

The relationships between lipophilicity, solubility and pK_a for ionizable molecules

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Talk presented at PhysChem Forum, November 2005

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- ★ Lipophilicity, solubility and pK_a (and other physicochemical parameters) are used to guide decision making in drug development
- ★ In an uncertain world ruled by biology, these parameters should be well-defined, and it should be possible to measure them accurately
- ★ Of all the data available to pharmaceutical scientists, this should be the most dependable

- ★ Lipophilicity has an IUPAC* definition
- ★ From **Glossary of Terms Used in Medicinal Chemistry** (IUPAC Recommendations 1998)
- ★ **Lipophilicity** represents the **affinity** of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid-liquid (e.g., **partition coefficient in octan-1-ol/water**) or solid/liquid (retention on reversed-phase high performance liquid chromatography (RP-HPLC) or thin-layer chromatography (TLC) system). (See also **Hydrophobicity**).
 - **Affinity** is the tendency of a molecule to associate with another.
 - **Hydrophobicity** is the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non polar molecules.

- ★ Unfortunately the IUPAC Glossary doesn't have definitions of solubility or pK_a

- ★ Solubility is a very wide topic
- ★ It covers the solubility of any type of substance in any other substance
 - The next few slides will discuss the need for clear definitions of solubility for use in medicinal chemistry

- ★ pK_a is a term derived from solution chemistry
- ★ The meaning of pK_a is extremely clear, but it's explained through equations that people don't always understand
 - Later, I'll try to explain it

The Experimental Determination of Solubilities, Glenn T. Hefter, Reginald P. T. Tomkins (editors), 2003

Contents

Introduction Quantities, Units and Conversions.

1. FUNDAMENTALS OF SOLUBILITY.

- Thermodynamics of Solubility.
- Kinetics and Mechanisms of Crystal Growth and Dissolution.

2. GASES.

- Solubility of Gases in Liquids.
- Solubility of Gases in Polymers.
- Solubility of Gases in Molten Salts.
- Solubility of Gases in Solid Metals.

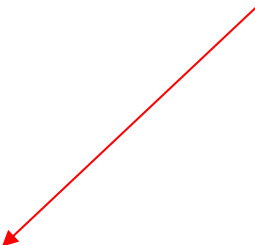
3. LIQUIDS.

- Liquid-Liquid Solubilities.

4. SOLIDS.

- **Solubility of Solids in Liquids.**
- **Solubility of Sparingly Soluble Solids in Liquids.**
- Solubility of Salt-Water Systems at High Temperatures and Pressures.
- Solubility of Metals and Non-metallic Substances in Liquid Metals.
- Solubility of Solids in Solids.

These are the more important topics in medicinal chemistry – but drugs are not covered



5. SPECIAL SYSTEMS.

- Solubility of Solids and Liquids in Supercritical Fluids.
- Solubility of Solids and Liquids in Cryogenic Liquids.
- Solubility of Polymers in Liquids.

- ✦ Halides
- ✦ Halates
- ✦ Oxyhalides
- ✦ Perchlorates
- ✦ Oxides
- ✦ Hydroxides
- ✦ Sulfites
- ✦ Selenites
- ✦ Tellurites
- ✦ Nitrates
- ✦ Phosphates
- ✦ Molten systems
- ✦ Special systems (mercury)
- ✦ Anions containing C, N and S: vol. 3
- ✦ Antibiotics: vols. 16/17, 34, 35, 36
- ✦ Organics: vols. 54, 58, 59

Volume 3: (1979)	<i>Silver Azide, Cyanide, Cyanamides, Cyanate, Selenocyanate and Thiocyanate</i>
Volume 16/17: (1985)	<i>1-Lactam Antibiotics</i>
Volume 34: (1988)	<i>4-Aminobenzenesulfonamides. Part I: Non-cyclic Substituents</i>
Volume 35: (1988)	<i>4-Aminobenzenesulfonamides. Part II: 5-membered Heterocyclic Substituents</i>
Volume 36: (1988)	<i>4-Aminobenzenesulfonamides. Part III: 6-membered Heterocyclic Substituents and Miscellaneous Systems</i>
Volume 54: (1994)	<i>Polycyclic Aromatic Hydrocarbons in Pure and Binary Solvents</i>
Volume 58: (1995)	<i>Polycyclic Aromatic Hydrocarbons: Binary Non-aqueous Systems, Part I: Solvents A-E</i>
Volume 59: (1995)	<i>Polycyclic Aromatic Hydrocarbons: Binary Non-aqueous Systems, Part II: Solvents F-Z</i>

- ★ **Kinetic solubility** - solubility of the fastest dissolving or fastest precipitating species
- ★ **Unbuffered solubility** - solubility of a saturated solution at whatever pH the solution ends up at. (self buffering)
- ★ **Thermodynamic solubility** – equilibrium solubility of all species
- ★ **Intrinsic solubility** - solubility of the neutral species regardless of physiological relevance of pH (similar to LogP)
- ★ **Buffered solubility** - solubility at a specified pH, 5 or 7.4 for example (similar to Log D – takes pK_a into account)

*Presented at Sirius User Meeting, Barcelona, April 2004

Barbara's terms...

- ★ **Kinetic solubility** (7 references in PubMed)
- ★ **Unbuffered solubility** (20 references in PubMed)
- ★ **Thermodynamic solubility** (24 references in PubMed)
- ★ **Intrinsic solubility** (51 references in PubMed)
- ★ **Buffered solubility** (349 references in PubMed)

Other terms are also used...

- ★ **Equilibrium solubility** (89 references in PubMed)
- ★ **Aqueous solubility** (998 references in PubMed)
- ★ **Natural solubility** (1,474 references in PubMed)
- ★ **Water solubility** (1,530 references in PubMed)
- ★ **Native solubility** (1,622 references in PubMed)
- ★ **Biological solubility** (7,146 references in PubMed)
- ★ **Solubility** (58,283 references in PubMed)

- ✦ At Sirius, we are making instruments to measure solubility
- ✦ We need to know what we are measuring
- ✦ We have created our own definitions (next slide)
- ✦ We'd like to encourage IUPAC to include solubility definitions in their next revision of the Glossary
- ✦ This PhysChem forum could help to revise and perfect the definitions before submission

- ★ **Kinetic Solubility** is the concentration of a compound in solution at the time when an induced precipitate first appears
- ★ **Equilibrium Solubility*** is the concentration of compound in a saturated solution when excess solid is present, and solution and solid are at equilibrium
- ★ **Intrinsic Solubility**** is the equilibrium solubility of the free acid or base form of an ionizable compound at a pH where it is fully un-ionized
- ★ Still required – definition of intrinsic solubility of zwitterions

* also called Thermodynamic Solubility

** Hörter, D.; Dressman, J. B. *Adv. Drug Deliv. Rev.*, 1997, 25, 3-14

- ★ Too difficult to define using words alone!
- ★ The term pK_a means $-\log_{10}K_a$
- ★ The term K_a is the ionization constant of a chemical reaction in solution in which a molecule accepts or loses a hydrogen ion in response to a change in pH

