Methods and Principles in Medicinal Chemistry

Dennis A. Smith, Charlotte Allerton, Amit Kalgutkar,
Han van de Waterbeemd, and Don K. Walker

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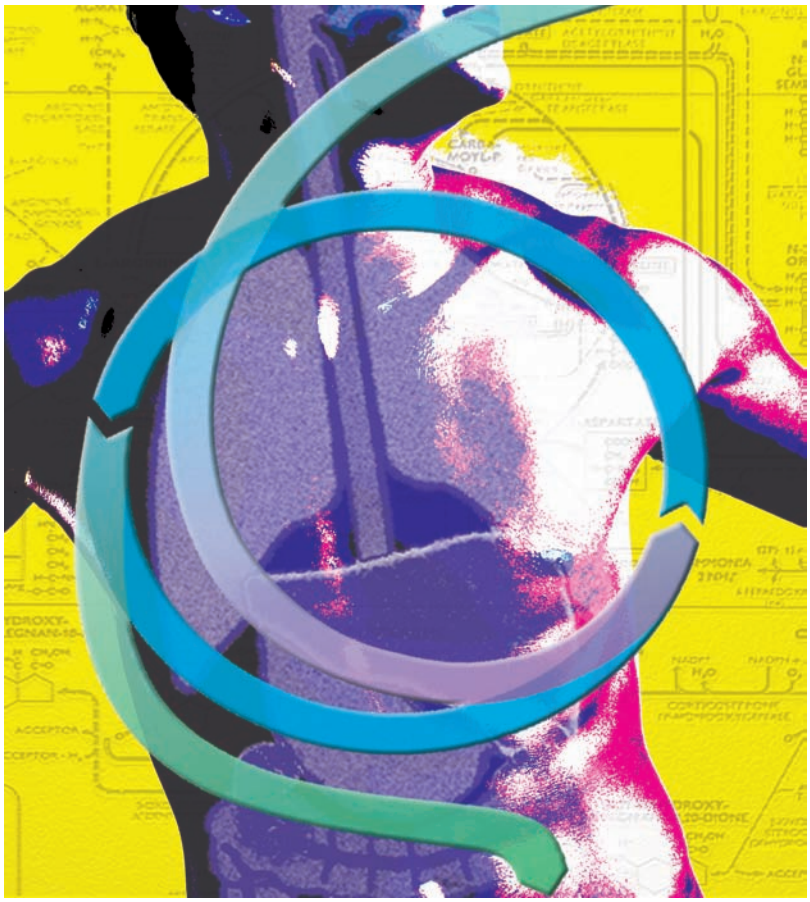
Pharmacokinetics and Metabolism in Drug Design

Third, Revised and Updated Edition



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R. Mannhold,
H. Kubinyi,
G. Folkers

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A Personal Foreword

Time seems to have passed so quickly since we updated the second edition, but as we began to assemble the list of changes and incorporations we needed to make for the third edition, we realized just how much the science has moved on. It really was time to renew the book and keep it at the forefront of the application of pharmacokinetics and metabolism in drug design. Not only the science had moved on, though, but also the people and finally the actual workplace.

In this edition, more attention is paid to the role of drug permeability in determining ADME fate. Much more emphasis is also placed on drug transport (in parallel to permeability). In addition, the role of free drug is described more fully. The latest concepts and knowledge in drug distribution, drug clearance, and drug toxicity are incorporated along with more descriptive sections on human prediction and screening methods. What has not changed is the layout of chapters, trying to condense the huge breadth of topics covered under a readable size. Each chapter can be read alone or in sequence. We hope readers who attempt the sequence find it not a sprint, not a marathon, but a satisfying middle distance: more magic mile than magic methyl.

Han and Don took retirement shortly before the third edition project began and Amit and Charlotte have stepped into their place as authors. Both have very strong chemistry backgrounds, with Charlotte a practicing and distinguished medicinal chemist before stepping across into the land of uncertainty and promise (ADME), and Amit is recognized worldwide for his experience in enzymology and metabolism chemistry. Don and Han have not disappeared, they have worked meticulously on the various chapter proofs, ensuring what we thought we had clearly written could actually be comprehended by the reader.

