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Review

Physicochemical profiling in drug research: a brief survey of the state-of-the-art of experimental techniques

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Abstract. This review begins with a general presentation of the new paradigm of drug discovery, with its emphasis on the rapid identification and elimination of compounds with unsuitable physicochemical and pharmacokinetic properties. The focus of the paper is on the various experimental methods used to determine such key physicochemical properties as ionization, lipophilicity and distribution in isotropic and anisotropic systems, solubility, and permeability across artificial membranes. Both traditional and high-throughput methods are presented and their limits highlighted. The text concludes with the trade-off between quantity/speed in high-throughput screening techniques versus greater data quality in the more labor-intensive methods.

Key words. Ionization; lipophilicity; distribution; solubility; permeability; screening; high-throughput method.

Physicochemical profiling in drug discovery

The 'new' drug research

The last years have seen remarkable advances in drug research, impressive developments in drug design, and a phenomenal explosion in drug discovery. To illustrate the discussion, we offer figure 1 as a schematic representation of drug research and development, beginning with 'hits' as the end-points of the new armamentarium [virtual screening, library screening, combinatorial chemistry and high-throughput (HT) screening (HTS) methodologies] [1]. In lead discovery and optimization, the traditional synergy between synthesis and rational design remains as fruitful as ever, particularly since its efficacy has been greatly enhanced by modern tools such as three-dimensional quantitative structure-activity relationships (3D-QSARs) and molecular modeling. In the very stringent preclinical and clinical phases, only a minuscule proportion of candidates will eventually emerge and reach the market.

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For decades, biological activity was equated almost exclusively with a pharmacodynamic (PD) response, i.e., the effects elicited in a biological system by a drug or any other xenobiotic. These effects include, among others, activation (agonism) or blockade (antagonism) of receptors, inhibition of enzymes, binding to nucleic acids, and stimulation or blockade of a functional biological response.
Progressively, a paradigm shift has taken place, such that the notion of biological response has been expanded to include also the pharmacokinetic (PK) response, i.e., all effects and influences a biological system exerts on a xenobiotic [2]. For many years, these effects have remained a lesser explored territory of drug design. More recently, however, the bottleneck of drug research has shifted from hit-and-lead discovery to lead optimization, and more specifically to PK lead optimization [3, 4]. Some major reasons for this state of affairs are (i) the imperative to reduce as much as feasible the extremely costly rate of attrition prevailing in the preclinical and clinical phases of drug development [4], and (ii) more stringent concerns for safety.