- ★ For example

Acids



$$K_a = \frac{[H^+][A^-]}{[HA]}$$

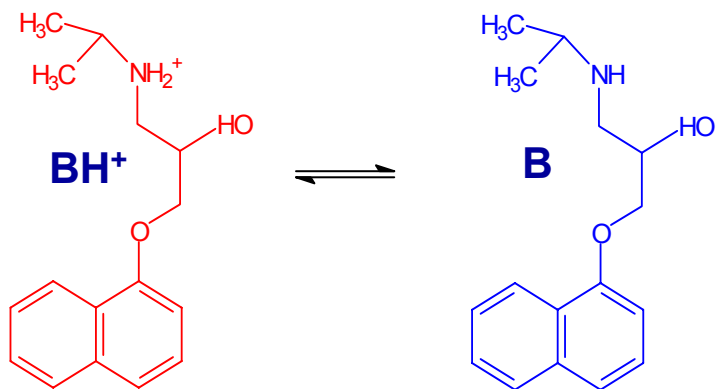
Bases



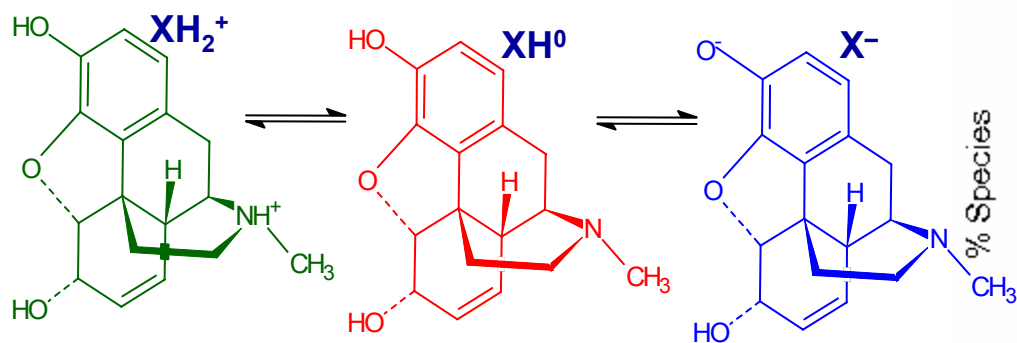
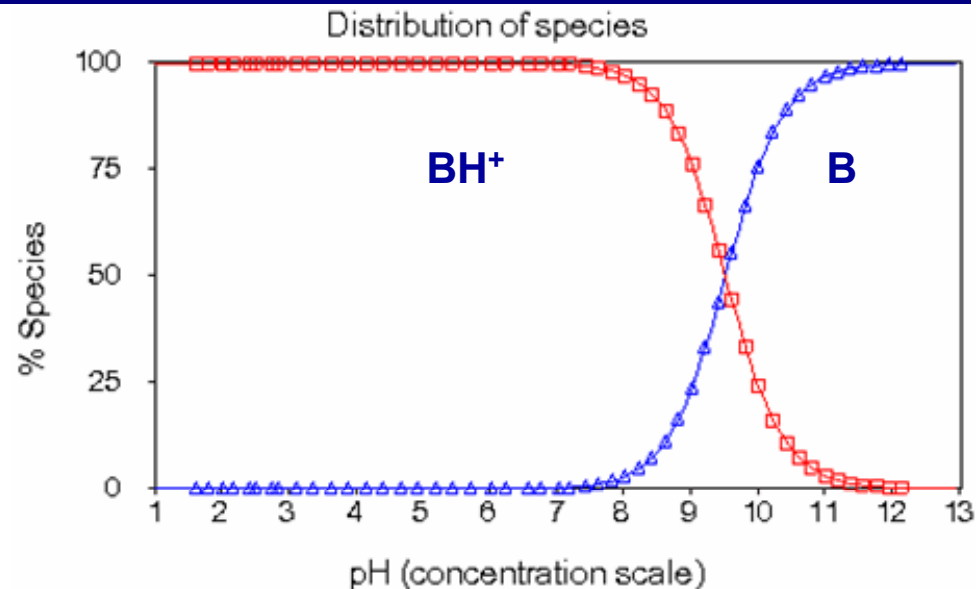
$$K_a = \frac{[H^+][B]}{[BH^+]}$$

- ★ This leads to a convenient statement for substances with only one pK_a – “ pK_a is the pH at which a substance is 50% ionized”
- ★ This definition is inadequate for substances with more than one pK_a

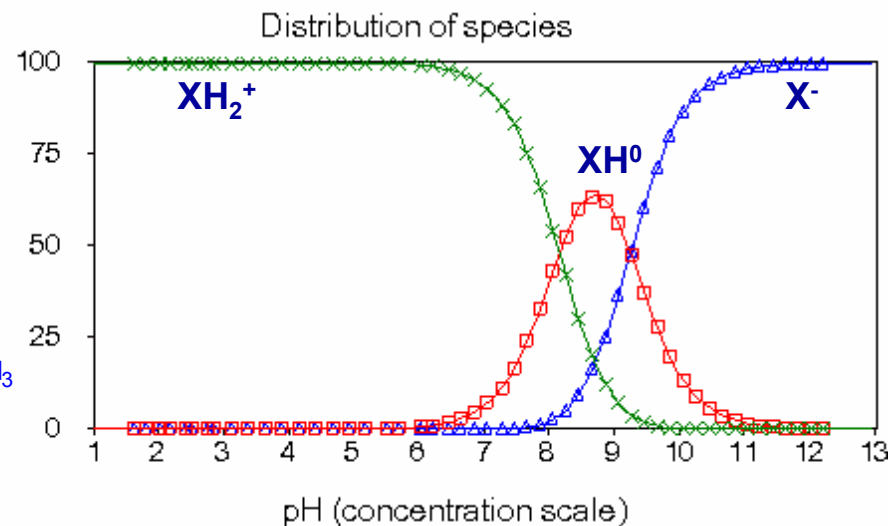
Simple definition doesn't work for morphine XH^0 species



Propranolol (a base): $pK_a = 9.53$



Morphine (an ampholyte): pK_a s = 9.26, 8.17



And now...

Relationships between lipophilicity, solubility and pK_a for ionizable molecules

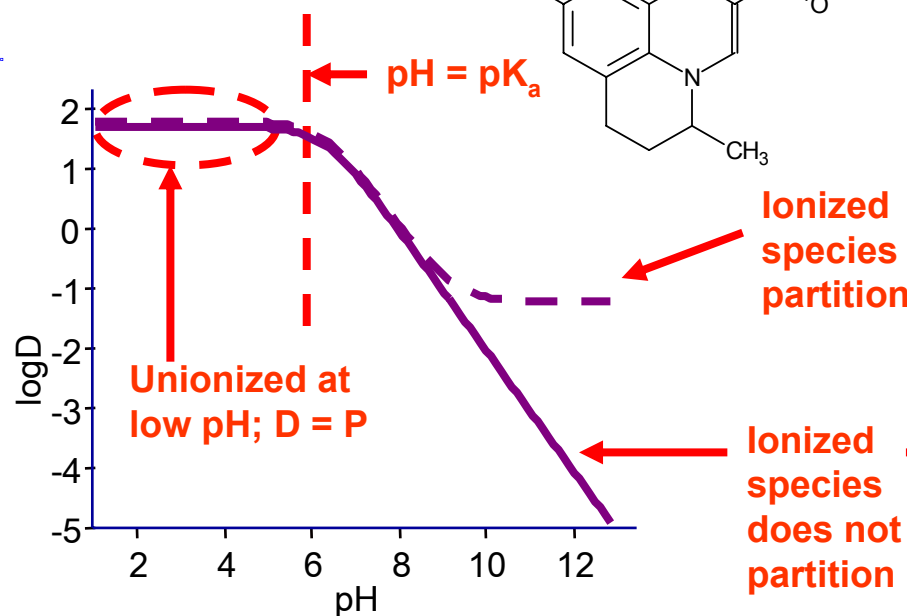
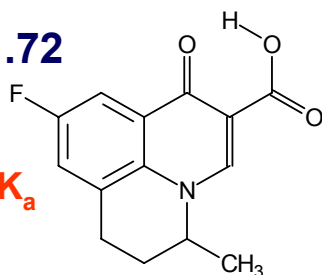
$$D = \frac{[\text{unionized} + \text{ionized species}]_{\text{octanol}}}{[\text{unionized} + \text{ionized species}]_{\text{water}}}$$

$$P = \frac{[\text{unionized species}]_{\text{octanol}}}{[\text{unionized species}]_{\text{water}}}$$

- ★ If there is no ionized species present, D = P
- ★ For ionizable drugs, concentration of both species changes with pH

