2
Physicochemical Properties in Drug Profiling

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
BBB	blood–brain barrier
BCS	Biopharmaceutics Classification Scheme
BMC	biopartitioning micellar chromatography
Caco-2	adenocarcinoma cell line derived from human colon
CNS	central nervous system
DMPK	drug metabolism and pharmacokinetics
FaSSIF	fasted-state simulated artificial intestinal fluid
HB	H-bonding
HDM	hexadecane membranes
HSA	human serum albumin
HTS	high-throughput screening
IAM	immobilized artificial membrane
ILC	immobilized liposome chromatography
MAD	maximum absorbable dose
MEKC	micellar electrokinetic chromatography
PAMPA	parallel artificial membrane permeation assay
PBPK	physiologically-based pharmacokinetic modeling
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
PPB	plasma protein binding
PSA	polar surface area (Å^2)
QSAR	quantitative structure–activity relationship
SPR	surface plasmon resonance

Symbols

A_D	cross-sectional area (Å^2)
Clog P	calculated logarithm of the octanol–water partition coefficient (for neutral species)

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D	distribution coefficient (often in octanol–water)
$diff(\log P^{N-I})$	difference between log P^N and log P^I
$\Delta\log P$	difference between log P in octanol–water and alkane–water
k_a	transintestinal rate absorption constant (min^{-1})
K_a	dissociation constant
Elog D	experimental log D based on a high-performance liquid chromatography method
log D	logarithm of the distribution coefficient, usually in octanol–water at pH 7.4
log $D^{7.4}$	logarithm of the distribution coefficient, in octanol–water at pH 7.4
log P	logarithm of the partition coefficient, usually in octanol–water (for neutral species)
log P^I	logarithm of the partition coefficient of a given compound in its fully ionized form, usually in octanol–water
log P^N	logarithm of the partition coefficient of a given compound in its neutral form, usually in octanol–water
MW	molecular weight (Da)
P	partition coefficient (often in octanol–water)
P_{app}	permeability constant measured in Caco-2 or PAMPA assay ($cm\,min^{-1}$)
pK_a	ionization constant in water
PPB%	percentage plasma protein binding
S	solubility ($mg\,mL^{-1}$)
SITT	small intestinal transit time (4.5 h = 270 min)
SIWV	small intestinal water volume (250 mL)
V	volume (mL or L)
V_{dss}	volume of distribution at steady state ($L\,kg^{-1}$)

2.1 Introduction

An important part of the optimization process of potential leads to candidates suitable for clinical trials is the detailed study of the absorption, distribution, metabolism and excretion (ADME) characteristics of the most promising compounds. Experience has shown that physicochemical properties play a key role in drug metabolism and pharmacokinetics (DMPK) [1–5]. In 1995, 2000 and 2004 specialized but very well attended meetings were held to discuss the role of log P and other physicochemical properties in drug research and lead profiling, and the reader is referred to the various proceedings for highly recommended reading on this subject [4, 6, 7].

The molecular structure is at the basis of physicochemical, DMPK, as well as safety/toxicity properties, as outlined in Fig. 2.1. Measurement and prediction of

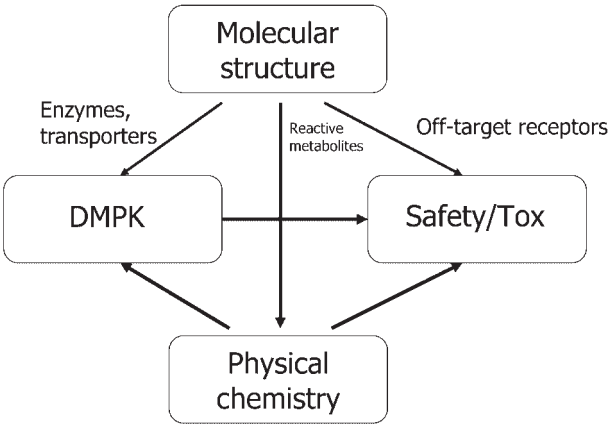

Fig. 2.1 Dependency of DMPK and safety/toxicity properties on structural and physicochemical properties.

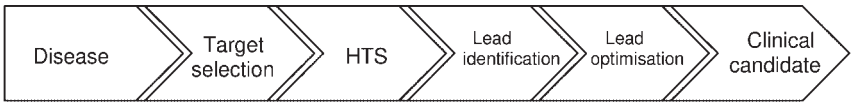

Fig. 2.2 The drug discovery process.

physicochemical properties is relatively easy compared to DMPK and safety properties, where biological factors come into play. However, DMPK and toxicity properties depend to a certain extent on the physicochemical properties of the compounds as these dictate the degree of access to biological systems such as enzymes and transporters.