Finally the workplace. What brought us together was Pfizer Drug Metabolism (or Pharmacokinetics, Dynamics and Metabolism as it became known later). The original trio of authors of the first and second editions worked together at Sandwich Laboratories. The year 2011 marked the closure of Drug Discovery at Sandwich. The laboratories that buzzed with the electrical energy of drug discovery creating doxazosin, fluconazole, amlodipine, voriconazole, eletriptan, sildenafil, maraviroc, and more now are almost deserted. I feel, as they do, that working at Sandwich during its almost magical productive period was a privilege that left us with a profound sense

of achievement. Charlotte too was Sandwich based, and I would like to dedicate the third edition to the people who made the Sandwich site so special in terms of drug discovery and drug metabolism. It will remain in the memory as a crucible of many of the thoughts and ideas in this book.

Dennis A. Smith

1
Physicochemistry

Abbreviations

CPC	Centrifugal partition chromatography
CoMFA	Comparative field analysis
CsA	Cyclosporine A
3D-QSAR	Three-dimensional quantitative structure–activity relationships
HDM	Hexadecane membrane
IUPAC	International Union of Pure and Applied Chemistry
MLP	Molecular lipophilicity potential
PAMPA	Parallel artificial membrane permeability assay
PGDP	Propylene glycol dipelargonate
PSA	Polar surface area
RP-HPLC	Reversed-phase high-performance liquid chromatography
SF	Shake flask, referring to traditional method of measuring log *P* or log *D*
TPSA	Topological polar surface are

Symbols

AP_{SUV}	Absorption potential measured in small unilamellar vesicles (SUV)
$\Delta\log D$	Difference between log *D* in octanol/water and log *D* in alkane/water
$\Delta\log P$	Difference between log *P* in octanol/water and log *P* in alkane/water
f	Rekker or Leo/Hansch fragmental constant for log *P* contribution
K_a	Ionization constant
Λ	Polarity term, mainly related to hydrogen bonding capability of a solute
$\log P$	Logarithm of the partition coefficient (P) of neutral species
$\log D$	Logarithm of the distribution coefficient (*D*) at a selected pH, usually assumed to be measured in octanol/water
$\log D_{oct}$	Logarithm of the distribution coefficient (*D*) at a selected pH, measured in octanol/water
$\log D_{chex}$	Logarithm of the distribution coefficient (*D*) at a selected pH, measured in cyclohexane/water

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$\log D_{7.4}$	Logarithm of the distribution coefficient (D) at pH 7.4
MW	Molecular weight
π	Hansch constant; contribution of a substituent to $\log P$
pK_a	Negative logarithm of the ionization constant K_a

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 principally comprised of the phospholipid bilayers that surround cells and form intracellular barriers around the organelles present in cells (mitochondria, nucleus, etc.). These are formed with the polar ionized head groups of the phospholipid facing toward 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 published IUPAC recommendations, both terms are best described as follows [2]:

> **Hydrophobicity** is the association of nonpolar groups or molecules in an aqueous environment that arises from the tendency of water to exclude nonpolar molecules.
>
> **Lipophilicity** represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behavior 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 or thin-layer chromatography (*TLC*) system).

Key physicochemical properties that are associated with hydrophobicity and lipophilicity include solubility, hydrogen bonding capacity, and the ionization state [3]. All these properties have a strong influence on membrane permeability that affects absorption [4], distribution, and balance of elimination by transporter-mediated processes and metabolism [5].

1.2 Partition and Distribution Coefficients as Measures of Lipophilicity

The inner hydrophobic core of a membrane can be modeled by using an organic solvent. Similarly, a water or aqueous buffer mimics the aqueous media surrounding

cells or present within cells. 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 and 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 the aqueous phases:

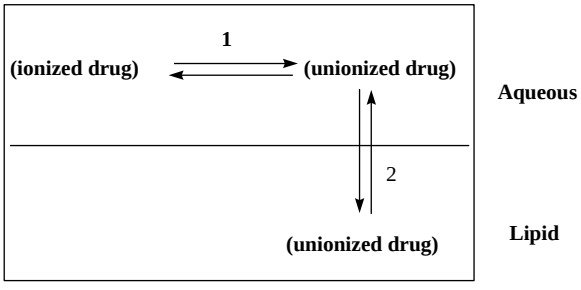
$$P = \frac{[\text{drug}]_{\text{organic}}}{[\text{drug}]_{\text{aqueous}}} \tag{1.1}$$

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 ionization*. 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 [8–11] (π-values), Rekker [12, 13] (f-values), and Leo and Hansch [8–10, 14, 15] (f'-values). These values break molecules down into fragments allowing the total lipophilicity to be calculated.