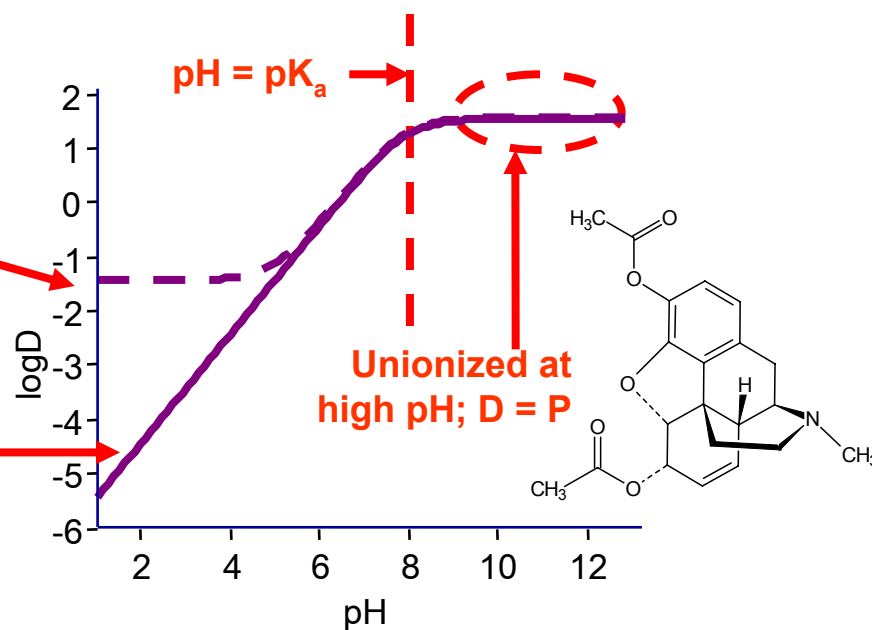
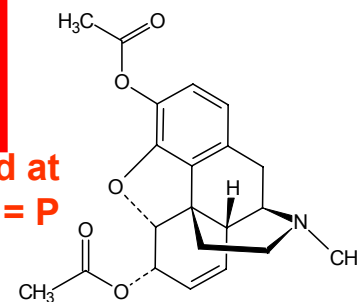
Flumequine (acid)

pK_a = 6.27, log P = 1.72

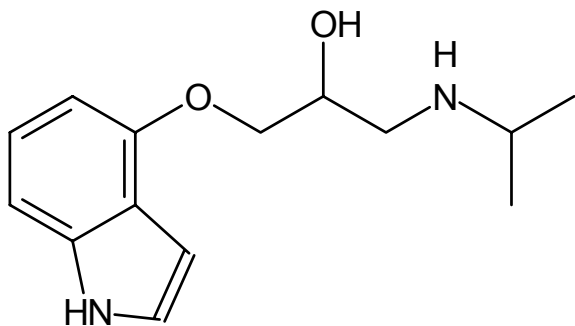


Diacetylmorphine (base)

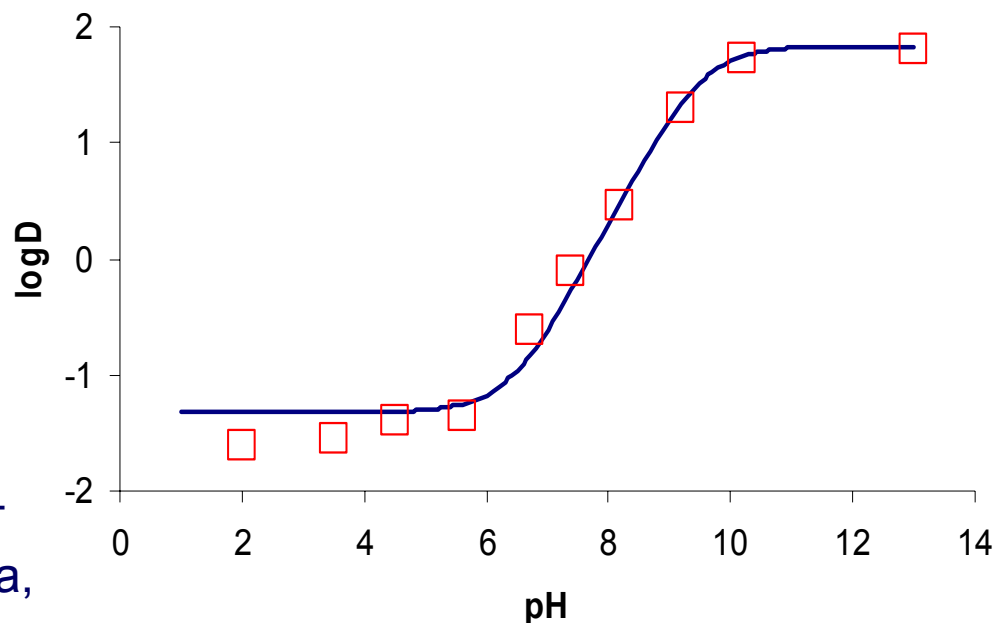
pK_a = 7.95, log P = 1.58



Pindolol



Line calculated using $pK_a = 9.54$,
 $\log P^{\text{neutral}} = 1.83$, $\log P^{\text{cation}} = -1.32$. pH-
 metric data obtained using Sirius GLpKa,
 0.15M KCl, 25°C [1]

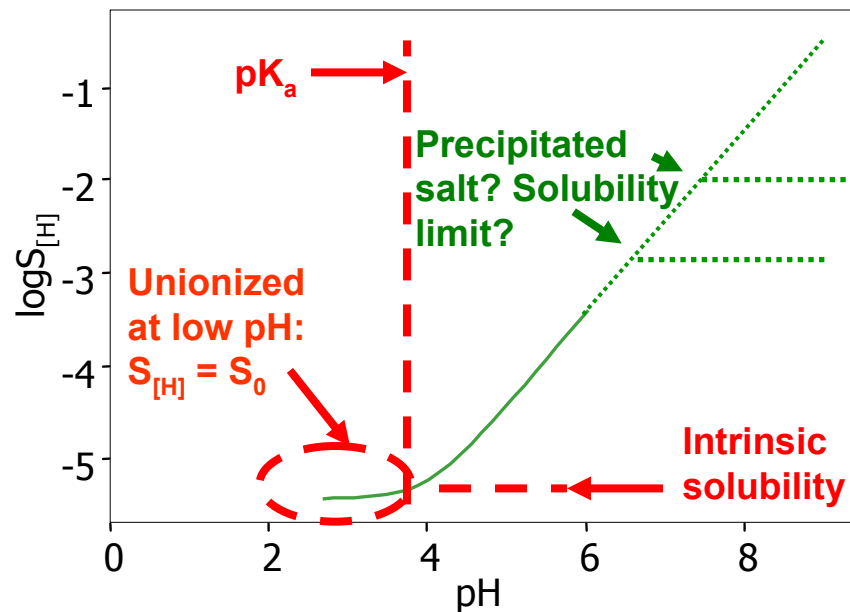


Points measured in buffered shake-flask experiments, 0.1M, 25°C [2]

[1] Caron, G., Steyaert, G., Pagliara, A., Reymond, F., Crivori, P., Gaillard, P., Carrupt, P.A., Avdeef, A., Comer, J., Box, K.J., Girault, H.H., Testa, B. *Helv Chim Acta*. 82, 1211-1222 (1999)

[2] Barbato, F., Caliendo, G., Larotonda, M.I., Morrica, P., Silipo, C., Vittoria, A. *Farmaco*. 45, 647-663 (1990)

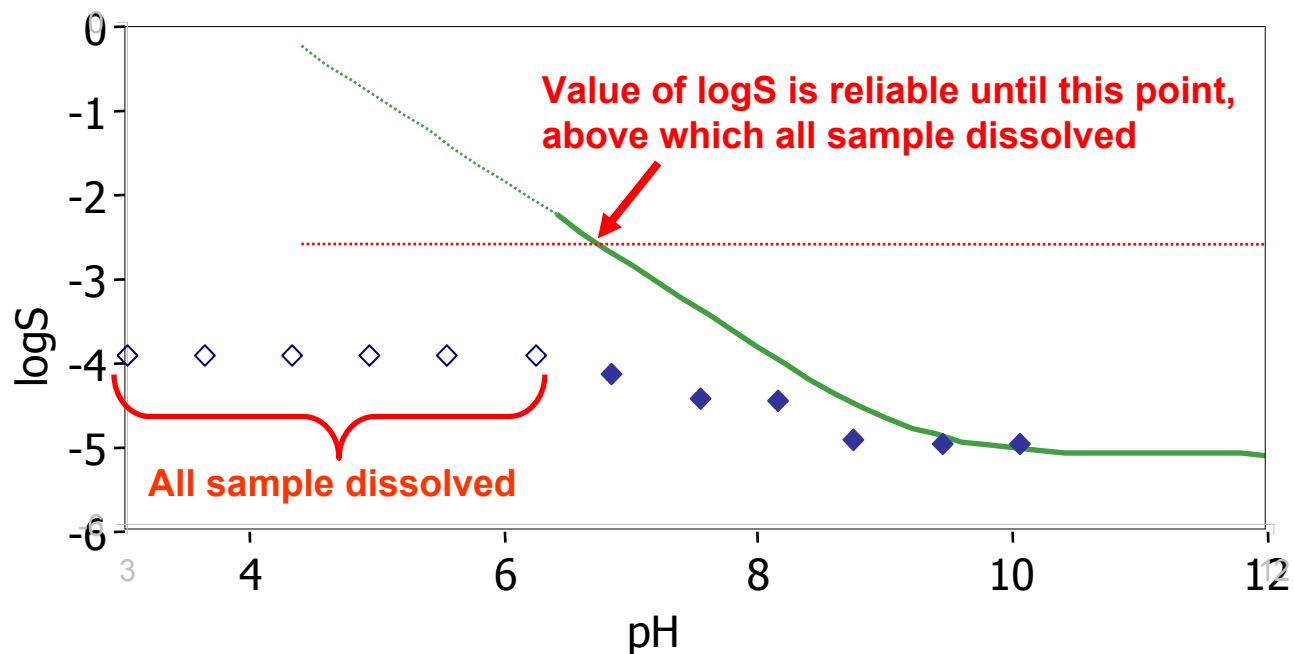
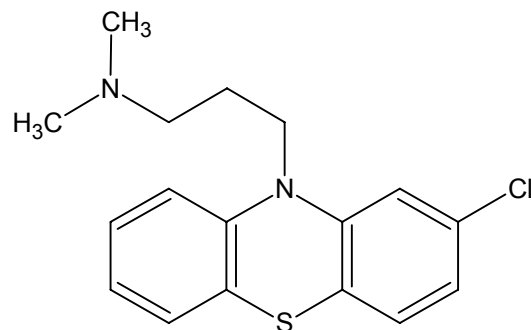
- ✦ Diagram shows solubility vs. pH for a weak acid with one pK_a (weak base is mirror-image)
- ✦ It shows the concentration $S_{[H]}$ of all species of sample in solution in equilibrium with excess undissolved sample
- ✦ Graph based on Henderson-Hasselbalch equation. To draw it, you need pK_a and intrinsic solubility S_0