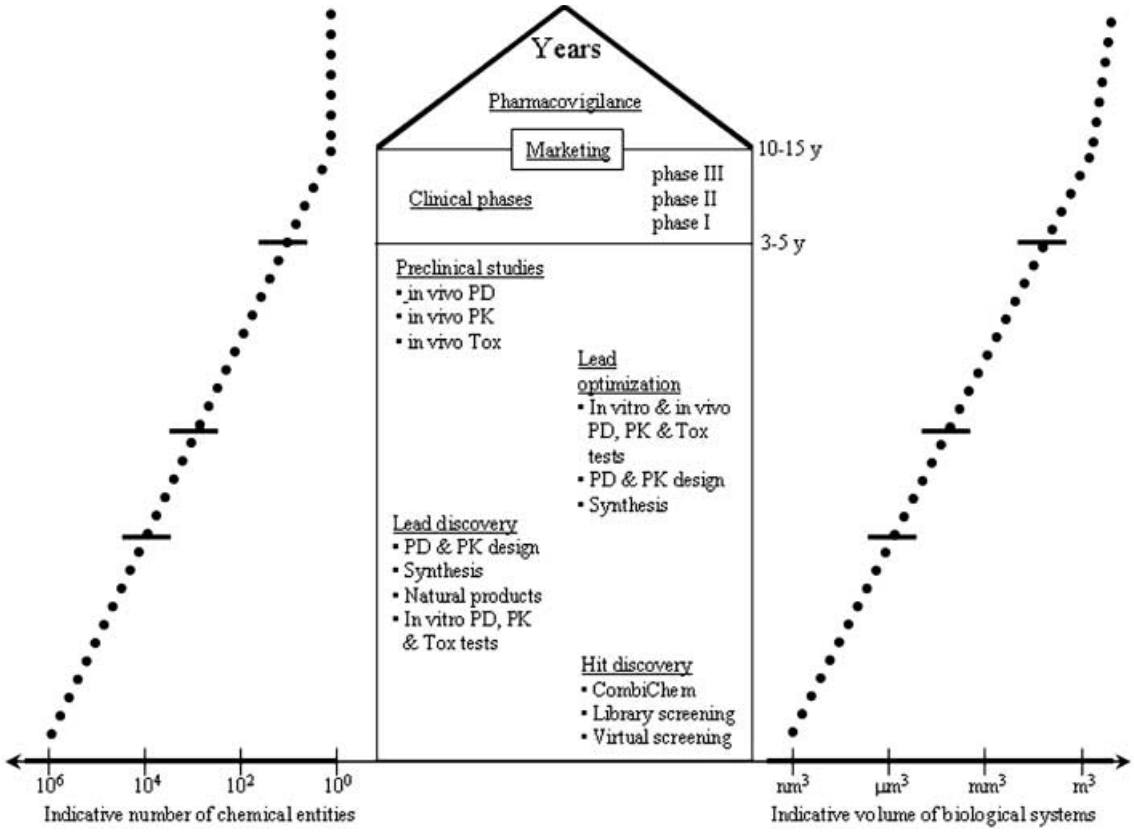

Figure 1. Highly schematic representation of the long road from hits and active compounds to actual (i.e., therapeutically used) drugs. The vertical axis is approximately ⟨log years⟩. PD, pharma codynamics; PK, pharmaco kinetics.

Predicting pharmacokinetic behavior

As a result of this (r)evolution, medicinal chemists now find themselves trying to relate molecular structure to the various components of the PK response, and have discovered that the actions of the body on a drug can be as varied and complex as the actions of a drug on the body.

1) At the *molecular level*, the PK response includes (i) low-energy processes such as membrane permeation, reversible binding to circulating and tissue-bound macromolecules, and uptake by transporters, and (ii) high-energy processes involving the cleavage and formation of covalent bonds, in other words biotransformation (metabolism) to form reactive or stable metabolites.
2) At the *level of the organism*, the PK response comprises all processes of drug disposition, namely absorption, distribution, storage, metabolism (chemical elimination), and excretion (physical elimination) [5].

The search for lead compounds and candidates with good PK properties coupled to outstanding PD effects has changed the priorities in drug discovery. Indeed, these two objectives may or may not be compatible within a given chemical series, a situation that should be clarified as early as possible in any project. Perhaps more importantly, project leaders must avoid investing too much time and resources in the development of active compounds ultimately doomed by PK defects. Early PK profiling of lead candidates is thus an imperative.

The major and obvious strategy for early PK profiling is in vitro biological screening using such tools as cell cultures (permeation, active transport), enzymatic preparations (metabolism, enzyme inhibition), and serum proteins (binding). PK screening, however, would be worthless if not complemented by physicochemical profiling, for two good reasons.

The first is that a physicochemical property such as solubility is an intrinsic determinant of absorption, as shown by the Biopharmaceutic Classification System (BCS) [6] which recognizes four types of bioavailability profile depending on whether solubility and membrane permeability are high or low.

A second reason, and a most relevant one in the present context, is that a number of physicochemical and structural properties are known to have a major influence on PK behavior and are therefore predictors of it [7–12]. In other words and in practical terms, very early determination of some relevant physicochemical properties may allow a fair prediction of the PK behavior of a new chem-

ical entity. These properties are ionization (i.e., acidity and basicity), water solubility, lipid/water partitioning (lipophilicity), and membrane/water permeability. Other molecular properties of significance in drug research include stereochemical attributes and chemical stability. These, however, lie outside the scope of this review.