Partitioning of a compound between aqueous and lipid (organic) phases is an equilibrium process. When in addition the compound is partly ionized in the aqueous phase, a further (ionization) equilibrium is set up since it is assumed that under normal conditions only the unionized form of the drug penetrates the organic phase [16]. This traditional view is shown schematically in Figure 1.1.

This model is consistent with many observations, but partitioning of some compounds into octanol has been shown to occur as an ion pair [17]. Such ion pairs include chloride with basic compounds and sodium with acidic compounds. Whether such behavior can occur with a biological membrane is still not clear, some evidence exists for this with the strongly acidic drug proxicromil. The lipophilicity of



1. Is a function of acid/base strength pK_a

2. Is a function of P (log P)

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

the drug above pH 6 in octanol–buffer partition experiments depends on ion pair formation and largely unaffected by change in pH in contrast to Eq. (1.5). Similar trends were demonstrated for the *in vitro* partition of the compound into rat gastrointestinal rings. Furthermore, the absorption of the compound from the perfused gastrointestinal tract of anesthetized rats *in vivo* was not consistent with classical nonionized drug absorption theories and supported ion pair formation as a mechanism of proxicromil absorption. While phenomena such as these are occasionally reported [18], it is probable that for 99% of examples the theory of unionized drug being the "lipophilic" species is sound.

The intrinsic lipophilicity (P) of a compound refers only to the equilibrium of the unionized drug between the aqueous phase and the organic phase. It follows that the remaining part of the overall equilibrium, that is, the concentration of ionized 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, ionized and unionized, 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 lipophilicity and its degree of ionization. The distribution coefficient (D) for a monoprotic acid (HA) is defined as

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

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

$$K_a = [\mathrm{H}^+][\mathrm{A}^-]/[\mathrm{HA}] \tag{1.3}$$

sometimes referred to as the Henderson–Hasselbalch relationship. The combination of Eqs. (1.1)–(1.3) gives the pH distribution (or "pH partition") relationship:

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

more commonly expressed for monoprotic organic acids in the form of Eqs. (1.5) and (1.6) as follows:

$$\log(\{P/D\} - 1) = \mathrm{pH} - \mathrm{p}K_a \tag{1.5}$$

or

$$\log D = \log P - \log(1 + 10^{\mathrm{pH}\text{-}\mathrm{p}K_a}) \tag{1.6}$$

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

$$\log(\{P/D\} - 1) = \mathrm{p}K_a - \mathrm{pH} \tag{1.7}$$

or

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

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 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) $pH_{7.4}$. Understanding the role of p*K*a and intrinsic lipophilicity (log *P*) in the lipoidal permeability of molecules is extremely important. Drugs can be rendered with more *effective* lipophilicity by

i. increasing intrinsic lipophilicity (log *P*) by addition of lipophilic fragments or the substitution of polar fragments for lipophilic ones;
ii. increasing for an acidic compound the p*K*a value of the acidic function, thus increasing the proportion of unionized drug available to distribute into the lipoidal medium;
iii. decreasing for a basic compound the p*K*a value of the basic function, thus increasing the proportion of unionized drug available to distribute into the lipoidal medium.

It is important to cross validate pK_a and log *D* and log *P* values using Eqs. (6) and (8) or the rule of thumb referred to above. This may illustrate badly measured values. For an ionizable compound, pK_a values can be determined by measuring log *D* across a pH range.

Obviously for compounds with multifunctional ionizable groups, the relationship between log *P* and log *D*, as well as log *D* as function of pH, becomes more complex [19]. Zwitterionic compounds are compounds with an acidic and basic function with p*K*a values separated to allow both to be ionized at a given pH. Such compounds are permanently ionized and are at their most lipophilic when both functions are ionized ($\log D_{max}$) see Figure 1.2. The permanent ionized state is explained by the seperation of p*K*a values such that even when one function is suppressed the other is ionized. The "lipophilic" charge neutral species occurs when both functions are ionized.

1.3
Limitations on the Use of 1-Octanol

Octanol is the most widely used model of a biological membrane [20, 21] and $\log D_{7.4}$ values above 0 normally correlate with effective transfer across the lipid core of the membrane, while values below 0 suggest an inability to traverse the hydrophobic barrier.