$$S_{[H]} = \frac{S_0(K_a + [H])}{[H]}$$

- ✦ Solubility increases as molecule gets more ionized
- ✦ The graph is questionable at higher $\log S_{[H]}$ values
- ✦ Two possible reasons:
 - The ionized sample may precipitate as a salt with a counter-ion
 - All the compound used in the experiment may dissolve (“Solubility Limit”)

Chlorpromazine

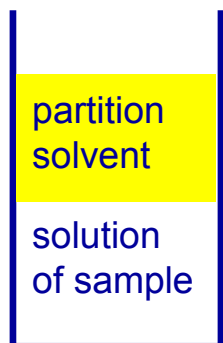


Line calculated using $pK_a = 9.24$,
 $\log S_0 = -5.07$, pH-metric data obtained using
 Sirius GLpKa, D-PAS and CheqSol. Sample
 weight 9.89mg [3]

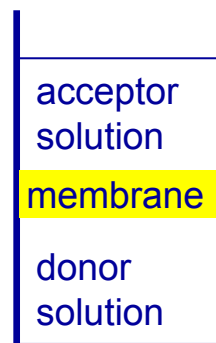
Points represent concentrations
 after precipitate removed from 12
 buffered solutions containing
 0.5% of 10mM sample in DMSO.
 Total sample weight 0.38mg

[3] Box, K J. Völgyi, G. Baka, E. Stuart, M. Takács-Novák, K. Comer, J. Equilibrium vs. kinetic measurements of aqueous solubility, and the ability of compounds to supersaturate in solution - a validation study. J Pharm Sci 2006 95(6) 1298-1307

- ★ People have used kinetic measurements to speed up data collection
- ★ Do they provide useful information?
- ★ Are they well defined and well characterised?
- ★ PAMPA
 - Parallel Artificial Membrane Permeability Assay
- ★ Kinetic solubility



Lipophilicity

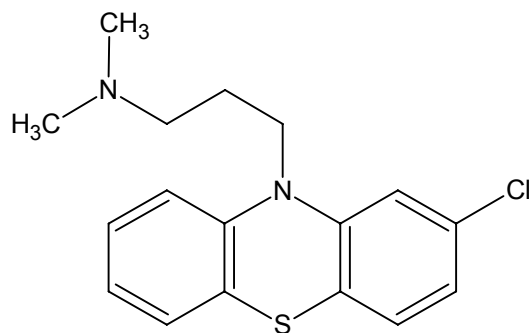


PAMPA

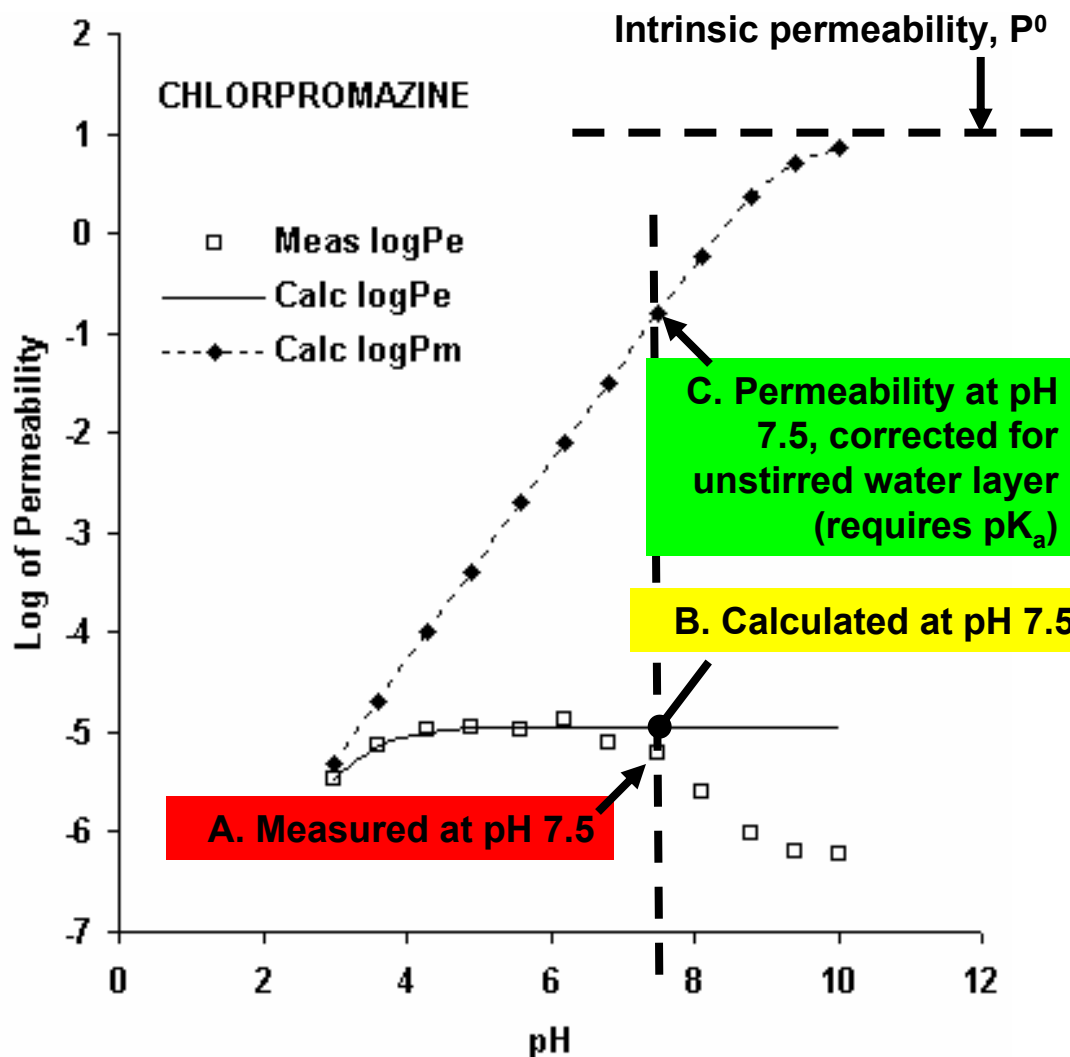
- ★ P is the ratio between the concentrations of a solute dissolved in two immiscible phases at equilibrium
 - ★ The result is a dimensionless number, normally expressed as $\log P$
 - ★ $\log P$ has been around for a long time, so it has IUPAC definition
 - ★ $\log P$ for a solute in a given solvent system (e.g. water-octanol, water-dodecane) is constant, regardless of the measurement technique used
- ★ PAMPA measures the rates at which compounds cross artificial lipid membranes. There are many system variables, such as
 - Membrane composition and thickness
 - Porosity of filter membrane support
 - pH of donor and acceptor solutions
 - Use of scavengers in acceptor solution
 - Rate of stirring in donor or acceptor
 - Incubation time
 - ★ The results are in cm/sec
 - ★ Intrinsic Permeability ($\log P^0$) values (typically between -7 and $+2$) can be calculated from data

Three different permeabilities can be chosen at a single pH.

