1
Physicochemistry

1.1
Physicochemistry and Pharmacokinetics

The body can be viewed as primarily composed of a series of membrane barriers dividing aqueous filled compartments. These membrane barriers are comprised principally of the phospholipid bilayers, which surround cells and also form intracellular barriers around the organelles present in cells (mitochondria, nucleus, etc.). These are formed with the polar ionised head groups of the phospholipid facing towards the aqueous phases and the lipid chains providing a highly hydrophobic inner core. To cross the hydrophobic inner core a molecule must also be hydrophobic and able to shed its hydration sphere. Many of the processes of drug disposition depend on the ability or inability to cross membranes and hence there is a high correlation with measures of lipophilicity. Moreover, many of the proteins involved in drug disposition have hydrophobic binding sites further adding to the importance of the measures of lipophilicity [1].

At this point it is appropriate to define the terms hydrophobicity and lipophilicity. According to recently published IUPAC recommendations both terms are best described as follows [2]:

- Hydrophobicity is the association of non-polar groups or molecules in an aqueous environment, which arises from the tendency of water to exclude non-polar molecules.
- Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid–liquid (e.g. partition coefficient in 1-octanol/water) or solid–liquid (retention on reversed-phase high-performance liquid chromatography (RP-HPLC) or thin-layer chromatography (TLC) system).

Further key physicochemical properties include solubility/dissolution and the ionisation state [3]. All these properties have a strong influence on absorption [4], membrane permeability, volume of distribution and to a certain extent metabolism [5]. The role of dissolution in the absorption process is also discussed under Section 3.2. Other properties closely linked to the physicochemical behaviour of

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molecules are the structural features molecular weight and hydrogen-bonding capacity, which can be seen as the main contributors to log *P*/*D*.

1.2 Partition and Distribution Coefficient as Measures of Lipophilicity

The inner hydrophobic core of a membrane can be modelled by the use of an organic solvent. Similarly a water or aqueous buffer can be used to mimic the aqueous filled compartment. If the organic solvent is not miscible with water then a two phase system can be used to study the relative preference of a compound for the aqueous (hydrophilic) or organic (hydrophobic, lipophilic) phase.

For an organic compound, lipophilicity can be described in terms of its partition coefficient *P* (or log *P* as it is generally expressed). This is defined as the ratio of concentrations of the compound at equilibrium between the organic and aqueous phases:

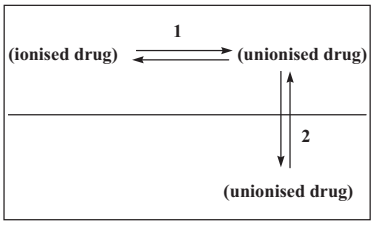
$$P = \frac{[\text{drug}]_{\text{organic}}}{[\text{drug}]_{\text{aqueous}}} \tag{1.1}$$

The partition coefficient (log *P*) describes the *intrinsic lipophilicity* of the collection of functional groups and carbon skeleton, which combine, to make up the structure of the compound, *in the absence of dissociation or ionisation*. Methods to measure partition and distribution coefficients have been described [6, 7].

Every component of an organic compound has a defined lipophilicity and calculation of partition coefficient can be performed from a designated structure. Likewise, the effect on log *P* of the introduction of a substituent group into a compound can be predicted by a number of methods as pioneered by Hansch (π values) [8–11], Rekker (*f* values) [12, 13], and Leo and Hansch (*f′* values) [8–10, 14, 15].

Partitioning of a compound between aqueous and lipid (organic) phases is an equilibrium process. When in addition, the compound is partly ionised in the aqueous phase a further (ionisation) equilibrium is set up, since it is assumed that under normal conditions only the unionised form of the drug penetrates the organic phase [16]. This traditional view is shown schematically in Fig. 1.1 below. However, the nature of the substituents surrounding the charged atom as well as the degree of delocalisation of the charge may contribute to the stabilisation of the ionic species and thus not fully exclude partitioning into an organic phase or membrane [17]. An example of this is the design of acidic 4-hydroxyquinolones (Fig. 1.2) as glycine/NMDA antagonists [18]. Despite a formal negative charge these compounds appear to be have considerable blood-brain barrier crossing.