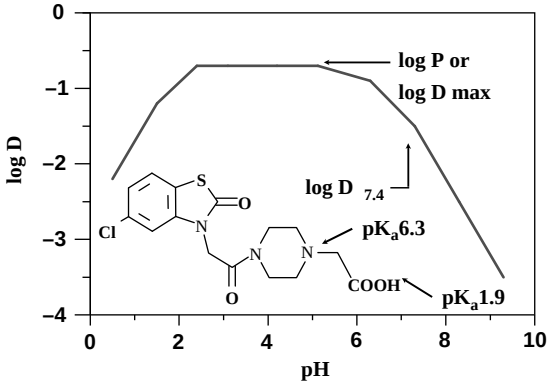


Figure 1.2 Example of a zwitterionic compound with basic and acidic functionality both ionized at a given pH.

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

The 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 ($\Delta\log P$) [22–24]. Unfortunately, cyclohexane is a poor solvent for many compounds and does not have the utility of octanol. Groups that bond with hydrogen and attenuate actual membrane crossing compared to their predicted ability based on octanol are listed in Figure 1.3. Later, various measures will be used to describe H bonding groups, but as a simple rule the presence of two or more amide functions in a molecule will significantly impact the membrane crossing ability and will need substantial intrinsic lipophilicity in other functions elsewhere in the molecule, to provide sufficient hydrophobicity and to penetrate the lipid core of the membrane.

1.4 Further Understanding of log *P*

1.4.1 Unraveling the Principal Contributions to log *P*

The concept that log *P* or log *D* is composed of two components [25], that of size and polarity, is a useful one. This can be written as Eq. (1.9),

$$\log P \text{ or } \log D = aV - \Lambda \tag{1.9}$$

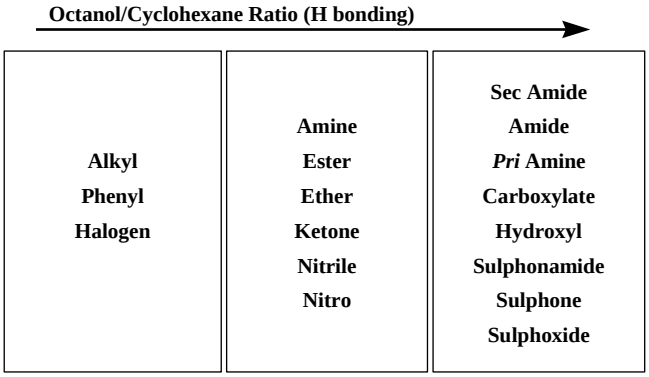


Figure 1.3 Functionality and H bonding.

where V is the molar volume of the compound, Λ is a general polarity descriptor, and a is a regression coefficient. Thus, the size component will largely reflect the carbon skeleton of the molecule (lipophilicity), while the polarity will reflect the hydrogen bonding capacity (normally oxygens and nitrogens). The positioning of these properties to the right and left of Figure 1.3 reflects their influence on the overall physicochemical characteristics of a molecule.

1.4.2 Hydrogen Bonding

Hydrogen bonding is recognized as an important property related to membrane permeation. Various scales expressing H bonding have been developed [26]. Some of these scales describe total hydrogen bonding capability of a compound, while others discriminate between donors and acceptors [27]. In this book, various measures of H bonding will be exemplified and referenced since similar correlations are obtained regardless of methods [28].

Of particular importance is the need to separate hydrogen bonds that form with solvent and internal H bonds. These are not exclusive and compounds can show a change of conformation that hides an "external H bond" by internal H-bonding within the interior of a membrane. Cyclic peptides have poor intrinsic membrane permeability, but they can be designed to have favorable properties. When the differing membrane permeabilities of a series of cyclic peptides were examined, these were found to be related to their ability to form internal hydrogen bonds [29]. Simply, the ability to form internal hydrogen bonds can promote passive membrane permeability by reducing the free energy cost of desolvating the compounds as they insert into the membrane. Cyclic peptides have poor membrane permeability, but there are exceptions, including cyclosporine A (CsA), a cyclic undecapeptide that is used as an orally active immunosuppressive drug. When the partition coefficient of cyclosporine A was measured in octanol/water and heptane/water, it was deduced

that the hydrogen-bonding capacity of CsA changed dramatically from an apolar solvent (where it is internally bonded to H) to a polar solvent (where it exposes its H-bonding groups to the solvent). The log *P* values were 2.9 in octanol and 1.4 in heptane, which is a remarkably small difference between the solvents. In comparison, cyclo (PHe-Phe) had values of 1.6 and < -3.0, respectively. Molecular dynamics simulations in water and CCl_4 showed that CsA underwent a solvent-dependent conformational change [30]. This interconversion process is slow on the molecular dynamics timescale.

