification of the plasma sample increases the percentage extracted from 47% to 89%.

Q *2.3(i) Native Indians in South America have for hundreds of years hunted animals using poison darts tipped in toxins from plants of the species* Chondodendron. *Using the structure of tubocurarine chloride in Figure 2.12 as a guide, explain how the Indians were able to eat the poisoned animals without themselves suffering any symptoms.*
(ii) What would happen if one of the Indians had a stomach ulcer?

A 2.3(i) Tubocurarine is a major component of the 'curare' type of arrow poison and has been used in Western medicine as a neuromuscular blocker prior to surgery. The drug is a competitive antagonist of nicotinic acetylcholine receptors and blocks the neuromuscular junction, causing paralysis. This is useful during surgery since it allows lower doses of anaesthetic to be used. When used as an

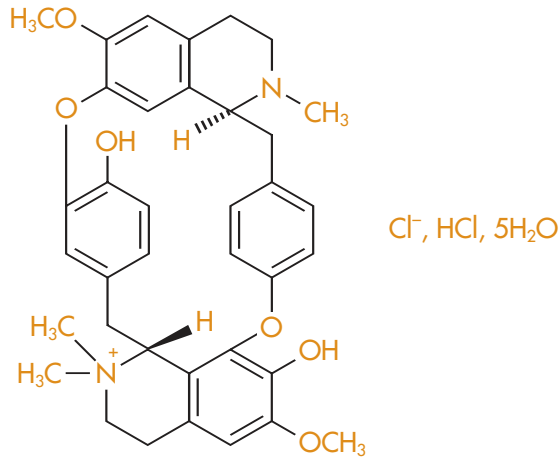

Figure 2.12 The structure of tubocurarine chloride.

arrow poison, the drug paralyses the animal, which either dies from asphyxia or is easily caught and killed by the hunters.

The drug is not destroyed by cooking, but the Indians can eat the hunted animal because tubocurarine is not able to cross the gut membrane by passive diffusion and therefore is not absorbed and does not accumulate in the bloodstream. This is because the drug is both a quaternary ammonium compound, ionised at all values of pH, and a tertiary amine which will exist predominately as the cation at gut pH. The drug is also a phenol, but phenols are normally too weakly acidic to ionise at the range of pH values found in the body.

(ii) An ulcer is a small hole in the muscle lining of the stomach. The hole is attacked by stomach acid, causing intense pain. If one of the Indians eating the poisoned animal has an ulcer, tubocurarine could be absorbed into the bloodstream and cause paralysis or even death – an example of dying for something to eat!

Problems

Q2.1 Explain the difference between the true partition coefficient and the apparent partition coefficient.

Outline how the true partition coefficient of a sparingly water-soluble drug such as sulfamethoxazole might conveniently be measured (see Figure 2.13).

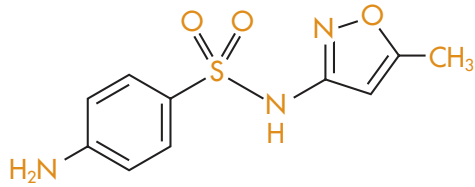

Figure 2.13 The structure of sulfamethoxazole, pK_a = 5.6, P (ether : water) = 125.

A 4 mL aliquot of plasma (pH 7.4) was taken from a patient receiving treatment with sulfamethoxazole and extracted with 2 × 5 mL aliquots of ether. The ether extracts were combined, evaporated to dryness and reconstituted in 2 mL of chloroform. The concentration of sulfamethoxazole in the chloroform was found to be 15.8 μg mL^{-1}.

Calculate the original concentration of drug in the plasma sample.

What percentage of the drug was extracted in the procedure outlined above and how might the procedure be changed to increase this value?

Q2.2 The structure of the β-blocker atenolol (pK_a = 9.6, P (ether : water) = 275) is shown in Figure 2.14.

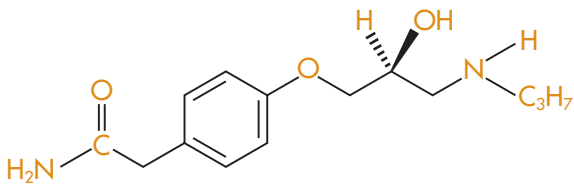

Figure 2.14 The structure of atenolol.

A 5 mL sample of plasma (pH 7.4) taken from a patient being treated with atenolol was extracted with 2 × 5 mL aliquots of ether. The ether layers were combined and evaporated to dryness and the residue was reconstituted in 5 mL of methanol. The concentration of atenolol in the methanol was found to be 0.604 μg mL^{-1}.

Calculate the original concentration of atenolol in the plasma sample and the percentage of the drug extracted.

How might the extraction procedure be altered to improve the percentage of the drug removed?

(Answers to problems can be found on pp. 254–255.)