

Why measure physicochemical parameters?

It is a major challenge to convert a compound binding with high affinity to a biological target (i.e. a Hit, Lead or Candidate molecule) into a successful drug on the market. Attrition during the drug development process is a serious economic problem for the pharmaceutical industry and it is often due to inappropriate physicochemical characteristics and related poor absorption and poor pharmacokinetics [1]. It is now widely recognised that the early drug discovery process needs to address in parallel not only potency (in relation to the primary target), but also other biological (selectivity), toxicological and physicochemical (pharmacokinetics related) properties [2]. One approach is called 'property-based design' [3] and it emphasises the importance of understanding how medicinal chemists can manipulate the critical combinations of physical and structural properties which contribute to 'drug-likeness'. Drug solubility and the ability of molecules to permeate barriers are two key properties that are often considered to be linked to lipophilicity. Excessive lipophilicity is a common cause of poor solubility and can lead to erratic and incomplete absorption following oral administration. A biopharmaceutics classification scheme has been proposed under which drugs can be categorised into four groups according to their solubility and permeability properties [4]. It should be noted that 60-70% of drug molecules contain ionisable groups [5], and that lipophilicity, permeability and solubility are pH-dependent for these molecules.

Why is pK_a important?

- most drugs ionise in solution
- other properties: lipophilicity, solubility, permeability are pK_a dependant
- in general, neutral molecules are more easily absorbed by membranes while ionised molecules remain in plasma and are cleared by renal excretion
- other reasons:
 - useful in formulation
 - can affect drug-receptor binding

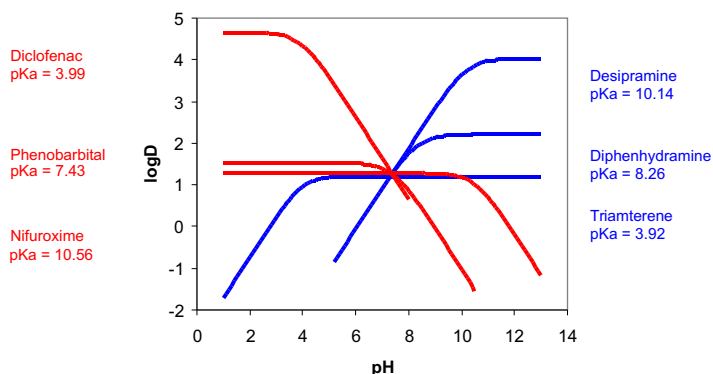
The table below shows how the ionisable groups are distributed between the 32,437 ionisable Compounds in World Drug Index (1999) (63% of total)

Combination of ionisable groups in the molecule	Percentage (from the above number)
1 base, no acid	42%
2 base, no acid	25%
1 acid, no base	12%
1 acid, 1 base	8%
2+ acid, no base	3%
1 acid, 2+ base	4%
2+ acid, 1 base	3%
others	3%

The majority of the commercially available drugs are ionisable molecules and approximately 2/3 of them are bases with no acid group.

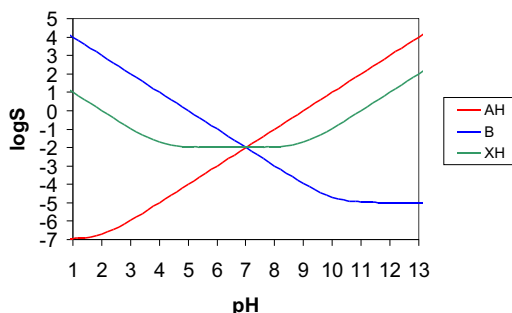
Lipophilicity is pK_a dependant!

The picture below shows that the lipophilicity value (expressed by LogD at pH 7.4) is the same for some acids and bases, but the lipophilicity - pH profile is very different. This information is important especially for the pH range that can be found in the human body. LogP is important because provides a rough guide to its pharmacokinetic behaviour and lipophilic molecules (high LogP) are more permeable through membranes (up to a point).