The larger the molecule, the process of separating inter- and intra-H bonds becomes more complex, and apart from natural products it is an area not well researched in terms of new drug design.

1.4.3 Polar Surface Area

Polar surface area is a concept that has gained popularity in recent years. PSA is a simple measure of total hydrogen bonding capacity [31]. The PSA of a molecule is defined as the area of its van der Waals surface that arises from all oxygen and nitrogen atoms plus the hydrogen atoms attached to them. Thus, PSA is also a measure of the ability of a compound to form hydrogen bonds and accounts for three-dimensional (3D) structural features such as shielding or burial of polar groups by other parts of the molecule. PSA has been used to predict passage across the blood–brain barrier [32, 33], flux across a Caco-2 monolayer [34], and human intestinal absorption [35, 36]. 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 in part, the energy involved in membrane transport. PSA depends on the conformation, and the original method of calculation [31] is based on a single minimum energy conformation. Others [32] have taken into account conformational flexibility and coined a dynamic PSA, in which a Boltzmann-weighted average PSA is computed. However, it was demonstrated that PSA calculated for a single minimum energy conformation is in most cases sufficient to produce a sigmoidal relationship to intestinal absorption (see Figure 3.14), differing very little from the dynamic PSA described above [36]. A fast calculation of PSA as a sum of fragment-based contributions, called toplogical polar surface area (TPSA), has been published [37], which no longer uses a 3D representation of the molecule. This technique brings TPSA to a similar footing to clog *P* and the two descriptors can be readily calculated for all molecules, even for large data sets such as combinatorial or virtual libraries.

Poorly absorbed compounds have been identified as those with a PSA $> 140\ \text{Å}^2$. Considering more compounds, more scatter was found around the sigmoidal curve observed for a smaller set of compounds [33]. This is partly due to the fact that compounds that are not of high permeability do not usually show simple passive diffusion as they are affected by active uptake carriers and efflux mechanisms involving P-glycoprotein (P-gp) and other transporter proteins.

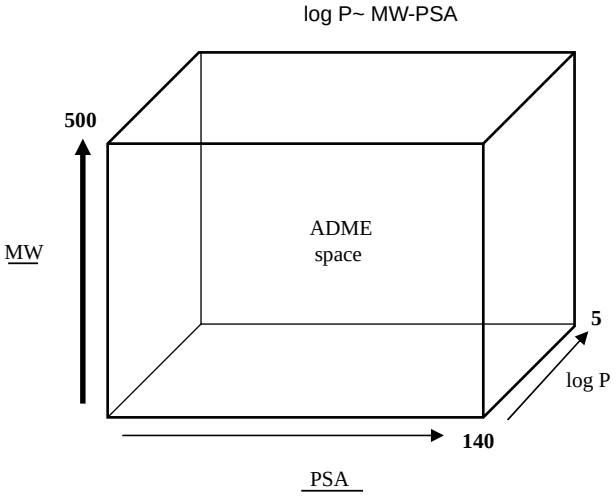


Figure 1.4 ADME space bounded by the interconnected physicochemical properties of molecular weight, polar surface area, and lipophilicity. Drugs with desirable pharmacokinetic properties such as absorption are much more likely to occupy the space. MW is not important per se but reflects that molecules of 500 MW or more are likely to exceed desirable PSA or clog *P* limits.

1.4.4 Molecular Size and Shape

The role of molecular size in oral absorption has been the subject of debate. The Lipinski rule-of-5 proposes an upper limit of MW 500 as acceptable for orally absorbed compounds [38]. Another view is that of Smith [40] who proposed that Eq. (1.9) is a virtual formula relating log *P* to MW–PSA and that MW is incidental to the two key terms log *P* and PSA [39]. Such a concept allows ADME (absorption, distribution, metabolism, and excretion) to be viewed in terms of a physicochemical space, which includes the properties most likely to be associated with drug-like properties. The dimensions of such a space and its boundaries are illustrated in Figure 1.4. The formula explains the interconnectivity of the physicochemical properties. Lipophilicity will increase membrane permeability but, at its upper limits, solubility will be so low that adequate dissolution will not be achieved at clinical doses. PSA as stated above is the energy cost in desolvation for the molecule to enter the membrane; so, as the higher values of PSA are reached, membrane permeability is energetically unfavorable. The interconnection with molecular weight has spawned a belief in that particular property being important per se. It is the belief of Smith and coworkers that drugs are largely products of carbon (lipophilicity), oxygen, and nitrogen (PSA) and that as molecular weight approaches 500, the chances of too high a lipophilicity or too great a PSA increases. In fact, it is difficult not to exceed a log *P* of 5 or a PSA of 140 Å^2 when synthesizing molecules of greater than 500 MW.