Such early physicochemical determinations must consume as little test material as possible, have HT, and be reasonably reliable without the need for high precision and accuracy. Two complementary approaches are open to medicinal chemists in this quest, namely experimental and virtual profiling. Experimental techniques are the scope of this survey, from the traditional ones to HTS. Some of their characteristics and advantages will be presented.

There are other important physicochemical properties, such as compound stability, safety, and integrity of compound libraries over long-term storage (in DMSO). These are receiving increasing attention in early discovery, and a recent review has addressed this [12]. However, these latter topics are beyond the scope of the present review.

Experimental determination of ionization constants (pK_a)

The pK_a of an ionizable compound is used to calculate the electrical state of the molecule at a particular pH. This is an all important property, since the charge state dramatically affects lipophilicity and PK behavior, not to mention affinity for drug-metabolizing enzymes and drug targets. The method of choice for the determination of ionization constants is potentiometry, but various circumstances can warrant the determination of pK_a by UV spectrophotometry or capillary electrophoresis (CE). In principle, UV and CE methods are more sensitive and less sample demanding than the pH-metric method. However, the latter method is often preferred because it is much better developed, and is very strongly supported commercially. Many other pK_a techniques have been used, but the above methods are best suited for pharmaceutical applications.

Potentiometric determinations: the workhorse method

The determination of pK_a values by the potentiometric (pH-metric) method is well-know to analytical chemists. Briefly, a precisely known volume of a standardized acid (e.g., 0.5 M HCl) or base (e.g., 0.5 M KOH or NaOH) are added to a vigorously stirred 2–20 ml solution of an ionizable compound (100 – 500 μM), during which the pH is continuously measured with a precision combination glass electrode, in a procedure covering an appropriate portion of the accessible pH 1.5–12.5. Often, the ionic strength is adjusted to a physiologically relevant 0.15 M (using NaCl or KCl) and the temperature is maintained constant at 25 °C, since pK_as depend on both conditions. Frequently, the aqueous solution (not containing added pH buffer) is mixed with an organic cosolvent, such as methanol or DMSO, and corrections for the effect are usually considered. Insoluble compounds require cosolvent use. Modern methods take advantage of Bjerrum plot analysis [13] in processing the data.

There are, however, a number of practical limits to the method that cannot be ignored. Thus, low water solubility can be a serious limitation. Other problems are the relatively high material consumption and low throughput of the traditional instrumentation. However, recent theoretical and experimental advances have gone a long way to help medicinal chemists push back these boundaries.

Thus, the problem of low solubility can often be solved by potentiometry using a mixed solvent approach. For example, the pK_a of the antiarrhythmic amiodarone, 9.06 ± 0.14, was determined from water-methanol mixtures, although the intrinsic solubility of the molecule is < 0.01 μM [13]. Mixed-solvent solutions (employing e.g., methanol, DMSO, dioxane) of various cosolvent-water proportions are titrated and psKa (the apparent pK_a) is determined in each mixture. The aqueous pK_a is deduced by extrapolation of the p_sK_a values to zero cosolvent. A more linear extrapolation is effected by the Yasuda-Shedlovsky procedure, a method that is widely used to determine the pK_a value of very sparingly soluble pharmaceutical compounds [14].

As the dielectric constant of the solvent mixture decreases, the apparent pK_a of an acid increases and that of a base decreases. In a multiprotic molecule, this can be a useful property in identifying the ionization groups. The dielectric constant for pure methanol is about 32, the same value associated with the surface of phospholipid bilayers in membranes [13], and this suggesting a possible predictive value of extrapolating to 100% methanol.

UV determination of pK_a for lower sample consumption

The most reliable spectrophotometric procedures for pK_a determination are based on the processing of whole absorption curves over a broad range of wavelengths, with data collected over a suitable range of pH. Most of the approaches are based on mass balance equations incorporating absorbance data and pH as dependent variables, wavelengths and titrant volumes as independent variables, and equilibrium constants and individual-species molar absorptivities at various wavelengths as parameters, determined by nonlinear least-squares refinement. Since more than one species may have nearly identical molar ab-

sorptivity curves, methods have been devised to determine the number of spectrally active components. With ill-conditioned equations, damping procedures are required. Gampp and coworkers [15] considered principal component analysis (PCA) and evolving factor analysis (EFA) methods in deciding the presence and stoichiometries of the absorbing species.