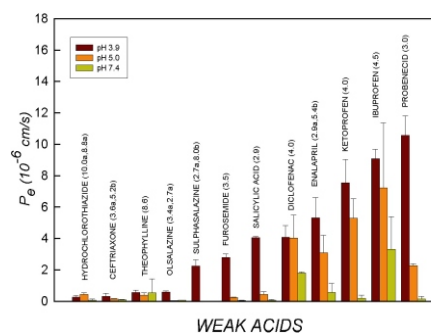
Solubility is pK_a dependant!

Molecules with high LogP are usually poorly soluble in water, but drugs need to be in solution before they can be absorbed. Molecules which are highly insoluble can make successful drugs but at a high cost. The figure below shows that 3 chemically different molecules, an acid, a base and an amphotile, can have the same solubility at pH 7.4 but very different solubility pH profiles.



Permeability is pK_a dependant!

Permeability is the rate at which a compound will pass through a membrane and is expressed in units of cm²/second. The graph below shows the pH dependence of a series of weak acids, all well-known drugs. The permeability measurements were performed by PAMPA (Parallel Artificial Membrane Permeability Assay) at 3 pH values.

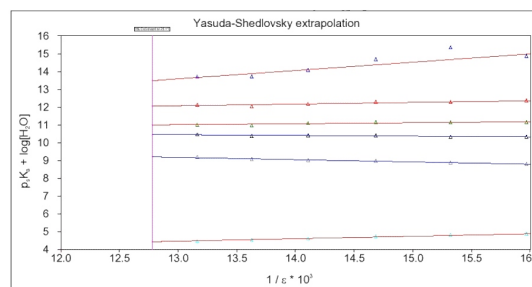


The neutral form of a drug molecule is most lipophilic, will be most permeable, but will be least soluble. The ionised form of a biologically active compound is the most soluble, but is less lipophilic, so will be less permeable. Finding the right combination between lipophilicity, solubility and permeability is a great challenge for today's drug discovery.

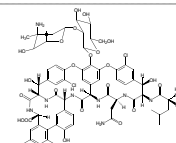
"Gold-standard" method for measuring pK_a

The pK_a value is very difficult to calculate, even for simple molecules and the prediction can also be unreliable. The measurement of pK_a values by potentiometry or by a combined method involving potentiometry and spectrophotometric detection is accurate and can provide very good experimental data for overlapping pK_as in complex molecules. The GLpKa instrument, manufactured by Sirius Analytical Instruments, is considered industry "gold standard" for pK_a measurements. The logP values can also be measured by a dual-phase titration in the presence of a partition solvent (usually octanol). The potentiometric titration method can be improved with UV-Vis detection (optional D-PAS module, Dip-Probe Absorption Spectroscopy). Low soluble samples (10⁻⁴ to 10⁻⁶ M), extreme pK_a values (in the range 1.2 to 12.0) and multiple overlapping pK_a values (up to 12 in a single molecule) are some of the method's technical characteristics.

The figure below shows 6 pK_a values obtained for Vancomycin, using a co-solvent titration and Yasuda-Shedlovsky extrapolation.



- pK_a1 = 11.86
- pK_a2 = 10.16
- pK_a3 = 9.26
- pK_a4 = 8.63
- pK_a5 = 7.49
- pK_a6 = 2.66



References

- [1] Hodgson, J. (2001) ADMET - turning chemicals into drugs. *Nature-Biotechnology* 19, 722-726
- [2] Baxter, A. D. and Lockey, P. M. (2001) 'Hit' to 'lead' and 'lead' to 'candidate' optimisation using multi-parametric principles. *Drug Discovery World* 3, 9-15
- [3] Van de Waterbeemd, H. et al. (2001) Property-Based Design: Optimisation of Drug Absorption and Pharmacokinetics. *J. Med. Chem.* 44, 1313-1333
- [4] Amidon et al. (1995) Theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* 12, 413-420
- [5] Comer, J.; Tam, K.; Lipophilicity Profiles: Theory and Measurement, in *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies*, E: Testa, B.; van de Waterbeemd, H.; Folkers, G.; Guy, R.; VHCA: Zurich, 2001, 275-304.