1.5
Alternative Lipophilicity Scales

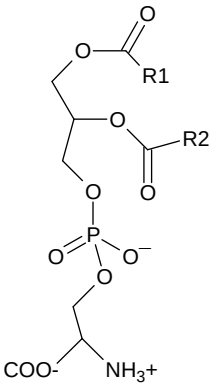
Since 1-octanol has certain limitations (see Section 1.3), many alternative lipophilicity scales have been proposed [40] (see Figure 1.3). A critical quartet of four solvent systems of octanol (amphiprotic), alkane (inert), chloroform (proton donor), and propylene glycol dipelargonate (PGDP) has been advocated [41, 42]. By measuring distribution in all four, a full understanding of partitioning properties should be obtained. Experience has indicated that the added value of more phases has not been sufficient and octanol has become the "gold standard" now universally adopted for both measurement and computational reference. Various solvents have been used in membrane systems such as PAMPA (see Chapter 10) including the standard *n*-hexadecane, 2% w/v dioleoylphosphatidylcholine in *n*-dodecane, and 20% w/v lecithin in *n*-dodecane [43]. These systems have also been compared with dodecane partitioning per se without really showing advancement on octanol-based systems.

1.6
Computational Systems to Determine Lipophilicity

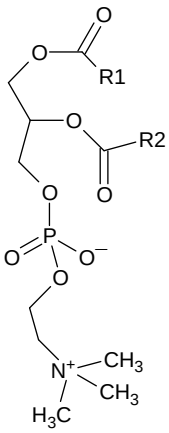
In the design of new compounds as well as the design of experimental procedures, an *a priori* calculation of log *P* or log *D* values is useful. Methods may be based on the summation of fragmental [44–46] or atomic contributions [47–49], or a combination of these [50, 51]. Many other methods continue to be advanced, but commercial software usually uses either fragment-based (clog *P*) or atom-based (alog *P*) methods and these have become standardized with clog *P* usually favored.

1.7
Membrane Systems to Study Drug Behavior

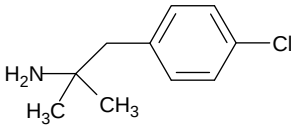
The standardization on octanol does not allow all aspects of compound behavior to be understood. Often acidic, neutral, and basic molecules with similar lipophilicities will show different behaviors in a biological system. To study some of these behaviors, different *in vitro* models have been utilized. 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 that includes the charged polar head group area (hydrated) and the highly lipophilic carbon chain region. Such studies indicate that for amine compounds ionized at physiological pH, partioning into the membrane is highly favored and independent of the degree of ionization. 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 [52]. Such ionic interactions between basic drugs are even more favored for membranes containing "acidic" phospholipids such as phosphatidylserine [53].



phosphatidylserine



phosphatidylcholine



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

Figure 1.5 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.

The structures of these two phospholipids are shown in Figure 1.5 together with the structure of the basic drug chlorphentermine.

Table 1.1 shows the binding of chlorphentermine to phosphatidylcholine and phosphatidylserine containing membranes.

The extent of binding of chlorphentermine and various amphiphilic drugs occurred in increasing order with liposomes prepared from sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS). The higher extent of binding to phosphatidylserine does not result from higher affinities for phosphatidylcholine but rather from higher capacity of phosphatidylserine liposomes compared to PC or SM liposomes. The divalent cationic drug

Table 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

chloroquine showed especially high binding to phosphatidylserine compared to monovalent drugs.

These systems potentially predict the actual 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 and relates in part to these interactions. This topic will be covered in Section 4.4.

1.8 Dissolution and Solubility

Each cellular membrane can be considered 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 the early stages of drug discovery. A compound only in solution is available for permeation across the gastrointestinal membrane. Solubility has long been recognized as a limiting factor in the absorption process leading to the implementation of solubility screens in early stages of drug design [54]. 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 [54].

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

In the drug design and discovery phase, simple solubility testing is the only practical method and this is often limited to kinetic solubility. This practice examines the solubility of a drug after mixing a small aliquot of a DMSO solution of the drug into aqueous media. Apart from solvent effects, the measurement does not measure the solubility of crystal forms.