Tam and coworkers [16–18] developed a very effective generalized method, using diode-array UV spectrophotometry, coupled to an automated pH titrator. Species selection was effected by target factor analysis (TFA). Multiprotic compounds with overlapping pK_a values were investigated [17]. Binary mixtures of ionizable compounds were considered. Determination of microconstants has been reported. The use of cosolvents allowed the deconvolutions of 12 microconstants of cetirizine, a three-pK_a molecule [18].

HTS pK_a determinations

A 96-well microtiter plate HT method, called spectral gradient analysis (SGA) and based on a pH gradient flow technique with diode-array UV detection, has recently been reported [19]. A universal buffer has been developed and can produce a pH gradient that is very linear in time. Similar flow-stream universal buffers have also been developed for determining kinetic parameters associated with pH-dependent hydrolysis degradations of drug substances [20].

pK_a Determination by CE with ultra-low sample consumption

CE has the advantage of being a universal method since different detection systems can be used [21, 22]. Being a separation technique, sample impurities are not generally a problem. A fused-silica capillary is filled with a dilute aqueous buffer solution. About 10 nl of a sample solution, whose concentration is about 50 µM, are gathered at one end of the capillary, and a 30-kV potential is applied between the ends of the capillary dipped into each of two buffer beakers. Sample consumption is roughly 0.2 ng per injection. Sample species migrate according to their charge and fluid drag. The mobility of ionizable compounds depends on the fraction of the compound in charged form. This in turn depends on the pK_a. The plot of the apparent mobility versus pH has a sigmoidal shape, with the midpoint pH equal to the pK_a. The practical range for buffer pH in CE is between 3 and 11. When UV detection is used, the limit of detection for a molecule having the molar absorptivity of benzoic acid at 220 nm is about 2 µM. This method has allowed the determination of the pK_a values of multiprotic molecules by CE, one molecule having seven ionization groups [21].

Experimental determination of lipophilicity (log k′, log P, log D)

Solvent/water partitioning

Solvent/buffer partitioning by a traditional shake-flask technique remains the method of reference. Two lipophilicity parameters can be determined:

1) the partition coefficient expressed as log P, which is valid for a single electrical species, often but not always the neutral form (i.e., log P^N);
2) the distribution coefficient expressed as log D^{pH}, which is pH dependent for ionizable solutes and results from the weighted contributions of all electrical forms present at this pH.

In such systems, the two liquid phases are isotropic. One is a buffered aqueous solution, the other a water-immiscible organic solvent. n-Octanol is often used as the prototypical H-bond donor and acceptor solvent. Non-H-bonding solvents include alkanes or 1,2-dichloroethane. Lipophilicity parameters measured in different solvent/water systems express different balances of hydrophobic forces, dipolarity/polarizability, and hydrogen bonding, as demonstrated by so-called solvatochromic analyses [23–25].

pH-metric method

The dual-phase titration method goes back five decades [26] and consists of two linked titrations. Typically, a preacidified solution of a weak acid is titrated with standardized KOH to some appropriately high pH; octanol or another solvent is then added, and the dual-solvent mixture is titrated with standardized HCl back to the starting pH. After each titrant addition, the pH is measured. If the ionizable substance partitions into the octanol phase, the two assays show nonoverlapping normalized titration curves. The greatest divergence between the two curves occurs in the buffer region. A large difference between the true pK_a and the apparent pK_a indicates a large value of log P. Determinations of log P values as low as –2 and as high as +8 have been documented. For ionizable solutes, a major advantage of the method over the shake-flask procedure is that it affords a distribution profile (i.e., a pH-log D curve) rather than single points [27].