These will be plotted vs. $\log D_{\text{alkane/water}}$ in the next slides



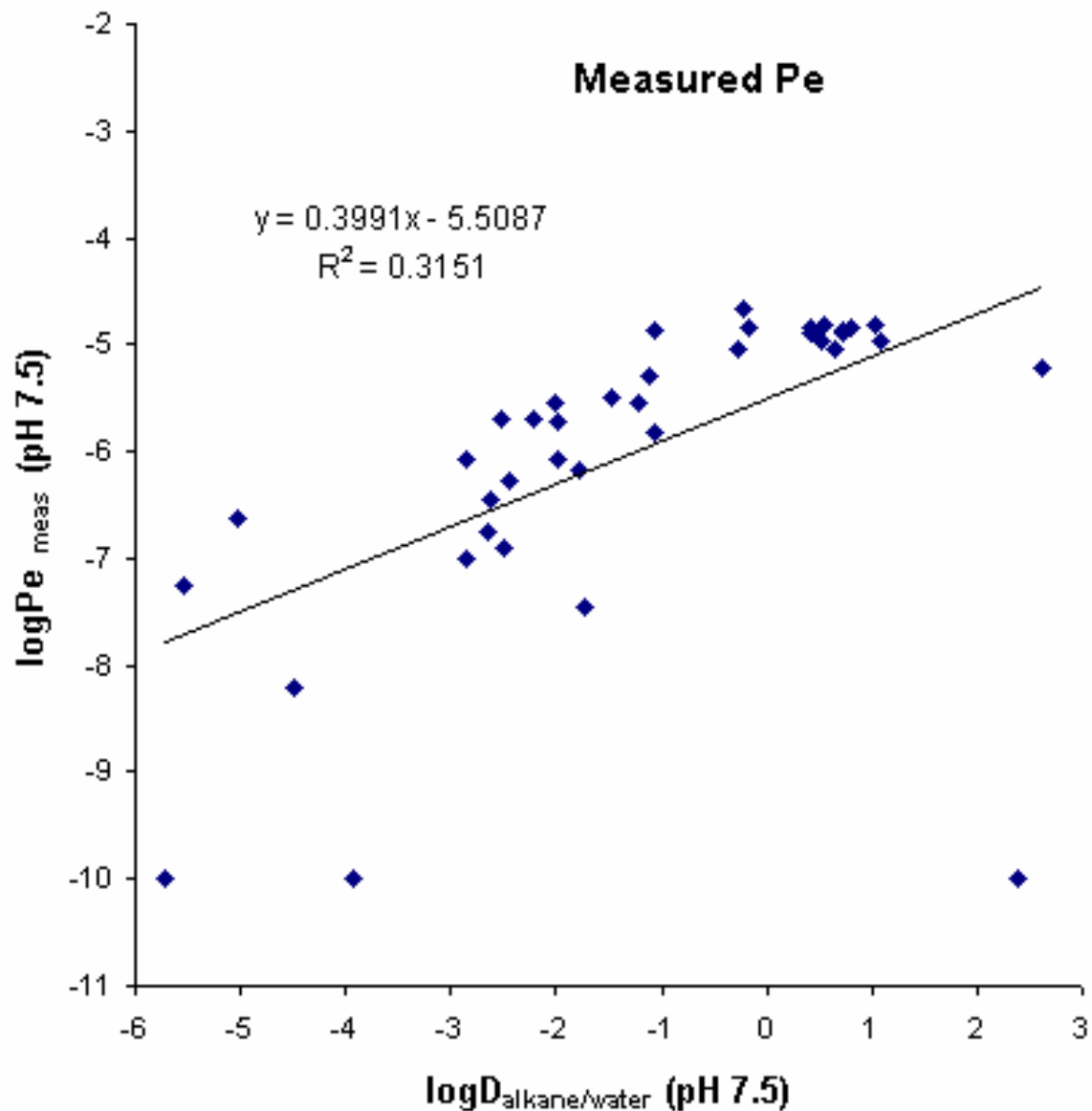
PAMPA permeabilities measured at Sirius under iso-pH conditions using membranes of 2% DOPC in dodecane



Huque, F T T. Box, K. Platts, J A. Comer, J. Permeability through DOPC/dodecane membranes: measurement and LFER modelling. *Eur. J. Pharm. Sci.* 2004, 23(3), 223-232