In a study of the permeability of alfentanil and cimetidine through Caco-2 cells (Fig. 1.3), a model for oral absorption, it was deduced that at pH 5 ca. 60% of the cimetidine transport and 17% of the alfentanil transport across Caco-2 monolayers can be attributed to the ionised form [19]. Thus the dogma that only neutral species can cross a membrane has been challenged recently.



1. Is a function of acid/base strength p*K*a
2. Is a function of *P* (log *P*)

Fig. 1.1 Schematic depicting the relationship between log *P* and log *D* and pK_a.

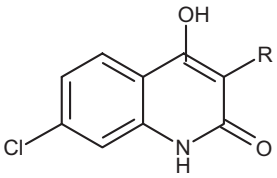
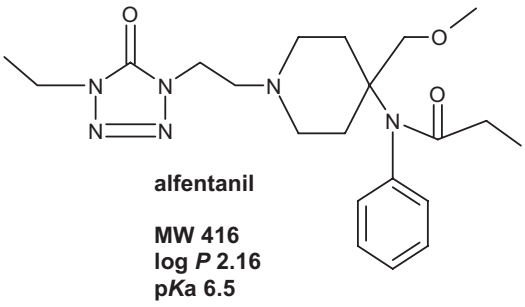


Fig. 1.2 4-Hydroxyquinolines with improved oral absorption and blood-brain barrier permeability [18].



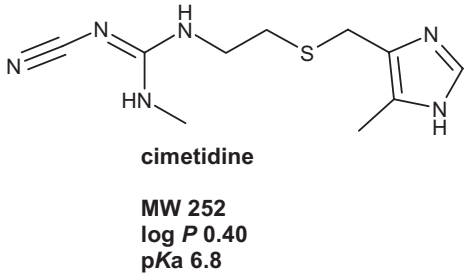


Fig. 1.3 Rapidly (alfentanil) and slowly (cimetidine) transported basic drugs across Caco-2 monolayers [19].

The intrinsic lipophilicity (*P*) of a compound refers only to the equilibrium of the unionised drug between the aqueous phase and the organic phase. It follows that the remaining part of the overall equilibrium, i.e. the concentration of ionised drug in the aqueous phase, is also of great importance in the overall observed partition ratio. This in turn depends on the pH of the aqueous phase and the acidity or basicity (pK_a) of the charged function. The overall ratio of drug, ionised and unionised, between the phases has been described as the *distribution coefficient* (*D*), to distinguish it from the intrinsic lipophilicity (*P*). The term has become widely used in recent years to describe, in a single term the *effective* (or *net*) *lipophilicity* of a compound at a given pH taking into account both its intrinsic lipophili-

city and its degree of ionisation. The distribution coefficient (D) for a monoprotic acid (HA) is defined as:

$$D = [HA]_{organic} / ([HA]_{aqueous} + [A^-]_{aqueous}) \quad (1.2)$$

where [HA] and [A^-] represent the concentrations of the acid in its unionised and dissociated (ionised) states, respectively. The ionisation of the compound in water is defined by its dissociation constant (K_a) as:

$$K_a = [H^+][A^-] / [HA] \quad (1.3)$$

sometimes referred to as the Henderson–Hasselbalch relationship. Combination of Eqs. 1.1–1.3 gives the pH distribution relationship:

$$D = P / (1 + \{K_a / [H^+]\}) \quad (1.4)$$

more commonly expressed for monoprotic organic acids in the form of Eqs. 1.5 and 1.6, below:

$$\log (\{P / D\} - 1) = pH - pK_a \quad (1.5)$$

or

$$\log D = \log P - \log (1 + 10^{pKa - pH}) \quad (1.6)$$

For monoprotic organic bases (BH^+ dissociating to B) the corresponding relationships are:

$$\log (\{P / D\} - 1) = pK_a - pH \quad (1.7)$$

or

$$\log D = \log P - \log (1 + 10^{pH - pKa}) \quad (1.8)$$

From these equations it is possible to predict the effective lipophilicity (log D) of an acidic or basic compound at any pH value. The data required in order to use the relationship in this way are the intrinsic lipophilicity (log P), the dissociation constant (pK_a), and the pH of the aqueous phase. The overall effect of these relationships is that the effective lipophilicity of a compound, at physiological pH; is the log P value minus one unit of lipophilicity, for every unit of pH the pK_a value is below (for acids) and above (for bases) pH of 7.4. Obviously for compounds with multifunctional ionisable groups the relationship between log P and log D, as well as log D as functions of pH become more complex [20]. For diprotic molecules there are already twelve different possible shapes of log D – pH plots.