The change in work practice towards high-throughput screening (HTS) in biology using combinatorial libraries has also increased the demands on more physicochemical and ADME data. There has been an increasing interest in physicochemical hits and leads profiling in recent years, using both *in vitro* and *in silico* approaches [8–11]. This chapter will review the key physicochemical properties, both how they can be measured as well as how they can be calculated in some cases. Chemical stability [12] is beyond the scope of this chapter, but is obviously important for a successful drug candidate.

The need and precision of a particular physicochemical property for decision making in a drug discovery project depends on the stage in the drug discovery process (see Fig. 2.2). Whilst calculated simple filters may be sufficient in library design, more experimental data are required in lead optimization. Striking the right balance between computational and experimental predictions is an important challenge in cost-efficient and successful drug discovery.

Physicochemical properties are considerably interrelated as visualized in Fig. 2.3. The medicinal chemist should bear in mind that modifying one often means

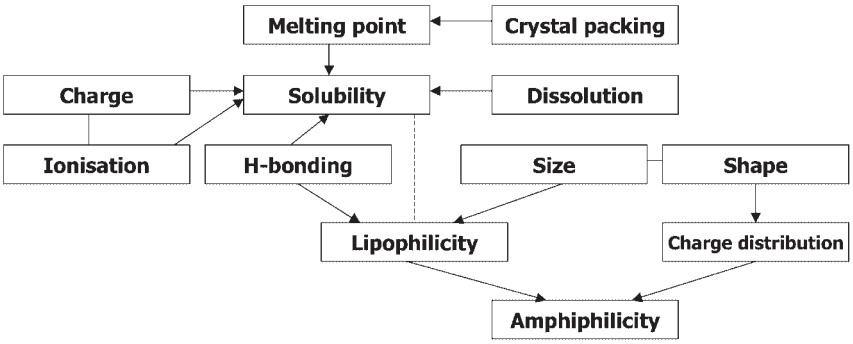

Fig. 2.3 Dependencies between various physicochemical properties.

also changes in other physicochemical properties, and hence indirectly influencing the DMPK and safety profile of the compound.

2.2 Physicochemical Properties and Pharmacokinetics

2.2.1 DMPK

The study of DMPK has changed from a descriptive to a much more predictive science [3]. This is driven by great progress in bioanalytics, development of *in vitro* assays and *in silico* modeling/simulation, and a much better basic understanding of the processes. Thus, and fortunately, ADME-related attrition has lowered from around 40% in 1990 to around 10% in 2005 [13].

2.2.2 Lipophilicity – Permeability – Absorption

As an example of the role of physicochemical properties in DMPK, the properties relevant to oral absorption are described in Fig. 2.4. It is important to note that these properties are not independent, but are closely related to each other. Oral absorption is the percentage of drug taken up from the gastrointestinal lumen into the portal vein blood. The processes involved are a combination of physical chemistry and biological (transporters, metabolizing enzymes). The transfer process through a membrane without any biological component is often called permeability. It can be mimicked in an artificial membrane such as the parallel artificial membrane permeation assay (PAMPA) set-up (see Section 2.8.1). However, *in vivo* permeability cannot be measured in isolation from biological events. All so-called *in vitro* measures for permeability are nothing else than different types of lipophilicity measures. In plotting oral absorption (percentage or fraction) against any

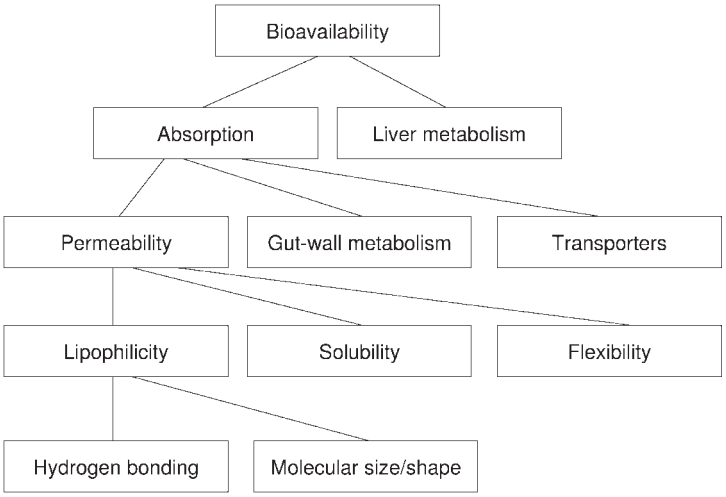

Fig. 2.4 Importance of physical chemistry properties on permeability, absorption and bioavailability [16]. (With kind permission of Elsevier.)