The lipophilicity of ionized species

If within the measurable window, the log P value of ionized species (i.e., log P^I) can be determined by the shake-flask or the pH-metric method. However, the nature and concentration of counterions markedly influence the results, which must be seen as mere apparent values. Cyclic voltammetry (CV) is the only method able to yield standard log P^I values, namely true log PI values independent of the Galvani potential [28, 29]. CV has the additional

advantage of having a very broad window, with partition coefficients reported as low as −9. The disadvantage of the method is the severe restriction on suitable organic solvents. In fact, only the 1,2-dichloroethane/buffer system is used in CV log P measurements.

HTS log P methods

Several efforts are being made to increase the throughput of the traditional log P methods, by scaling down to a 96-well microtiter plate format [30]. Parallel methods using scanning 96/384-well plate UV spectrophotometers are inherently faster. They will become 50-fold faster with the imminent introduction of diode-array plate readers.

Liposomes/buffer partitioning and other anisotropic biphasic systems

Liposomes, which are vesicles formed from phospholipid bilayers, have been used as models of biological membranes. The method requires that the sample be equilibrated with a suspension of liposomes, followed by a separation procedure, before the sample is quantitated in the fraction free of the lipid component. Ultrafiltration/centrifugation or equilibrium dialysis can be used to separate the drug-laden liposomes from the aqueous solution [31]. A recently reported HT method based on phospholipid-impregnated porous resin may speed things up [32]. Drug samples are allowed to equilibrate with the suspended particles and then the solution is simply filtered. The filtrate is assayed for the unbound sample. The pH-metric method, which also requires no phase separation, has been used to determine drug-liposome partitioning [33].

CE has been used to determine partition coefficients [34, 35]. Lipid vesicles or micelles are added to the buffer whose pH is adjusted to different values. Since drug molecules partition to different extents as a function of pH, analysis of mobility versus pH data yields log P values.

Liquid chromatography techniques

Reversed-phase (RP)-HPLC techniques, first described by Mirrlees et al. [36] and Unger et al. [37], remain popular methods for determining log P. The directly measured retention parameters (capacity factors) are lipophilicity indices in their own right, but they can also be converted to a log P scale through the use of standards and correlation equations, and they contain comparable information on intermolecular interactions [38]. The newest variants, breadths of scope and limitations have been recently reviewed. The generic fast-gradient RP-HPLC methods look promising [39]. Methods based on octanol-coated resins with eluants saturated with octanol have been refined [40, 41], and a commercially supported instrument based on a similar technique is expected to be launched shortly.

An HPLC method was developed by Pidgeon and coworkers [42], where silica resin beads were modified by covalent attachment of phospholipid-like groups to the surface. These immobilized artificial membranes (IAMs) yield retention parameters that mimic the partitioning of drugs into phospholipid bilayers. The topic has been well reviewed [43].

Experimental determination of solubility

Saturation shake-flask methods

Classical approaches for measuring solubility are based on the saturation shake-flask method. The techniques are slow and not easily adapted to the HT needs of modern drug discovery research, and so new methods have been developed. Solubility measurement at a single pH under equilibrium conditions is largely a labor-intensive but straightforward procedure, requiring long equilibration times (up to 7 days). The analyte is added to a standard buffer solution until saturation occurs, indicated by undissolved excess drug. The thermostated saturated solution is shaken as equilibration between the two phases establishes. After microfiltration or centrifugation, the concentration of the substance in the supernatant solution is determined. If a solubility-pH profile is required, the measurements need to be performed in several different pH buffers. Care must be taken to avoid interferences due to buffer components.

Complications in measurement of aqueous solubility

Solubility reactions, being heterogeneous, are often slow. Furthermore, surface-active compounds, when dissolved in water under conditions of saturation, form self-associated aggregates which can perturb the determination of the true aqueous solubility. Use of cosolvents such as DMSO can offset these effects. However, the presence of these solvents can in some cases interfere with determination of the true aqueous solubility. If measurements are done in the presence of simple surfactants, bile salts, complexing agents such as cyclodextrins, or 'ion-pair forming' counterions, complicated experimental designs and computations need to be applied in attempting to extract the true aqueous solubility from the data [13, 44].