1.3
Limitations on the Use of 1-Octanol

Octanol is the most widely used model of a biological membrane [21, 22] and log $D_{7.4}$ values above zero normally correlate with effective transfer across the lipid core of the membrane, whilst values below zero suggest an inability to traverse the hydrophobic barrier.

Octanol, however, supports H-bonding. Besides the free hydroxyl group, octanol also contains 4% v/v water at equilibrium. This obviously conflicts with the exclusion of water and H-bonding functionality at the inner hydrocarbon core of the membrane. Therefore, for compounds that contain functionality capable of forming H-bonds, the octanol value can over-represent the actual membrane crossing ability. These compounds can be thought of as having a high hydration potential and have difficulty in shedding their water sphere.

Use of a hydrocarbon solvent such as cyclohexane can discriminate these compounds either as the only measured value or as a value to be subtracted from the octanol value ($\Delta\log P$) [23–25]. Unfortunately, cyclohexane is a poor solvent for many compounds and does not have the utility of octanol. Groups which hydrogen bond and attenuate actual membrane crossing compared to their predicted ability based on octanol are listed in Fig. 1.4. The presence of two or more amide, carboxyl functions in a molecule will significantly impact membrane crossing ability and will need substantial intrinsic lipophilicity in other functions to provide sufficient hydrophobicity to penetrate the lipid core of the membrane.

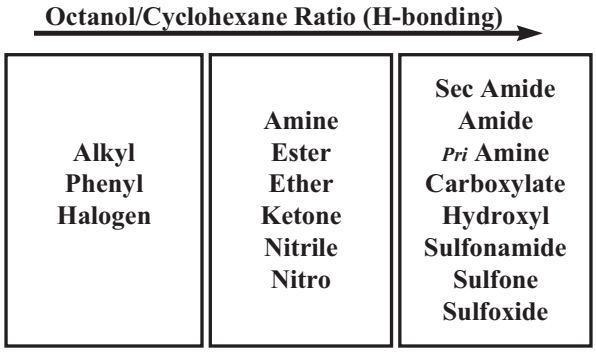


Fig. 1.4 Functionality and H-bonding.

1.4 Further Understanding of Log *P*

1.4.1 Unravelling the Principal Contributions to Log *P*

The concept that log P or log D is composed of two components [26], that of size and polarity is a useful one. This can be written as

$$\log P \text{ or } \log D = a \cdot V - \Lambda \tag{1.9}$$

where V is the molar volume of the compound, Λ a general polarity descriptor, and a is a regression coefficient. Thus the size component will largely reflect the carbon skeleton of the molecule (lipophilicity) whilst the polarity will reflect the hydrogen-bonding capacity. The positioning of these properties to the right and left in Fig. 1.4 reflects their influence on the overall physicochemical characteristics of a molecule.

1.4.2 Hydrogen Bonding

Hydrogen bonding is now seen as an important property related to membrane permeation. Various scales expressing H-bonding have been developed [27]. Some of these scales describe total hydrogen-bonding capability of a compound, while others discriminate between donors and acceptors [28]. It was demonstrated that most of these scales show considerable intercorrelation [29].

Lipophilicity and H-bonding are important parameters for uptake of drugs in the brain [30]. Their role has been studied in a series of structurally diverse, sedating and non-sedating, histamine H_1 receptor antagonists [31]. From these studies a decision tree guideline for the development of non-sedative anti-histamines was designed (see Fig. 1.5).