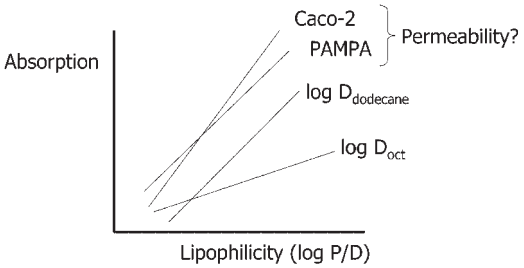

Fig. 2.5 Trendships between oral absorption and permeability/lipophilicity. In reality these relationships are most likely sigmoidal, i.e. more complex than these trends indicate.

"permeability" or lipophilicity scale (see Fig. 2.5) one observes a trend indicating that higher permeability or lipophilicity leads to better absorption. Often a plateau is observed too, indicating that such relationships are in fact nonlinear and can be approached by a sigmoidal function. Several lipophilicity scales can be related to each other via a Collander (Eq. 1) or an extended Collander relationship (Eq. 2) by adding a parameter for the difference in H-bonding (HB) between the two solvent systems. The equivalent for relating, for example, PAMPA scales to each other or PAMPA with Caco-2 has also been published [14, 15].

$$\log P_1 = a \log P_2 + b \tag{1}$$

$$\log P_1 = p \log P_2 + q\mathrm{HB} + r \tag{2}$$

Instead of using surrogate measures for oral absorption with a lipophilicity or permeability assay *in vitro*, oral absorption can also be estimated *in silico* by using

human oral absorption data from the literature [16]. This data is rather sparse because oral absorption is not systematically measured in clinical trials. The data is also skewed towards high absorption compounds. In addition, interindividual variability is important (around 15%). Of course absorption can also be dose and formulation dependent. Therefore, early estimates are only rough guides to get the ballpark right.

2.2.3
Estimation of Volume of Distribution from Physical Chemistry

The distribution of a drug in the body is largely driven by its physicochemical properties and in part for some compounds by the contribution of transporter proteins [17]. By using the Oie–Tozer equation and estimates for ionization (pK_a), plasma protein binding (PPB) and lipophilicity (log $D^{7.4}$) quite robust predictions for the volume of distribution at steady state (V_{dss}), often within 2-fold of the observed value, can be made [18].

2.2.4
PPB and Physicochemical Properties

The percentage of binding to plasma proteins (PPB%) is an important factor in PK and is determinant in the actual dosage regimen (frequency), but not important for the daily dose size [3]. The daily dose is determined by the required free or unbound concentration of drug required for efficacy [3]. Lipophilicity is a major driver to PPB% [19, 20]. The effect of the presence of negative (acids) or positive (bases) charges has different impacts on binding to human serum albumin (HSA), as negatively charged compounds bind more strongly to HSA than would be expected from the lipophilicity of the ionized species at pH 7.4 [19, 20] (see Fig. 2.6).

2.3
Dissolution and 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 [21]. 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. Only compound 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 high-throughput solubility screens in early stages of drug design [22–26]. Excessive lipophilicity is a common cause of poor solubility and can lead to erratic and incomplete absorp-

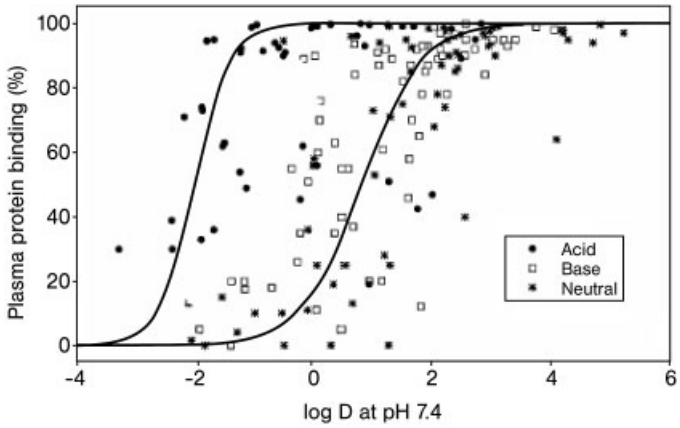

Fig. 2.6 Trendships between percentage human PPB (hPPB%) and octanol–water log $D^{7.4}$ [20]. Note the around 2 log units downshift of the sigmoidal relationship for acids as compared to neutrals and basics. (With kind permission of Springer-Kluwer.)

Tab. 2.1 Desired solubility ($\mu g\,mL^{-1}$) needed for expected doses [26].