Turbidimetric ranking assays

Turbidity detection-based methods popularized by Lipinski and others [45] have partly met some HT needs of drug discovery research. In the analyte addition method, small aliquots of a DMSO solution of drug are added at 1-min intervals to a pH 7 buffer solution. If turbidity is detected, a few additional aliquots are added, and the plot of volume versus turbidity is back extrapolated to zero turbidity, to

indicate the point of the onset of precipitation. About 50–300 compounds may be analyzed each day. The selected upper limit of the method reflects that 87% of marketed drugs have a solubility greater than 65 µg/ml. Solubilities below this value are the problematic ones. An improved procedure with an expanded solubility range has been described. However, the presence of DMSO exaggerates solubility to an unknown extent.

The turbidity approaches, although not strictly rigorous, are an attempt to rank compounds according to predicted solubilities. Usually, the measurements are done at one pH. Various implementations of the basic method are practiced using custom-built equipment. Detection systems based on 96-well microtiter plate nephelometers are well established. An automated solubility analyzer incorporating such a detector usually requires the user to develop an appropriate chemistry procedure and to integrate a robotic fluidic system in a customized way. The possible shortcomings of the turbidity methodology are (i) poor reproducibility for very sparingly water soluble compounds, (ii) use of excessive amounts of DMSO (5% v/v in some implementations, compared to 0.2% v/v in others), and (iii) the apparent lack of standardization. Although such standardization may not be implemented in discovery, most practitioners would find it desirable.

HPLC- and LC/MS-based assays

In an effort to increase throughput, several pharmaceutical companies have transferred the classical saturation shake-flask method to 96-well plate technology using a robotic liquid-dispensing system [46]. Analyses are performed with fast generic gradient RP-HPLC. Data handling and report generation are often the rate-limiting steps in the operations. The above procedure has been adapted to fast LC/MS detection (3 min/well). Ultrafiltration is used to separate the solid, micelles, and high-molecular components. The throughput is about 200 compounds per day [47].

Modified-equilibrium method

A method has been described where 1 mg of solid is weighed into a filter chamber and a small volume of aqueous buffer is added. Filter caps are firmly attached and the vials are shaken in an HPLC sampling block for 24 h. The filter-containing cap compartments are then depressed to effect separation of the solid, and the top compartment solutions are analyzed by HPLC. About 50 compounds can be processed per day [48].

Potentiometric method

A novel approach, called dissolution template titration (DTT), has been recently introduced [49, 50]. Such a procedure has been cited by the FDA as being acceptable for applications for assessing solubility in the BCS [51]. The procedure takes as input parameters the pK_a and log P_{oct}. The latter parameter is used to estimate the intrinsic solubility using the Hansch-type expression. Using the pK_a and the estimated intrinsic solubility, the DTT procedure simulates the entire titration curve *before* the assay begins. The simulated curve serves as an assay procedural template. Titration of the sample suspension is done in the direction of dissolution, even well past the point of complete dissolution, at a cautious data acquisition rate not exceeding that predicted by the classical Noyes-Whitney expression. Typically, 3–10 h are required for an entire solubility-pH profile. Although the potentiometric method can be used in discovery settings to calibrate HT solubility methods and computational procedures, it is too slow for direct HTS applications. In fact, the procedure described in the next section was 'calibrated' in just such a manner.