GABA (γ-aminobutyric acid) is a major neurotransmitter in mammals and is involved in various CNS (central nervous system) disorders. In the design of a series of GABA uptake inhibitors a large difference in *in vivo* activity between two compounds with identical IC_{50} values was observed, one compound being devoid of activity [32]. The compounds have also nearly identical pK_a and log D_{oct} values (see Fig. 1.6) and differ only in their distribution coefficient of cyclohexane/water (log D_{chex}). This results in a Δlog D of 2.71 for the *in vivo* inactive compounds, which is believed to be too large for CNS uptake. The active compound has a Δlog D of 1.42, well below the critical limit of around 2. Besides this physicochemical explanation, further evaluation of metabolic differences should complete this picture. It should be noted that the concept of using the differences between solvent systems was originally developed for compounds in their neutral state (Δlog P values, see Section 2.2). In this case two zwitterions are being compared, which are considered at pH of 7.4 to have a net zero charge, and thus the Δlog P concept seems applicable.

Considerable interest is focused on the calculation of hydrogen-bonding capability for use in quantitative structure–activity relationships (QSAR) studies, design of combinatorial libraries, and for correlation with absorption and permeability data [33–35]. A number of different descriptors for hydrogen bonding have been discussed [36], one of the simplest being the count of the number of hydrogen bond-forming atoms [37].

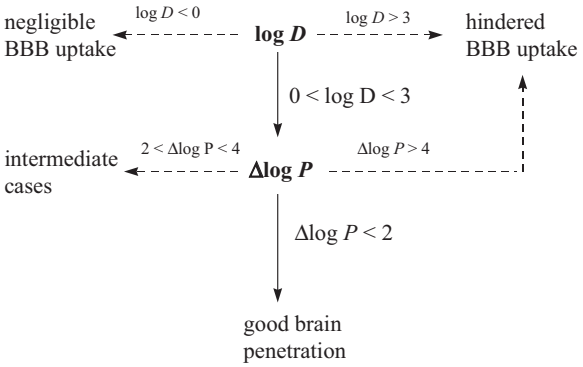
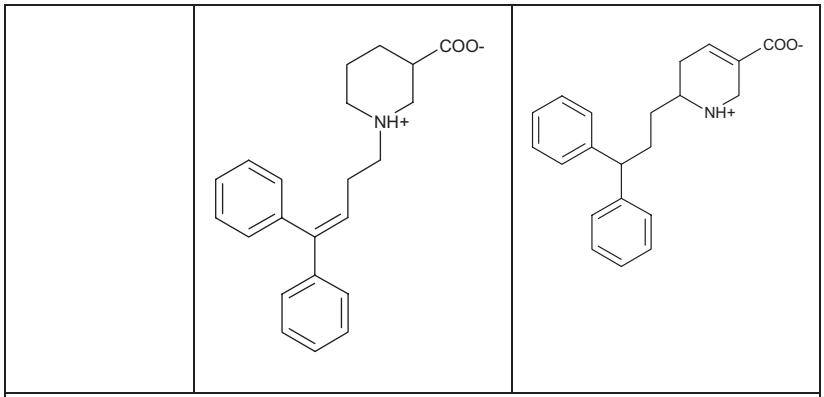


Fig. 1.5 Decision tree for the design of non-sedative H_1 antihistaminics. Log *D* is measured at pH 7.4, while Δlog *P* refers to compounds in their neutral state (redrawn from [31]).



IC_{50} *in vivo*	0.11 µM active	0.1 µM inactive
p*K*a	3.57 / 9.23	3.39 / 9.25
log D_{oct}	0.99	0.71
log D_{chex}	-0.43	-2.00
Δlog *D*	1.42	2.71

Fig. 1.6 Properties of GABA-uptake inhibitors [32].