Dose ($mg\,kg^{-1}$)	*Permeability*		
	High	*Medium*	*Low*
0.1	1	5	21
1	10	52	210
10	100	520	2100

tion 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 2.1 [26]. 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.

The concept of maximum absorbable dose (MAD) relates drug absorption to solubility via [27, 28]:

$$\mathrm{MAD} = S \times k_a \times \mathrm{SIWV} \times \mathrm{SITT} \tag{3}$$

where S = solubility ($mg\,mL^{-1}$) at pH 6.5, k_a = transintestinal absorption rate constant (min^{-1}), SIWV = small intestinal water volume (mL), assumed to be around 250 mL, and SITT = small intestinal transit time (min), assumed to be 4.5 h = 270 min.

Dissolution testing has been used as a prognostic tool for oral drug absorption [29]. A Biopharmaceutics Classification Scheme (BCS) has been proposed under which drugs can be categorized into four groups according to their solubility and permeability properties [30]. As 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 H-bonding [31]. The BCS has been adopted as a regulatory guidance for bioequivalence studies.

2.3.1 Calculated Solubility

As a key first step towards oral absorption, considerable effort went into the development of computational solubility prediction [32–39]. 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 [40]. Further fine-tuning of the models can be expected now high-throughput data has become available to construct such models. Models will be approximate since they do not take into account the effect of crystal packing, ionic force, type of buffer, temperature, etc. Solubility is typically measured in an aqueous buffer only partly mimicking the physiological state. More expensive fasted-state simulated artificial intestinal fluid (FaSSIF) solutions have been used to measure solubility, which in some cases appear to give better predictions in physiologically based pharmacokinetic (PBPK) modeling than solubility data using a simpler aqueous buffer [41].

2.4 Ionization (pK_a)

It was assumed for a long time that molecules can only cross a membrane in their neutral form. This dogma, based on the pH-partition theory, has been challenged [42, 43]. Using cyclic voltammetry it was demonstrated that compounds in their ionized form pass into organic phases and might well cross membranes in this ionized form [44].

The importance of drug ionization using cell-based methods such as Caco-2 in the *in vitro* prediction of *in vivo* absorption was discussed [45]. It was observed that when the apical pH used in Caco-2 studies was lowered from 7.4 to 6.0 a better correlation was obtained with *in vivo* data, demonstrating that careful selection of experimental conditions *in vitro* is crucial to produce a reliable model. Studies with Caco-2 monolayers also suggested that the ionic species might contribute considerably to overall drug transport [46].

Various ways that a charged compound may cross a membrane by a "passive" mechanism have been described [42]. These include transport as ion (*trans*- and/or paracellular), ion-pair or protein-assisted (using the outer surface of a protein spanning a membrane).

Therefore a continued interest exists in the role of pK_a in oral absorption, which often is related to its effect on lipophilicity and solubility. Medicinal chemists can modulate these properties through structural modifications [47]. Various methods to measure pK_a values have been developed [47–50] and considerable databases are now available.

The difference between the log *P* of a given compound in its neutral form (log P^N) and its fully ionized form (log P^I) has been termed *diff*(log P^{N-I}) and contains series-specific information, and expresses the influence of ionization on the intermolecular forces and intramolecular interactions of a solute [44, 51, 52].

2.4.1
Calculated pK_a

A number of approaches to predict ionization based on structure have been published (for a review, see [53]) and some of these are commercially available. Predictions tend to be good for structures with already known and measured functional groups. However, predictions can be poor for new innovative structures. Nevertheless, pK_a predictions can still be used to drive a project in the desired direction and the rank order of the compounds is often correct. More recently training algorithms have also become available which use in-house data to improve the predictions. This is obviously the way forward.

2.5
Molecular Size and Shape

Molecular size can be a further limiting factor in oral absorption [54]. The Lipinski "Rule-of-5" proposes an upper limit of molecular weight (MW) of 500 as acceptable for orally absorbed compounds [25]. High-MW compounds tend to undergo biliary excretion. 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 [55].

2.5.1
Calculated Size Descriptors

MW 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 properties are equally simple to calculate, and may offer a better guide to estimate 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 compounds which can access the brain ($A_D < 80\,Å^2$) of those that are too large to cross the blood–brain barrier (BBB) [55]. Similar studies have been performed to define a cut-off for oral absorption [56].

2.6 H-bonding

Molecular size and H-bonding have been unraveled as the two major components of log *P* or log *D* [57–59]. It was found that H-bonding capacity of a drug solute correlates reasonably well with passive diffusion. Δlog *P*, the difference between octanol–water and alkane–water partitioning, was suggested as a good measure for solute H-bonding [58, 60, 61]. However, this involves tedious experimental work and it appeared that calculated descriptors for H-bonding could most conveniently be assessed, in particular also for virtual compounds.