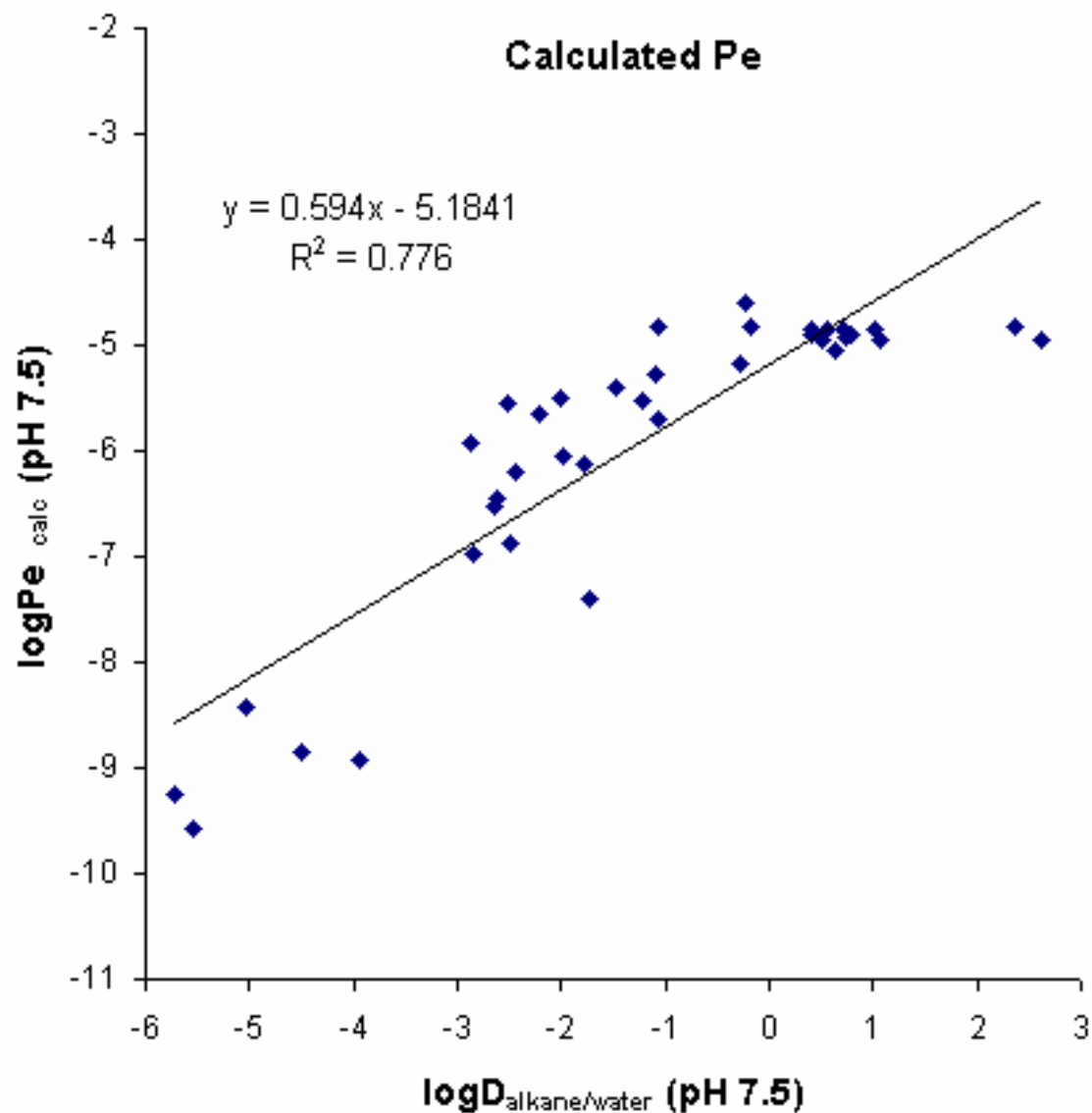
Poor correlation, many outliers

Verdict - unreliable



Some correlation, still
some outliers

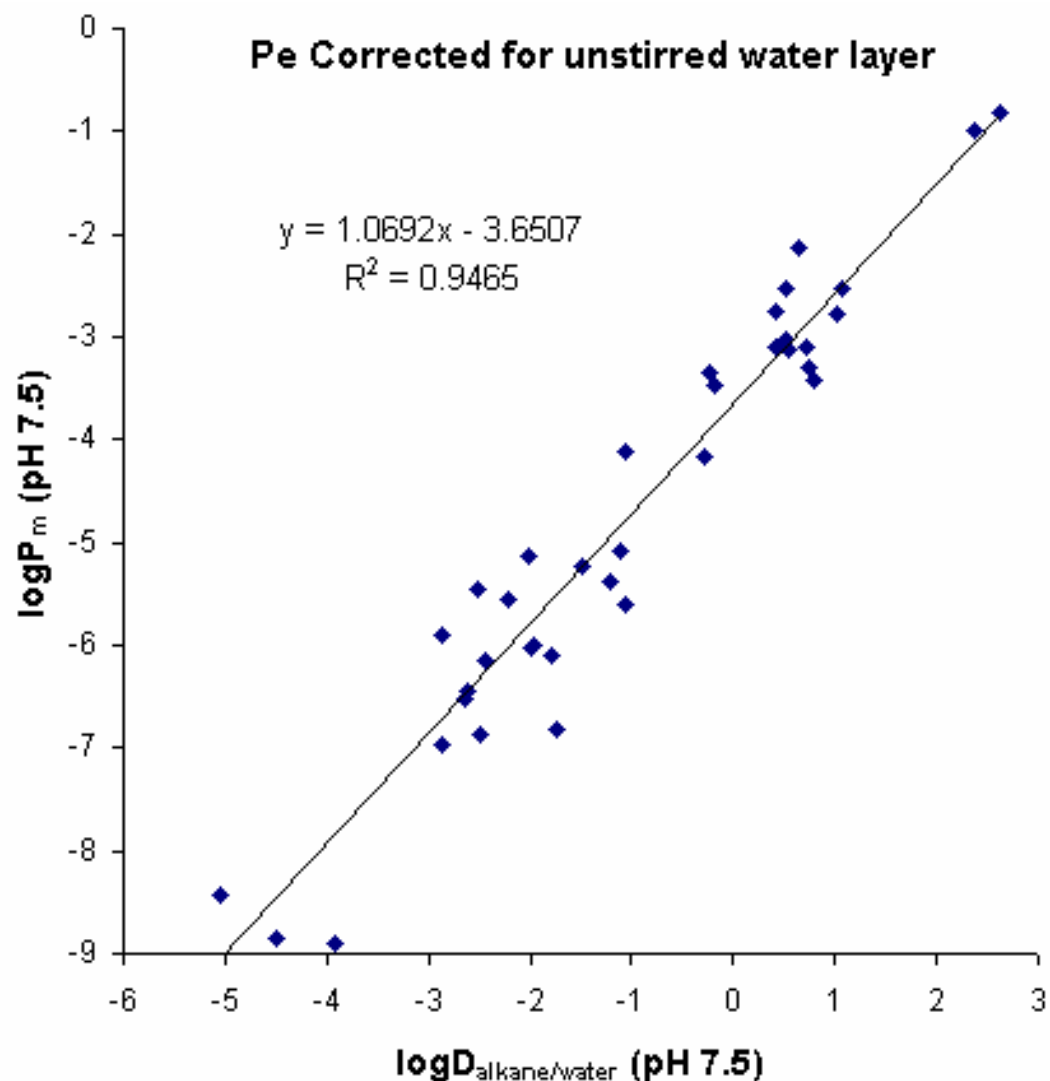
Verdict – could be better



Excellent correlation

Verdict – these logD values could be used to estimate permeability

NOTE: Measured pK_a values will be required



K. Box, J. Comer, F. Huque, Correlations between PAMPA permeability and log P. In Pharmacokinetic profiling in drug research: biological, physicochemical and computational strategies. Wunderli, H. Kraemer, S. Folkers, G. Testa, B., editors, Verlag Helvetical Chimica Acta, *in press*

Our results suggest that PAMPA and logD alkane water results convey similar information about compound behaviour.

Which is the best parameter to measure?

If a compound appears in the PAMPA acceptor well, it must be permeable!

High throughput possible using automation

LogD dodecane-water values show if compounds should be permeable.

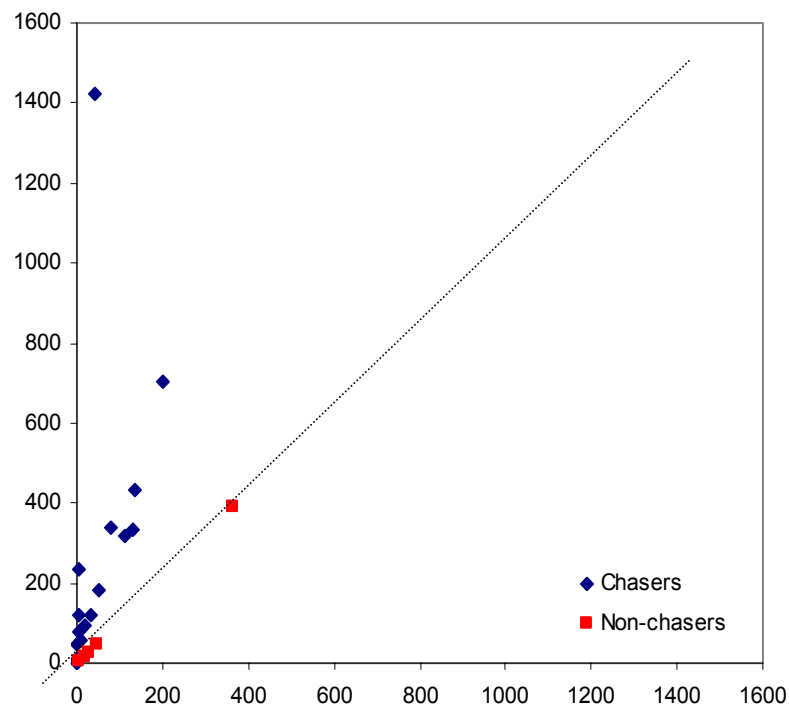
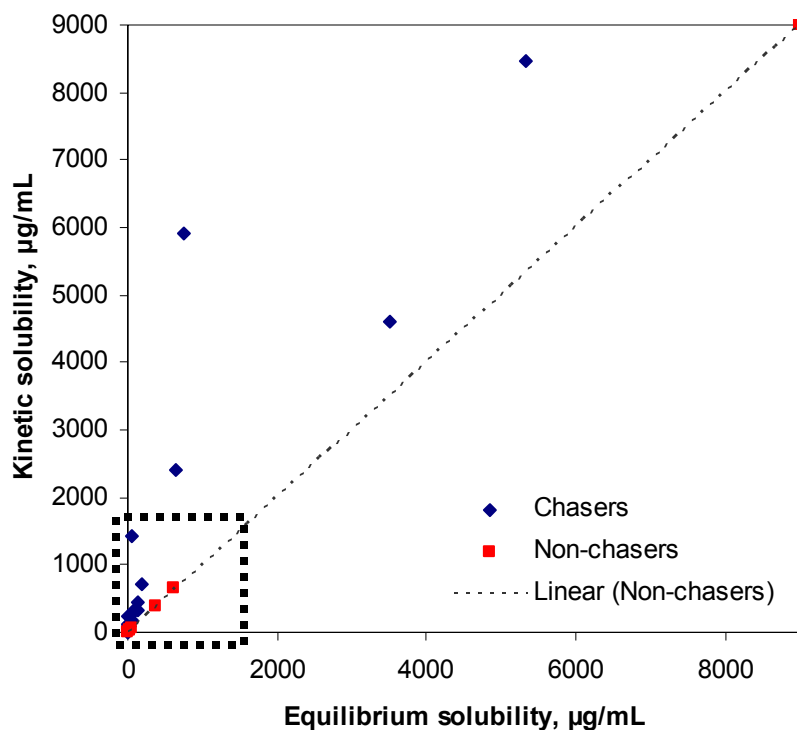
LogD dodecane-water can be measured pH-metrically on Sirius GLpKa.