As the key, first step toward oral absorption, considerable effort went into the development of computational solubility prediction [55–59]. 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 [60]. Further fine-tuning of the models can be expected as now high-throughput data have become available to construct such models.

Table 1.2 Desired solubility correlated with expected doses [54].

Dose (mg/kg)	Permeability high	Medium	Low
0.1	1*	5	21
1	10	52	207
10	100	520	2100

* μg/mL.

1.9
The BCS Classification and Central Role of Permeability

Despite the apparent complexity of the drug absorption process, which combines physicochemical properties of the drug, physiological factors of the gastrointestial tract and the physics of the dosage form the work of Amidon and coworkers [61] who revealed that the fundamental events controlling oral drug absorption are the permeability of the drug through the GI membrane and the solubility/dissolution of the drug dose in the GI milieu. These key parameters have been characterized in the Biopharmaceutics Classification System (BCS) by three dimensionless numbers: absorption number (A_n), dissolution number (D_n), and dose number (D_0). Importantly in this, the solubility is related to the dose size so drugs with high potencies that have adequate solubility for their low dose size may be classed differently to lower potency drugs with better solubility but still inadequate relative to dose size. These numbers take into account both physicochemical and physiological parameters and are fundamental to the oral absorption process. On the basis of their solubility and intestinal membrane permeability characteristics, drug substances have been classified into one of the four categories according to the BCS (Figure 1.6).

Wuand and Benet [62] extended the influence of the BCS classification much further when they correlated the high-permeability characteristics of BCS class I and II with metabolic fate. Highly permeable drugs will not be subjected to major transporter influences and will have ready access to metabolizing enzymes within the hepatocytes; moreover, these drugs will not be eliminated renally due to tubular absorption. This analysis showed that if the drug was highly permeable, then the drug will be cleared by metabolism, whereas if the drug is of low permeability the major route of clearance will be renal and/or hepatobiliary. Such is the nature of the

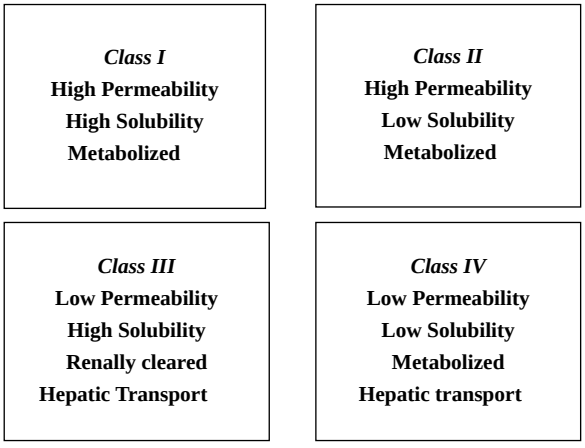


Figure 1.6 BCS classification of drugs. The classification not only allows to understand absorption behavior but also, particularly around permeability, provides a framework for all drug dispositions to be referenced.

relationship with permeability that it can be used to characterize the clearance fate of the drug be it via passive permeability and metabolism or via carrier-mediated transport. These properties can also be related to ADME space illustrated in Figure 1.5. The relationship between PSA/log *P* can rationally be grouped with the categories of drugs in the following classification:

Class 1	PSA/log *P*	Medium
Class 2	PSA/log *P*	Low
Class 3	PSA/log *P*	High
Class 4	PSA and log *P*	High

This can be exemplified by

Class 1 Propranolol	41/3.1
Class 2 Phenytoin	58/1.4
Class 3 Aliskiren	146/2.7
Class 4 Nelfinavir	127/7.3

Propanolol is a basic compound with nM potency and a dose size around 100 mg. In contrast phenytoin is a neutral compound with μM potency and dose size up to 1 g explaining the characterization of solubility. Both are cleared exclusively by metabolism. Because the BCS classification includes solubility (which is relevant only in absorption), an altered form can be made to understand disposition and include the physicochemical descriptor PSA/log *P* ratio and its

Table 1.3 Relationship of the disposition fate of a compound with its permeability across a biological membrane. Permeability of a biomembrane is favored by lipophilicity and attenuated by polar functionality (PSA).