2.6.1 Calculated H-bonding descriptors

Considerable interest is focused on the calculation of H-bonding capability in the design of combinatorial libraries, for assessing the potential for oral absorption and permeability [16, 62–65]. A number of different descriptors for H-bonding have been discussed [66], one of the simplest being the count of the number of H-bond forming atoms [67].

A simple measure of H-bonding capacity, originally proposed by Van de Waterbeemd and Kansy, is the polar surface area (PSA), defined as the sum of the fractional contributions to surface area of all nitrogen and oxygen atoms and hydrogens attached to these [68]. PSA was used to predict passage of the BBB [69–71], flux across a Caco-2 monolayer [72] and human intestinal absorption [73, 74]. 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 [68] is based on a single minimum energy conformation. Others [73] 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, differing very little from the dynamic PSA described above [74]. A fast calculation of PSA as a sum of fragment-based contributions has been published [75], allowing use of these calculations for large datasets such as combinatorial or virtual libraries. The sigmoidal relationship can be described by $A\% = 100/[1 + (PSA/PSA_{50})^{\gamma}]$, where $A\%$ is percentage of orally absorbed drug, PSA_{50} is the PSA at 50% absorption level and γ is a regression coefficient [76].

Poorly absorbed compounds have been identified as those with a $PSA > 140\,Å^2$. Considering more compounds, considerable more scatter was found around the sigmoidal curve observed for a smaller set of compounds [74]. 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. These factors also con-

tribute to the considerable inter-individual variability of human oral absorption data. A further refinement in the PSA approach is expected to come from taking into account the strength of the H-bonds, which in principle already is the basis of the HYBOT approach [63–65].

2.7 Lipophilicity

Octanol–water partition (log *P*) and distribution (log *D*) coefficients are widely used to make estimates for membrane penetration and permeability, including gastrointestinal absorption [77, 78], BBB crossing [60, 69] and correlations to pharmacokinetic properties [1]. The two major components of lipophilicity are molecular size and H-bonding [57], which each have been discussed above (see Sections 2.5 and 2.6).

According to published International Union of Pure and Applied Chemistry recommendations the terms hydrophobicity and lipophilicity are best described as follows [79]:

- *Hydrophobicity* is the association of nonpolar groups or molecules in an aqueous environment which 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 system).

The *intrinsic lipophilicity* (*P*) of a compound refers only to the equilibrium of the unionized (neutral) drug between the aqueous phase and the organic phase. It follows that the remaining part of the overall equilibrium, i.e. 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:

$$D = [HA]_{organic}/([HA]_{aqueous} + [A^-]_{aqueous}) \quad (4)$$

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:

$$K_a = [H^+][A^-]/[HA] \tag{5}$$

sometimes referred to as the Henderson–Hasselbalch relationship. The combination of Eqs. (4)–(6) gives the pH-distribution (or "pH-partition") relationship:

$$D = P/(1 + \{K_a/[H^+]\}) \tag{6}$$

more commonly expressed for monoprotic organic *acids* in the form:

$$\log(\{P/D\} - 1) = \mathrm{pH} - \mathrm{p}K_a \tag{7}$$

or

$$\log D = \log P - \log(1 + 10^{\mathrm{pH}-\mathrm{p}K_a}) \tag{8}$$

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

$$\log(\{P/D\} - 1) = \mathrm{p}K_a - \mathrm{pH} \tag{9}$$

or:

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

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 approximately 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. Obviously for compounds with multifunctional ionizable groups the relationship between log *P* and log *D*, as well as log *D* as a function of pH become more complex [65, 68, 70]. For diprotic molecules there are already 12 different possible shapes of log *D*–pH plots.

Traditional octanol–water distribution coefficients are still widely used in quantitative structure–activity relationship (QSAR) and in ADME/PK studies. However, alternative solvent systems have been proposed [80]. To cover the variability in biophysical characteristics of different membrane types a set of four solvents has been suggested, sometimes called the "critical quartet" [81]. The 1,2-dichloroethane–water system has been promoted as a good alternative to alkane–water due to its far better dissolution properties [82, 83], but may find little application because of its carcinogenic properties.

Several approaches for higher throughput lipophilicity measurements have been developed in the pharmaceutical industry [50] including automated shake-plate methods [84] and immobilized artificial membranes [85]. A convenient method to

measure octanol–water partitioning is based on potentiometric titration, called the pH method [86]. Methods based on chromatography are also widely used, e.g. chromatographic hydrophobicity indices measured on immobilized artificial membranes (IAM) [19, 87]. Another chromatography-based method is called Elog *D* giving log *D* values comparable to shake-flask data [88].