A simple measure of hydrogen-bonding capacity is polar surface area (*PSA*), summing up the fractional contributions to surface area of all nitrogen and oxygen atoms [38]. This was used to predict passage of the blood-brain barrier [39, 40], flux across a Caco-2 monolayer [41], and human intestinal absorption [42, 43]. The physical explanation is that polar groups are involved in desolvation when they move from an aqueous extracellular environment to the more lipophilic interior of membranes. *PSA* thus represents, at least part, of the energy involved in membrane transport. *PSA* is dependent on the conformation and the original method is based on a single minimum energy conformation [38]. Others have taken into account conformational flexibility and coined a dynamic *PSA*, in which a Boltzmann-weighted average *PSA* is computed [42]. However, it was demonstrated that the *PSA* calculated for a single minimum energy conformation is in most cases sufficient to produce a sigmoidal relationship to intestinal absorption (see Fig 3.12), differing very little from the dynamic *PSA* described above [43]. A fast calculation of *PSA* as a sum of fragment-based contributions, called topological polar surface area (*TPSA*), has been published [44], allowing the use of these calculations for large data sets such as combinatorial or virtual libraries. The sigmoidal relationship can be described by $A\% = 100 / [1 + (PSA / PSA_{50})^{\gamma}]$, where $A\%$ is the percentage of orally absorbed drug, PSA_{50} is the *PSA* at 50% absorption level, and γ is a regression coefficient. Others have used a Boltzmann sigmoidal curve given by $y = \text{bottom} + (\text{top} - \text{bottom}) / (1 + \exp[(x_{50} - x)/\text{slope}])$ [43].

Poorly absorbed compounds have been identified as those with a $PSA > 140\,\text{Å}^2$. Considering more compounds, considerably more scatter was found around the sigmoidal curve observed for a smaller set of compounds [43]. This is partly due to the fact that many compounds do not show simple passive diffusion only, but are affected by active carriers, efflux mechanisms involving P-glycoprotein (P-gp) and other transporter proteins, and gut wall metabolism. A further refinement in the *PSA* approach is expected to come from taking into account the strength of the hydrogen bonds, which in principle is the basis of the hydrogen bond thermodynamics approach.

1.4.3 Molecular Size and Shape

Molecular size can be a limiting factor in oral absorption. The Lipinski *rule of five* proposes an upper limit of (molecular weight) *MW* 500 as acceptable for orally absorbed compounds [45]. Molar volume as used in Eq. 1.9 is another way to express the size of a compound. It is very much related to molecular surface area. For convenience often the molecular weight is taken as a first estimate of size. It is also useful to realise that size is not identical to shape. Size and shape parameters are generally not measured, but rather calculated. A measured property is the so-called cross-sectional area, which is obtained from surface activity measurements [46].

Molecular weight is often taken as the size descriptor of choice, while it is easy to calculate and is in the chemist's mind. However, other size and shape proper-

ties are equally as simple to calculate and may offer a better guide to estimate the potential for permeability. Thus far no systematic work has been reported investigating this in detail. Cross-sectional area A_D obtained from surface activity measurements have been reported as a useful size descriptor to discriminate between compounds, which can access the brain ($A_D < 80\ Å^2$) of those that are too large to cross the blood-brain barrier [46]. Similar studies have been performed to define a cut-off for oral absorption [47].

Many companies have tried to develop peptidic renin inhibitors. Unfortunately these are rather large molecules and not unexpectedly poor absorption was often observed. The role of physicochemical properties has been discussed for this class of compounds. One of the conclusions was that compounds with higher lipophilicity were better absorbed in the intestine [48]. Absorption and bile elimination rate both are *MW*-dependent. Lower *MW* gives better absorption and less bile excretion. The combined influence of molecular size and lipophilicity on absorption of a series of renin inhibitors can be seen in Fig. 1.7. The observed isosize curves are believed to be part of a general sigmoidal relationship between permeability and lipophilicity [49–51] (see Chapter 3).