Lipoidal permeability	**Low**	**Medium**	**High**
PSA/log *P*	**High**	**Medium**	**Low**
Absorption	Low (e.g., aliskiren) unless MWt less than 250 Da and absorbed by paracellular route (e.g., atenolol)	Variable. Influenced by permeability and transporters (e.g., nelfinavir)	High via transcellular route (e.g., propranolol)
Bioavailability	As for absorption	As for absorption and metabolism	Variable. Influenced by metabolism
Clearance	Renal or biliary (possible transporter involvement)	Transporters and metabolism	Metabolism

correlation with permeability. In this, low-permeability compounds cross membranes paracellularly (aqueous pore) and medium- and high-permeability compounds cross membranes by the transmembrane lipoidal route. This classification is exemplified in Table 1.3 [39].

References

1 Pliska, V., Testa, B., and Van de Waterbeemd, H. (eds) (1996) *Lipophilicity in Drug Action and Toxicology*, Wiley-VCH Verlag GmbH, Weinheim.

2 Van de Waterbeemd, H., Carter, R.E., Grassy, G., Kubinyi, H., Martin, Y.C., Tute, M.S., and Willett, P. (1998) *Annual Reports in Medicinal Chemistry*, **33**, 397–409.

3 Van de Waterbeemd, H. (2002) Physicochemical properties, in *Medicinal Chemistry: Principles and Practice*, 2nd edn (ed. F.D. King), RSC, London.

4 Van de Waterbeemd, H., Lennernas, H., and Artursson, P. (2003) *Drug Bioavailability*, Wiley-VCH Verlag GmbH, Weinheim.

5 Van de Waterbeemd, H., Smith, D.A., Beaumont, K., and Walker, D.K. (2001) *Journal of Medicinal Chemistry*, **44**, 1313–1333.

6 Dearden, J.C. and Bresnen, G.M. (1988) *Quantitative Structure–Activity Relationships*, **7**, 133–144.

7 Hersey, A., Hill, A.P., Hyde, R.M., and Livingstone, D.J. (1989) *Quantitative Structure–Activity Relationships*, **8**, 288–296.

8 Hansch, C. and Leo, A. (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley-Interscience, New York.

9 Hansch, C., Leo, A., and Hoekman, D. (1995) *Exploring QSAR. Hydrophobic, Electronic and Steric Constants*, ACS, Washington.

10 Hansch, C. and Leo, A. (1995) *Exploring QSAR. Fundamentals and Applications in Chemistry and Biology*, ACS, Washington.

11 Fujita, T., Iwasa, J., and Hansch, C. (1964) *Journal of the American Chemical Society*, **86**, 5175–5180.

12 Rekker, R.F. and De Kort, H.M. (1979) *European Journal of Medicinal Chemistry*, **14**, 479–488.

13 Rekker, R.F. and Mannhold, R. (1992) *Calculation of Drug Lipophilicity*, Wiley-VCH Verlag GmbH, Weinheim.

14 Leo, A. and Abraham, D.J. (1988) *Proteins: Structure, Function and Genetics*, **2**, 130–152.

15 Leo, A., Hansch, C., and Elkins, D. (1971) *Chemical Reviews*, **71**, 525–616.

16 Manners, C.N., Payling, D.W., and Smith, D.A. (1988) *Xenobiotica: The Fate of Foreign Compounds in Biological Systems*, **18**, 331–350.

17 Davis, M.G., Manners, C.N., Payling, D.W., Smith, D.A., and Wilson, C.A.J. (1984) *Journal of Pharmaceutical Sciences*, **73**, 949–953.

18 Reymond, F., Carrupt, P.A., Testa, B., and Girault, H.H. (1999) *Chemistry: A European Journal*, **5**, 39–47.

19 Smith, R.N., Hansch, C., and Ames, M.M. (1975) *Journal of Pharmaceutical Sciences*, **64**, 599–605.

20 Avdeef, A. (1996) Assessment of distribution-pH profiles, in *Lipophilicity in Drug Action and Toxicology* (eds V. Pliska, B., Testa, and H. Van de Waterbeemd), Wiley-VCH Verlag GmbH, Weinheim, pp. 109–139.

21 Avdeef, A. (2003) *Absorption and Drug Development*, Wiley-Interscience, Hobroken, NJ.

22 Young, R.C., Mitchell, R.C., Brown, T.H., Ganellin, C.R., Griffiths, R., Jones, M., Rana, K.K., Saunders, D., Smith, I.R., Sore, N.E., and Wilks, T.J. (1988) *Journal of Medicinal Chemistry*, **31**, 656–671.

23 El Tayar, N., Tsai, R.S., Testa, B., Carrupt, P.A., and Leo, A. (1991) *Journal of Pharmaceutical Sciences*, **80**, 590–598.