2.7.1 Calculated log *P* and log *D*

A number of rather comprehensive reviews on lipophilicity estimation have been published and are recommended for further reading [89–91]. Due to its key importance, a continued interest is seen to develop good log *P* estimation programs [82–94]. Most log *P* approaches are limited due to a lack of parameterization of certain fragments. For the widely used CLOGP program (Daylight/Biobyte computer program for the calculation of log *P*), a version making estimates for missing fragments has become available [95].

With only few exceptions, most log *P* programs refer to the octanol–water system. Based on Rekker's fragmental constant approach, a log *P* calculation for aliphatic hydrocarbon–water partitioning has been reported [96]. Another more recent approach to alkane–water log *P* and log *D* is based on the program VolSurf [97]. It is believed that these values may offer a better predictor for uptake in the brain. The group of Abraham investigated many other solvent systems and derived equations to predict log *P* from structure for these solvent systems, which are also commercially available [94].

Log *D* predictions are more difficult as most approaches rely on the combination of estimated log *P* and estimated pK_a. Obviously, this can lead to error accumulation and errors of 2 log units or more can be found. Some algorithms, however, are designed to learn from experimental data so that the predictions improve over time. An interesting approach is also the combination of a commercial log *D* predictor with proprietary descriptors using a Bayesian neural network approach [98].

2.8 Permeability

An overview of permeability assays is presented in Table 2.2. As discussed earlier in this chapter, these permeability scales are correlated to each other as well as the various lipophilicity scales via extended Collander equations.

2.8.1 Artificial Membranes and PAMPA

When screening for absorption by passive membrane permeability, artificial membranes have the advantage of offering a highly reproducible and high-throughput system. Artificial membranes have been compared to Caco-2 cells and for passive

Tab. 2.2 *In vitro* models for membrane permeability.

Permeability model	*Reference*
Solvent–water partitioning	
octanol–water distribution	52
Chromatography	
IAM	99–103
ILC	104
MEKC	105
BMC	106
Vesicles	
phospholipid vesicles	107
liposome binding	108, 109
Transil particles	110–112
fluorosomes	113
SPR biosensor	114, 115
colorimetric assay	116
Artificial membranes	
impregnated membranes	72
PAMPA	117–123
filter IAM	121–123
hexadecane-coated polycarbonate filters (HDM)	124, 125
Other	
surface activity	126
Cell-based assays	
Caco-2	76, 78
Madin-Darby canine kidney	127

diffusion found to behave very similar [72]. This finding was the basis for the development of the PAMPA for rapid prediction of transcellular absorption potential [117–120]. In this system the permeability through a membrane formed by a mixture of lecithin and an inert organic solvent on a hydrophobic filter support is assessed. Whilst not completely predictive for oral absorption in humans, PAMPA shows definite trends in the ability of molecules to permeate membranes by passive diffusion, which may be valuable in screening large compound libraries. This system is commercially available [121], but can easily be set up in-house. Further optimization of the experimental conditions has been investigated, concluding that predictability increases when a pH of 6.5 or 5.5 is used on the donor side [122, 123]. It was also demonstrated that the effect of a cosolvent such as dimethylsulfoxide (DMSO) could have a marked effect depending on the nature, basic/acid, of the compound [123]. Stirring of the donor compartment to limit the contribution of the unstirred water layer appears to be important to get meaningful results. There have been so far no reports in the literature about using PAMPA data in a drug discovery project.

A similar system has been reported based on polycarbonate filters coated with hexadecane, also called hexadecane membranes (HDM) [124, 125]. Thus, this

system consists of a 9- to 10-μm hexadecane liquid layer immobilized between two aqueous compartments. Also here it was observed that in this set-up for lipophilic compounds the diffusion through the unstirred water layer becomes the rate-limiting step. To mimic the *in vivo* environment permeability measurements were repeated at different pH values in the range 4–8 and the highest transport value used for correlation with percentage absorbed in human. This gives a sigmoidal dependence, which is better than when taking values measured at a single pH, e.g. 6.8.