The measurement is information-rich, but not very fast (say 10 per day)

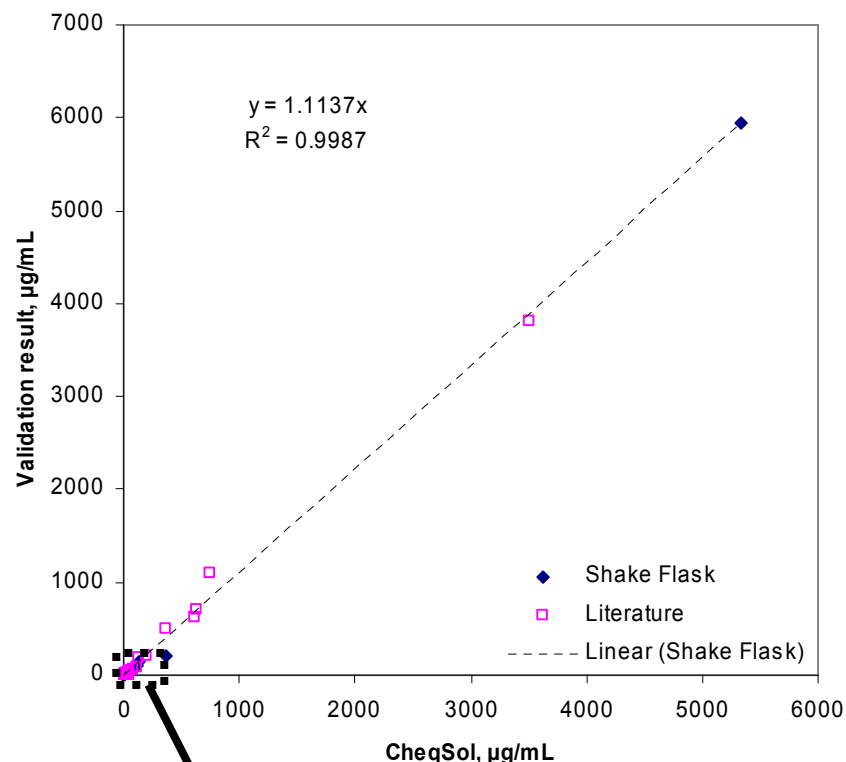
Much higher throughput possible using automation

Neither technique can predict paracellular or active transport, or efflux.

- ★ Non-chasers can't form super-saturated solutions
 - When the pH is right, they fall out of solution immediately
 - Their kinetic and equilibrium solubilities are identical
- ★ Chasers form super-saturated solutions
 - Their kinetic solubilities are higher than their equilibrium solubilities



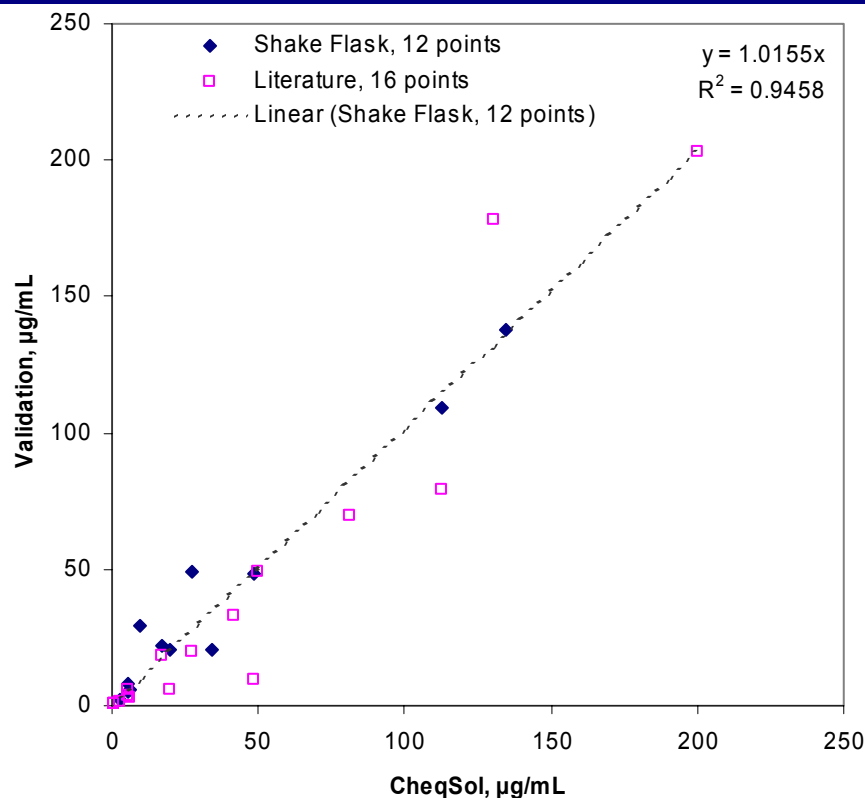
Name	Equilibrium solubility			Kinetic Solubility Chaser	Kinetic Solubility non-chaser
	CheqSol	Shake-Flask	Literature		
All results in µg/mL					
1 Phthalic Acid	5330	5950		8462	
2 Quinine	363	201			391
3 Trazodone	134.6	138.0		435	
4 Nitrofurantoin	112.5	109.5		319	
5 Nortriptyline	27.0	49.3	615.2		27.3
6 Verapamil	48.5	48.5	491.0		47.8
7 Niflumic Acid	9.53	29.5		59	
8 Imipramine	17.2	21.7	18.1		17.3
9 Flumequine	34.2	20.7		121	
10 Furosemide	19.7	20.4	78.9	96	
11 Maprotiline	5.80	8.05		77	
12 Piroxicam	5.92	5.95		233	
13 Warfarin	5.30	5.25	5.60	120	
14 Chlorpromazine	2.70	2.41			2.70
15 Lidocaine	3500		3810	4600	
16 Famotidine	740.0		1100	5900	
17 Hydrochlorothiazide	630.0		700.0	2400	
18 Chlorpheniramine	608.3		18.1		668
19 Sulfamerazine	200.3		203.0	701	
20 Ketoprofen	130.6		178.0	336	
21 Propranolol	81.0		70.0	340	
22 Ibuprofen	50.0		49.0	180	
23 Pindolol	41.7		32.7	1424	
24 Miconazole	1.00		0.67		
25 Diclofenac	0.90		0.80	45	
26 Amodiaquin	0.41			8.8	
27 Pamoic acid	0.0003			0.019	



19 compounds in this group – see next slide

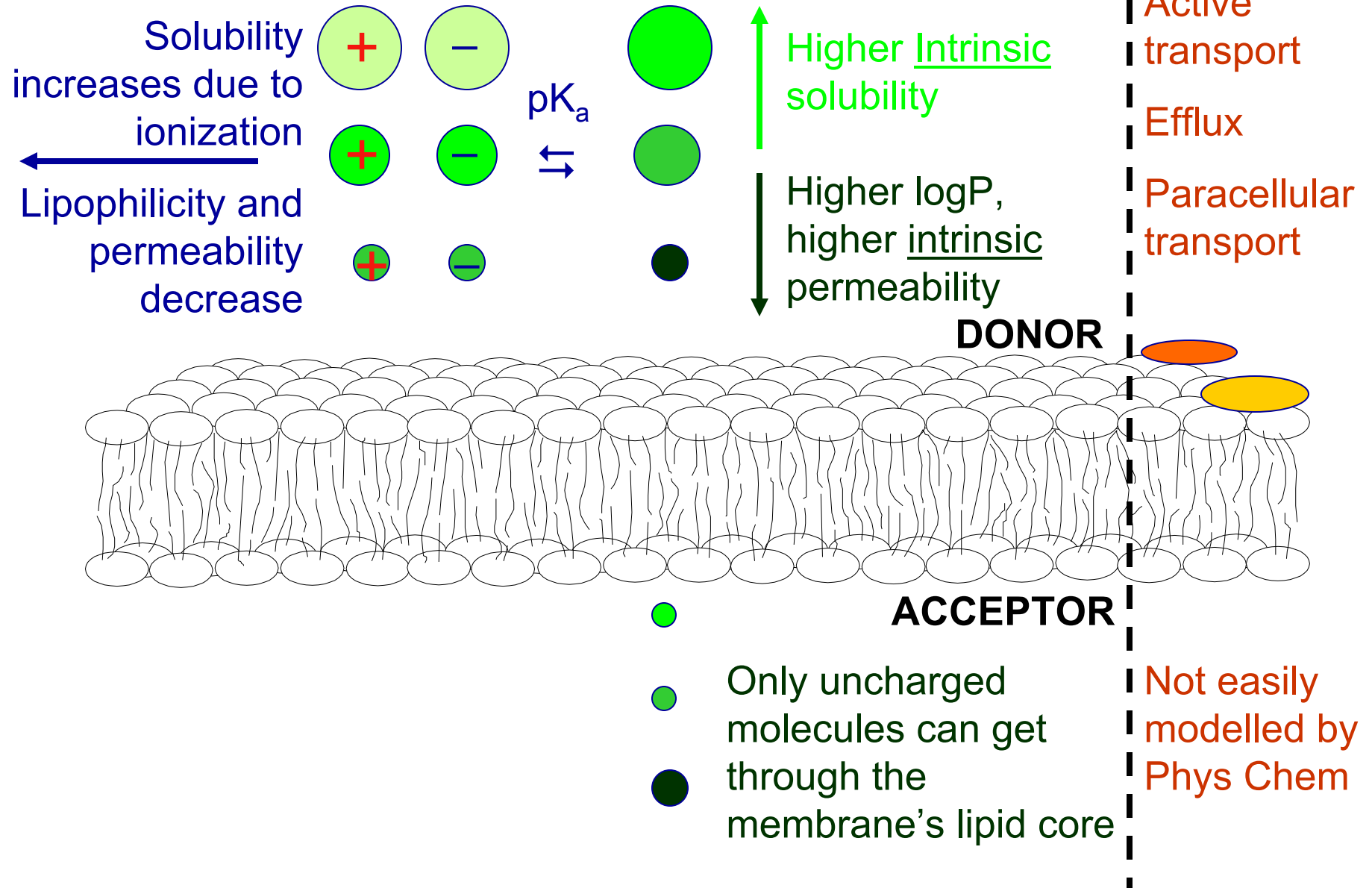
CheqSol and Shake-flask results from our second paper for compounds 1 – 14