Fast UV plate spectrophotometer method

An HT method using a 96-well microtiter plate format and plate UV spectrophotometry has been recently described [13, 44]. Solubilities at a single or more pH values can be determined. A known quantity of sample (either as dry powder or as DMSO solution) is added to a known volume of a universal buffer solution of known pH. The amount of sample must be sufficient to cause precipitation to occur in the solution. After waiting a period of time to allow the saturated solution to reach equilibrium, the solution is filtered to remove the solid. But before the UV spectra are taken, a quantity of a water-miscible cosolvent is added to the sample solution. Suitable cosolvents are ones with the lowest vapor pressure, the greatest capability in dissolving a solute, and the lowest UV absorption. The spectrum of the solution is then taken by the UV spectrophotometer. For the reference solution, a known quantity of sample is added to a known volume of system solution of known pH, with the amount of sample being comparable to that found in the sample plate, and no effort is made in this step to suppress precipitation in the formed solution, nor is the solution filtered. A volume of the cosolvent is then added to a volume of reference solution and its UV spectrum recorded. Mathematical treatment of the spectral data yields the area-under-the-curve of the cosolvent reference solution. The solubility of the sample compound is then calculated from the ratio of the reference spectrum and the sample spectrum, adjusted for dilution factors. This HTS method can go down to 0.1 µg/ml. If done at one pH, about 400 compounds can be processed per day.

Figure 2 shows the solubility-pH profile for piroxicam, determined by the fast-UV method, showing evidence of aggregation/DMSO effects. The main evidence for the lat-

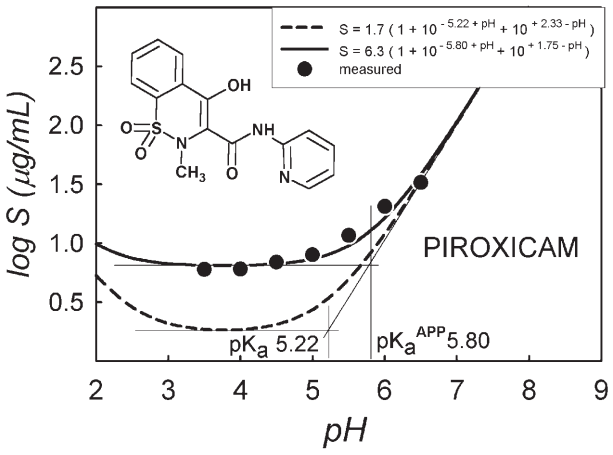

Figure 2. Solubility-pH profile of piroxicam, determined by the fast-UV method. The apparent curve (solid line) is shifted from the true aqueous curve (dashed line) due to the presumed formation of aggregates, as evidenced by the shift in the pK_a value of the drug.

ter is indicated by the shift in the pK_a of piroxicam, from 5.22 to 5.80. This illustrates the value of knowing the pK_a well, even at the discovery stage of research.

Experimental determination of permeability using artificial membranes

The established in vitro assay to assess intestinal permeability coefficients is based on Caco-2 cultured cell monolayers, which are outside the scope of this review. However, there now exist some biophysical methods based on artificial lipid membranes which can be classified at the interface between biological and physicochemical methods.

Black-lipid membranes

Mueller et al. [52] discovered in 1962 that when a small quantity of a phospholipid (2% w/v alkane solution) was carefully placed over a pin-hole in a thin sheet of plastic, a thin film gradually forms at the center of the hole, with excess lipid flowing toward the perimeter. Eventually, the central film turns black as a single black-lipid membrane (BLM) forms over the hole. Unfortunately, BLMs are extremely fragile and tedious to make. The permeation of a series of simple carboxylic acids across eggPC/decane BLMs was studied [53]. Permeability coefficients (P_e) were calculated from tracer fluxes. A linear relationship was observed between log P_e and hexadecane/water log P (partition coefficient). Using a similar system, Xiang and Anderson [54] studied the pH-dependent transport of a series of homologues of p-toluic acid, comparing the permeabilities to partition coefficients determined in octanol/water, alkane/water and alkene/water systems. The lowest correlation was found with octanol. Correction for the unstirred water layer was key to these analyses.

Parallel artificial membrane permeability assay

Efforts to overcome the limitations of the fragile membranes have evolved with the use of membrane supports, e. g., using polycarbonate filters or other more porous microfilters. Kansy et al. [55] published a widely cited study using such a method. Their PAMPA (parallel artificial membrane permeability assay) method has attracted favorable attention in the pharmaceutical community, and has spurred the development of a commercial instrument. The Roche investigators were able to relate their measured fluxes to human absorption values, much like that indicated by Caco-2 screening. The outliers in their assays were solutes known to be actively transported. Since the artificial membranes have no active transport systems and no metabolizing enzymes, the assay would not be expected to model actively transported molecules.