2.8.1.1 *In Silico* PAMPA

The experimental P_{app} data have been used to build predictive models. However, since PAMPA is already a model, an *in silico* model based on this is a model of a model. The predictability for *in vivo* permeability or absorption of such *in silico* PAMPA model can be questioned (see Eq. 11), since it is two steps from reality:

$$\text{model} \times \text{model} = \text{random} \tag{11}$$

2.8.2 IAM, Immobilized Liposome Chromatography (ILC), Micellar Electrokinetic Chromatography (MEKC) and Biopartitioning Micellar Chromatography (BMC)

IAM columns are another means of measuring lipophilic characteristics of drug candidates and other chemicals [99–103]. IAM columns may better mimic membrane interactions than the isotropic octanol–water or other solvent–solvent partitioning system. These chromatographic indices appear to be a significant predictor of passive absorption through the rat intestine [128].

A related alternative is called ILC [104, 105]. Compounds with the same log *P* were shown to have very different degrees of membrane partitioning on ILC depending on the charge of the compound [105].

Another relatively new lipophilicity scale proposed for use in ADME studies is based on MEKC [106]. A further variant is called BMC and uses mobile phases of Brij35 [polyoxyethylene(23)lauryl ether] [129]. Similarly, the retention factors of 16 β-blockers obtained with micellar chromatography with sodium dodecyl sulfate as micelle-forming agent correlates well with permeability coefficients in Caco-2 monolayers and apparent permeability coefficients in rat intestinal segments [130].

Each of these scales produce a lipophilicity index related but not identical to octanol–water partitioning.

2.8.3 Liposome Partitioning

Liposomes, which are lipid bilayer vesicles prepared from mixtures of lipids, also provide a useful tool for studying passive permeability of molecules through lipid. This system has, for example, been used to demonstrate the passive nature of the absorption mechanism of monocarboxylic acids [131]. Liposome partitioning of

ionizable drugs can be determined by titration and has been correlated with human absorption [108, 109, 132]. Liposome partitioning is only partly correlated with octanol–water distribution and might contain some additional information.

A further partition system based on the use of liposomes, and commercialized under the name Transil [110, 111], has shown its utility as a lipophilicity measure in PBPK modeling [112]. Fluorescent-labeled liposomes, called fluorosomes, are another means of measuring the rate of penetration of small molecules into membrane bilayers [113, 120]. Similarly, a colorimetric assay amenable to HTS for evaluating membrane interactions and penetration has been presented [116]. The platform comprises vesicles of phospholipids and the chromatic lipid-mimetic polydiacetylene. The polymer undergoes visible concentration-dependent red–blue transformations induced through interactions of the vesicles with the studied molecules.

2.8.4
Biosensors

Liposomes have been attached to a biosensor surface, and the interactions between drugs and the liposomes can be monitored directly using surface plasmon resonance (SPR) technology. SPR is measuring changes in refractive index at the sensor surface caused by changes in mass. Drug–liposome interactions have been measured for 27 drugs and compared to fraction absorbed in humans [114]. A reasonable correlation is obtained, but it is most likely that this method represents just another way of measuring "lipophilicity". The throughput was 100 substances per 24 h, but further progress seems possible. In more recent work using this method it is proposed to use two types of liposomes to separate compounds according to their absorption potential [115].

2.9
Amphiphilicity

The combination of hydrophilic and hydrophobic parts of a molecule defines its amphiphilicity. A program has been described to calculate this property and calibrated against experimental values obtained from surface activity measurements [133]. These values can possibly be used to predict effect on membranes leading to cytotoxicity or phospholipidosis, but may also contain information, not yet unraveled, on permeability. Surface activity measurements have also been used to make estimates of oral absorption [126].

2.10
Drug-like Properties

The various properties described above are important for drugs, in particular for those given orally. The important question arises whether such properties of drugs

are different from chemicals used in other ways. This has been subject of a number of investigations [134, 135]. Using neural networks [136, 137] or a decision tree approach [138], a compound can be predicted as being "drug-like" with an error rate of around 20%. A further approach to predict drug-likeness consists of training of the program PASS (prediction of activity spectra for substances) [139], which originally was intended to predict activity profiles and thus is suitable to predict potential side effects.

From an analysis of the key properties of compounds in the World Drug Index the now well accepted "Rule-of-5" has been derived [25, 26]. It was concluded that compounds are most likely to have poor absorption when MW > 500, calculated octanol–water partition coefficient Clog $P > 5$, number of H-bond donors >5 and number of H-bond acceptors >10. Computation of these properties is now available as a simple but efficient ADME screen in commercial software. The "Rule-of-5" should be seen as a qualitative absorption/permeability predictor [43], rather than a quantitative predictor [140]. The "Rule-of-5" is not predictive for bioavailability as sometimes mistakenly is assumed. An important factor for bioavailability in addition to absorption is liver first-pass effect (metabolism). The property distribution in drug-related chemical databases has been studied as another approach to understand "drug-likeness" [141, 142].