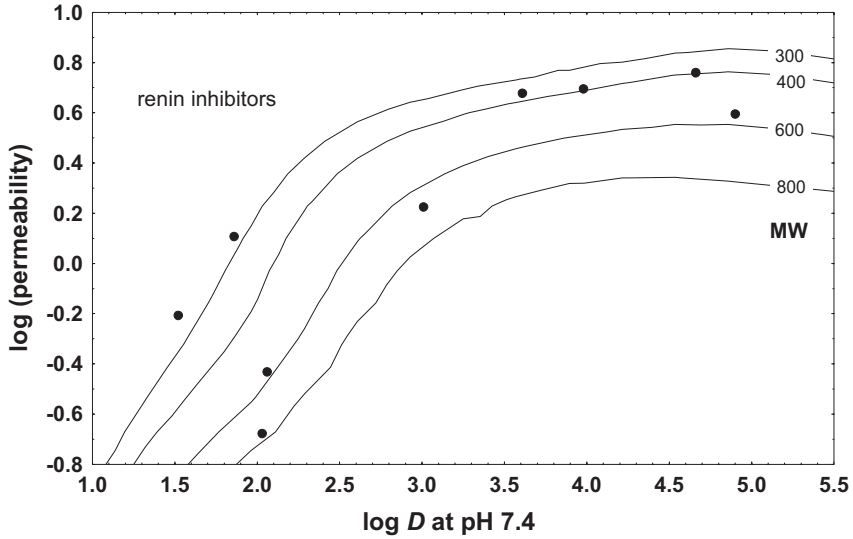


Fig. 1.7 Isomolecular weight curves showing the influence of molecular size on membrane permeability with increasing lipophilicity [51].

1.5 Alternative Lipophilicity Scales

1.5.1 Different Solvent Systems

Since 1-octanol has certain limitations (see Section 1.3) many alternative lipophilicity scales have been proposed [52] (see Fig. 1.3). A critical quartet of four solvent systems of octanol (amphiprotic), alkane (inert), chloroform (proton donor) and propylene glycol dipelargonate (PGDP) has been advocated [53, 54]. By measuring distribution in all four a full coverage of partitioning properties should have been obtained. Also non-aqueous systems such as heptane/acetonitrile [55] or heptane/glycol [56] may be of use. This latter system appears to offer a direct measure for hydrogen bonding. Alkane/water partitioning is thought to be a good imitation of the blood-brain barrier. Hexadecane/water partitioning and distribution can be measured in a PAMPA-like set-up using hexadecane membranes (HDM) [57] (for PAMPA see Chapter 3).

1.5.2 Chromatographic Approaches

In order to increase throughput over the traditional shake flask and related methods, various chromatographic techniques can be used [1]. Particularly, immobilised artificial membranes (IAM) had considerable attention [58, 59]. IAMs consist of phospholipids grafted on a solid phase HPLC support intended to mimic membrane character (see Fig. 1.8). It appears that IAM retention times are highly correlated with shake flask log *D* octanol/water coefficients and thus do not really measure something different.

1.5.3 Liposome Partitioning

Several groups have suggested that studying partitioning into liposomes may produce relevant information related to membrane uptake and absorption [59, 60]. Liposomes, which are lipid bilayer vesicles prepared from mixtures of lipids, provide a useful tool for studying passive permeability of molecules through lipids. This system has been used to demonstrate the passive nature of the absorption mechanism of monocarboxylic acids [61]. Liposome partitioning of ionisable drugs can be determined by titration and has been correlated with human absorption [62]. A new absorption potential parameter has been suggested, as calculated from liposome distribution data and the solubility-dose ratio, which shows an excellent sigmoidal relationship with human passive intestinal absorption (Eq. 1.10) [62–64].

$$AP_{SUV} = \log (\text{Distribution} \times \text{Solubility} \times V / \text{Dose}) \quad (1.10)$$