In the PAMPA assay, a 'sandwich' is formed from a 96-well microtiter plate and a 96-well filter plate, such that each composite well is divided into two chambers: donor at the bottom and acceptor at the top, separated by a 125 µm-thick microfilter disc (0.45-µm pores), coated with a 10% (w/v) dodecane solution of egg lecithin, under conditions where multilamellar bilayers form inside the filter channels when the system contacts an aqueous buffer solution. The use of lipids other than egg lecithin has also been explored [56–58].

Figure 3 shows the permeability-pH profile of propranolol, determined in charge-neutral phosphatidylcholine/-dodecane membranes (filled circles), and also in nega-

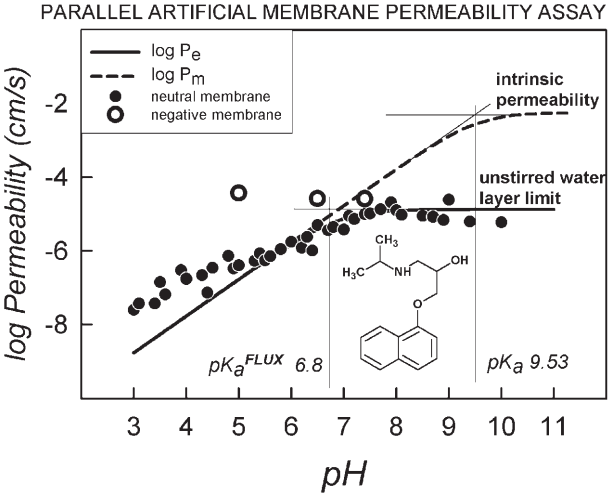

Figure 3. Permeability-pH profile of propranolol determined by the PAMPA method. The observed effective permeability (P_e) curve is limited by the effect of the unstirred water layer for pH > 7. Knowing the true pK_a allows one to determine the membrane permeability (P_m) curve, and the intrinsic permeability of the drug.

tively charged soy lecithin/dodecane membranes (open circles at pH 5, 6.5, 7.4). Membrane charge appears to play a role in passive diffusion across phospholipid-based membranes, as shown by the lack of pH dependence in the soy-based membrane. The microtiter plate was not stirred during the PAMPA experiment. Note that the unstirred water layer resistance [53] is the rate-limiting factor in transport above pH 7, as indicated by the shift in the pK_a from 9.53 to 6.8. Knowing the pK_a, one can determine the intrinsic permeability of propranolol. This again illustrates the value of knowing the pK_a well, even at the discovery stage in drug research.

Conclusion

In drug research and development, the physicochemical profiling of lead candidates, leads, and preclinical candidates is an essential step in the optimization of PD, PK, and pharmaceutical properties. To this end, efficient physicochemical methods have been developed and they continue to be improved at an impressive pace [59]. Such methods are *virtual* (i.e., in silico) or *experimental* (i.e., 'wet') ones. They can also be classified according to the objective they serve, namely screening or understanding. *Screens* (superficial probes) aim at acquiring a limited amount of information on a maximum number of compounds. They must be fast and relevant, but semi-quantitative or even qualitative (yes/no) responses may suffice. Nor is precision a stringent condition.

In-depth methods (deep probes) must yield a broad and deep knowledge on a limited number of compounds. Here, precision, accuracy, and reproducibility are conditions of success.

Clearly, there is a trade-off between quantity on the one hand and greater quality on the other. Yet, and as shown in this review, the experimental methods used in physicochemical profiling fall not into two exclusive classes but along a continuum whose extremes are deep and superficial probes. What is available to medicinal (physico)-chemists is a large box of tools, some more quantity oriented, others yielding data of higher quality. Researchers can thus select those methods best adapted to their current problem and objectives, and in so doing maximize their chances of success.

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