Other attempts have been made to try to define good leads. In general lead-like properties are lower/smaller than drug-like properties. Thus, MW < 350 and Clog $P < 3$ should be good starting points for leads [143, 144]. A "Rule-of-3" has been proposed [145] for screening of small fragments, which says the good lead fragments have MW < 300, Clog $P < 3$, H-bond donors and acceptors <3 and rotatable bonds <3.

Similarly, in a study on drugs active as central nervous system (CNS) agents and using neural networks based on Bayesian methods, CNS-active drugs could be distinguished from CNS-inactive ones [145]. A CNS rule-of-thumb says that if the sum of the nitrogen and oxygen (N + O) atoms in a molecule is less than 5 and if the Clog $P - (N + O) > 0$, then compounds are likely to penetrate to the BBB [146]. Another "rule" is PSA < 90 Å^2, MW < 450 and log D at pH 7.4 of 1–3 [147]. In designing CNS drugs it is important to distinguish BBB penetration and CNS efficacy. The latter is a subtle balance between permeability, effect of BBB transporters, lipophilicity, and free fraction in blood and brain [148].

These aforementioned analyses all point to a critical combination of physicochemical and structural properties [149], which to a large extent can be manipulated by the medicinal chemist. This approach in medicinal chemistry has been called property-based design [2]. Under properties in this context we intend physicochemical as well as PK and toxicokinetic properties. These have been neglected for a long time by most medicinal chemists, who in many cases in the past only had the quest for strongest receptor binding as the ultimate goal. However, this strategy has changed dramatically, and the principles of drug-like compounds are now being used in computational approaches towards the rational design of combinatorial libraries [150] and in decision making on acquisition of outsourced libraries.

2.11 Computation versus Measurement of Physicochemical Properties

2.11.1 QSAR Modeling

Calculation of many different one-, two- and three-dimensional descriptors for building predictive QSAR models for physicochemical (and ADME/toxicity) properties is possible using a range of commercially available software packages, such as ACD, SYBYL, Cerius^2, Molconn-Z, HYBOT, VolSurf, MolSurf, Dragon, MOE, BCUT, etc. Several descriptor sets are based on quantification of three-dimensional molecular surface properties [151, 152] and these have been explored for the prediction of, for example, Caco-2 permeability and oral absorption [16]. It is pointed out here that a number of these "new" descriptors are often strongly correlated to the more traditional physicochemical properties. An aspect largely neglected so far is the concept of molecular-property space looking at the conformational effects on physicochemical properties [153].

Numerous QSAR tools have been developed [152, 154] and used in modeling physicochemical data. These vary from simple linear to more complex nonlinear models, as well as classification models. A popular approach more recently became the construction of consensus or ensemble models ("combinatorial QSAR") combining the predictions of several individual approaches [155]. Or, alternatively, models can be built by running the same approach, such as a neural network of a decision tree, many times and combining the output into a single prediction.

To build robust predictive models good quality training set and sound test set are required. Criteria for a good set include sufficient coverage of chemical space, good distribution between low- and high-end values of the property studied, and a sufficiently large number of compounds. Models can be global (covering many types of chemistry) or local (project-specific). There are many reasons why predictions can fail [156] and medicinal chemists need to be aware of these. There is also a difference between a useful model and a perfect model. The latter does not exist!

In-house physicochemical data collections are growing rapidly through the use of HTS technologies [157]. Therefore, the need for rapidly building and updating is also increasing. Systems for automatic and regular updating of QSAR predictive models have been reported [158] and we expect these to become more widespread. A consequence of regularly updated *in silico* models is that the predicted values will change too. This will require adapted ways of working by the chemists and DMPK scientists in projects using more dynamic data generation and interpretation tools.

2.11.2 *In Combo*: Using the Best of two Worlds

In modern drug discovery speed and cost control are important in addition to high quality. *In silico* virtual screening for drugability [159] is a good first step in library

design and compound acquisition. Once compounds have been made for a targeted project a well-balanced approach using both *in silico* predictions and *in vitro* screening will be a good strategy to guide the programme in a cost-efficient manner. New experimental data can be used to update predictive models regularly so that the ongoing projects can benefit from the latest local and global models available [158, 160].

2.12 Outlook

Physical chemistry plays a key role in the behavior of drugs. Measurement of the key properties has been automated and industrialized to high throughput. The data can and are used to build robust predictive models. These can in turn be used to limit the use of experiments when not strictly needed. This is of course compound saving and more cost-effective. Predictive models are also great tools in virtual screening, prioritization decision making and guiding projects. The rest of this book provides in-depth insight into some of the properties briefly discussed in this introductory chapter.

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