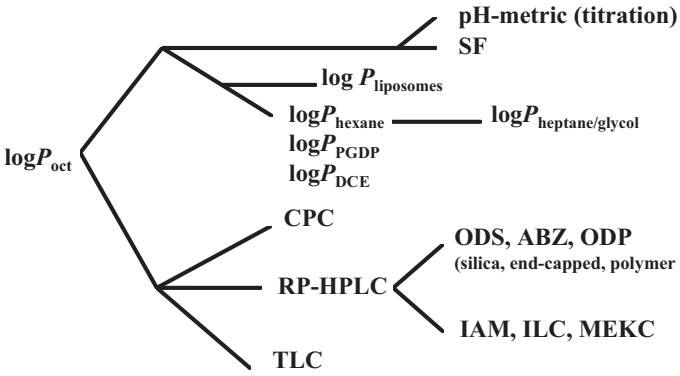


Fig. 1.8 Experimental methods to measure lipophilicity (modified after [26]). Key of abbreviations-log P_{oct}:1-octanol/water partition coefficient, log $P_{liposomes}$: partition coefficient between liposomes and buffer, log P_{hexane}: 1-hexane/water partition coefficient, log P_{PGDP}: propyleneglycol dipelargonate/water partition coefficient, log $P_{heptane/glycol}$: a non-aqueous partitioning system, SF: shake flask, pH-metric: log *P* determination based on potentiometric titration in water and octanol/water, CPC: centrifugal partition chromatography, RP-HPLC: reversed-phase high-performance liquid chromatography, TLC: thin-layer chromatography, ODS: octadecylsilane, ABZ: end-capped silica RP-18 column, ODP: octadecylpolyvinyl packing, IAM: immobilised artificial membrane, ILC: immobilised liposome chromatography, MEKC: micellar electrokinetic capillary chromatography.

Here, AP_{SUV} is the absorption potential measured from the distribution in small unilamellar vesicles (SUV) at pH 6.8, the solubility was measured at pH 6.8 in simulated intestinal fluid, *V* is the volume of intestinal fluid, and dose is a mean single oral dose. Liposome partitioning is only partly correlated with octanol/water distribution.

A further partition system based on the use of liposomes, and commercialised under the name Transil, has been investigated [52, 65, 66].

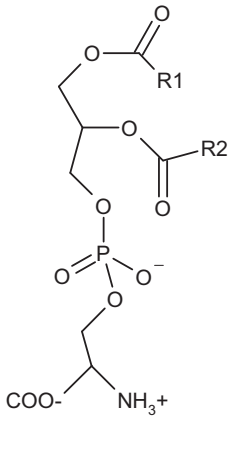
1.6 Computational Approaches to Lipophilicity

In the design of new compounds as well as the design of experimental procedures an a priori calculation log *P* or log *D* values may be very useful. Methods may be based on the summation of fragmental [67–69], or atomic contributions [70–72], or a combination [73, 74]. Reviews of various methods can be found in Refs. [67, 75–78]. Further approaches based on the use of structural features have been suggested [75, 79]. Atomic and fragmental methods suffer from the problem that not all contributions may be parameterised. This leads to the observation that for a typical pharmaceutical file ca. 25% of the compounds cannot be computed. Recent efforts try to improve the *missing value* problem [80].

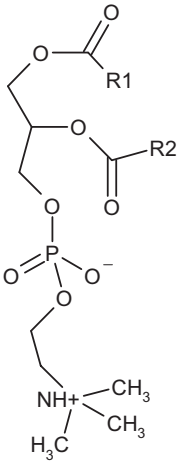
Molecular lipophilicity potential (MLP) has been developed as a tool in 3D-QSAR, for the visualisation of lipophilicity distribution on a molecular surface and as an additional field in CoMFA studies [76]. MLP can also be used to estimate conformation-dependent log *P* values.

1.7 Membrane Systems to Study Drug Behaviour

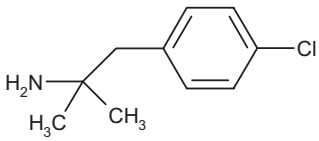
In order to overcome the limitations of octanol, other solvent systems have been suggested. Rather than a simple organic solvent, actual membrane systems have also been utilised. For instance the distribution of molecules has been studied between unilamellar vesicles of dimyristoylphosphatidylcholine and aqueous buffers. These systems allow the interaction of molecules to be studied with the whole membrane, which includes the charged polar head group area (hydrated)



phosphatidylserine



phosphatidylcholine



chlorphentermine **log *P* 2.6, log *D* 0.4, pK_a 9.6**

Fig. 1.9 Structures of charge neutral (phosphatidylcholine) and acidic (phosphatidylserine) phospholipids together with the moderately lipophilic and basic drug chlorphentermine. The groupings R1 and R2 refer to the acyl chains of the lipid portions.

and the highly lipophilic carbon chain region. Such studies indicate that partitioning for amine compounds ionised at physiological pH, partitioning into the membrane is highly favoured and independent of the degree of ionisation. This is believed to be due to electrostatic interactions with the charged phospholipid head group. This property is not shared with acidic compounds even for the *electronically neutral* phosphatidylcholine [81]. Such ionic interactions between basic drugs are even more favoured for membranes containing *acidic* phospholipids such as phosphatidylserine [82]. The structures of these two phospholipids are shown in Fig. 1.9, together with the structure of the basic drug chlorphentermine.

Table 1.1 shows the preferential binding of chlorphentermine for phosphatidylcholine-containing membranes, the phospholipid with overall acidic charge. These systems predict actually affinity for the membrane, rather than the ability to transfer across a membrane. Membrane affinity, and hence tissue affinity, is particularly important in the persistence of drugs within the body. This topic will be covered under volume of distribution in Chapter 4.

Tab. 1.1 Affinity (k) and capacity (moles drug/moles lipid) of chlorphentermine for liposomes prepared from phosphatidylcholine and phosphatidylserine.

Phospholipid	k [10^{-4}] M	n_{max}
Phosphatidylserine	2.17	0.67
Phosphatidylcholine	1.26	0.05

1.8 Dissolution and Solubility

1.8.1 Why Measure Solubility?

Each cellular membrane can be considered as a combination of a physicochemical and biological barrier to drug transport. Poor physicochemical properties may sometimes be overcome by an active transport mechanism. Before any absorption can take place at all, the first important properties to consider are dissolution and solubility. Many cases of solubility-limited absorption have been reported and therefore solubility is now seen as a property to be addressed at early stages of drug discovery. Only a compound in solution is available for permeation across the gastrointestinal membrane. Solubility has long been recognised as a limiting factor in the absorption process leading to the implementation of solubility screens in early stages of drug design [45, 83]. High-throughput solubility measurements have been developed, which can be used in early discovery [45, 84–86].

Excessive lipophilicity is a common cause of poor solubility and can lead to erratic and incomplete absorption following oral administration. Estimates of desired solubility for good oral absorption depend on the permeability of the compound and the required dose, as illustrated in Table 1.2 [83].

Tab. 1.2 Desired solubility correlated to expected doses [83].

Dose (mg kg^{-1})	Permeability		
	High	Medium	Low
0.1	1*	5	21
1	10	52	207
10	100	520	2100

* μg mL^{-1}

The incorporation of an ionisable centre, such as an amine or similar function, into a template can bring a number of benefits including water solubility (see Chapter 3).

Dissolution testing has been used as a prognostic tool for oral drug absorption [87]. A biopharmaceutical classification scheme (BCS) has been proposed, under which drugs can be categorised into four groups according to their solubility and permeability properties [88]. Because both permeability as well as solubility can be further dissected into more fundamental properties, it has been argued that the principal properties are not solubility and permeability, but rather molecular size and hydrogen bonding [89]. The BCS has been adopted as a regulatory guidance for bioequivalence studies.

1.8.2 Calculated Solubility

As a key first step towards oral absorption, considerable effort went into the development of computational solubility prediction [90–94]. However, partly due to a lack of large sets of experimental data measured under identical conditions, today's methods are not robust enough for reliable predictions [95]. Further fine-tuning of the models can be expected now as high-throughput data becomes available to construct such models.

1.9 Ionisation (pK_a)

Drug ionisation has an important effect in the *in vitro* prediction of *in vivo* absorption [96]. Various ways an ion may cross a membrane have been described [97]. These include transport as ion (trans- and/or para-cellular), ion pair, or protein-assisted (using the outer surface of a protein spanning a membrane).

The dogma based on the pH-partition theory that only neutral species cross a membrane has been challenged [98]. An example was already discussed above for studies with Caco-2 monolayers that suggested that the ionic species may contribute considerably to overall drug transport [18]. Using cyclic voltammetry it was also demonstrated that compounds in their ionised form pass into organic phases and might well cross membranes in this ionised form [99].

Therefore a continued interest exists in the role of pK_a in absorption, which often is related to its effect on lipophilicity and solubility. New methods to measure pK_a values are being explored, e.g. using electrophoresis, and an instrument for high-throughput pK_a measurement has been developed [100].

The difference between the log *P* of a given compound in its neutral form (log P^N) and its fully ionised form (log P^I) has been termed *diff*(log P^{N-I}) and contains series-specific information, and expresses the influence of ionisation on the intermolecular forces and intramolecular interactions of a solute [99]. It is unclear at present how these latter concepts can be used in drug design.

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