Box, K J. Völgyi, G. Baka, E. Stuart, M. Takács-Novák, K. Comer, J E A. Equilibrium vs. kinetic measurements of aqueous solubility, and the ability of compounds to supersaturate in solution - a validation study. J. Pharm. Sci. 2005, *submitted*



- ✦ Equilibrium solubility is the only true measure of aqueous solubility
- ✦ Results are identical, regardless of the technique used
- ✦ Kinetic solubility results may be too high

Solubility and lipophilicity



If drug is permeable and soluble, OK

If it's very permeable but poorly soluble, OK

If it's poorly permeable but very soluble, OK

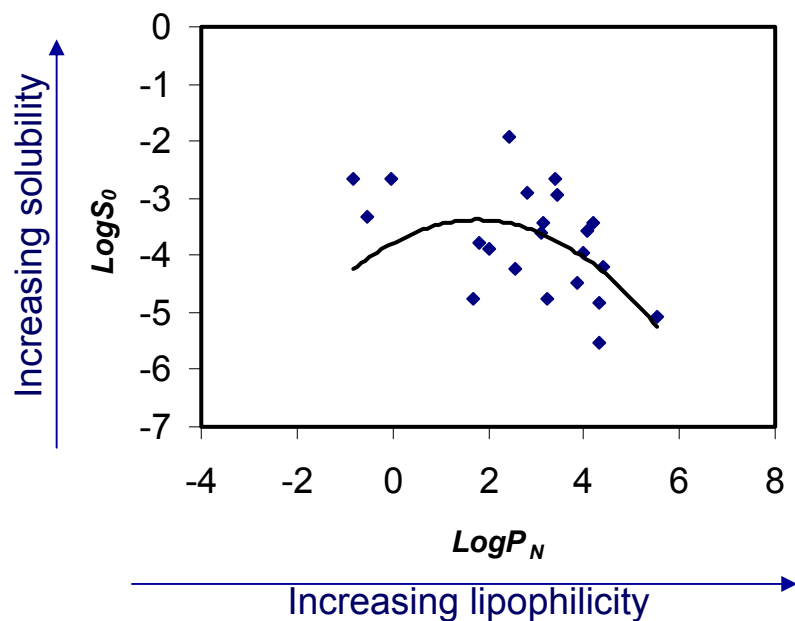
If it's not permeable and not soluble, BAD!

Don't rely on ionization to make drug soluble

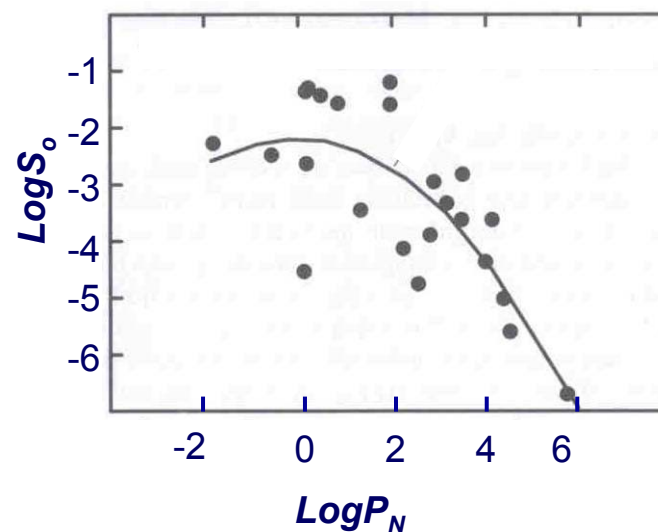
★ In general

- Molecules are more soluble when ionized
- Molecules are less lipophilic when ionized
- Lipophilic molecules are poorly water-soluble (below)

★ Exceptions to all these rules can be found



Above data measured at Sirius

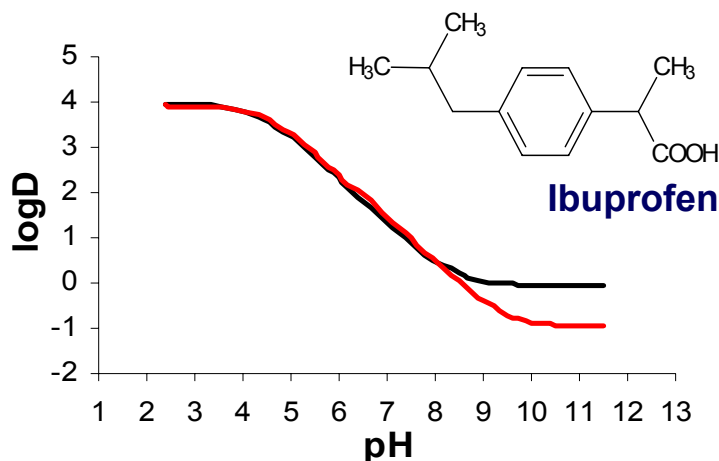


Above graph from Avdeef, A. Physicochemical profiling (solubility, permeability and charge state). Current Topics in Medicinal Chemistry, 2001, 1, 277-351

- ★ Solubility generally increases as molecules become more ionized
- ★ Poorly soluble samples like diclofenac and hexachlorophene (both acids) will dissolve in strong aqueous KOH, where they are fully ionized
- ★ Most samples prepared as salts with soluble counter-ions will also dissolve when ionized
- ★ However, the salts of some samples (e.g. miconazole, amiodarone) are so poorly soluble that neither the unionized nor the ionized sample will dissolve in aqueous solution at extreme pH
- ★ Also, some counter-ions are so poorly soluble that they prevent ionized samples from dissolving

★ Lipophilicity generally decreases as molecules become more ionized – however...

★ Ionizable species form neutral ion-pairs with counter-ions. Their lipophilicity increases with salt concentration



High salt

$$\log P_{\text{neutral}} = 3.97$$

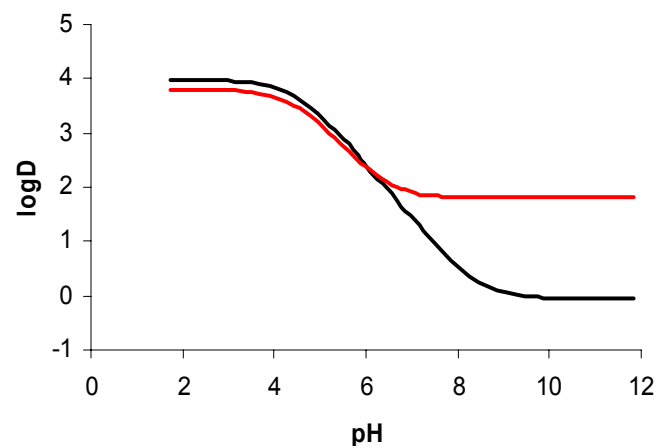
$$\log P_{\text{ion}} = -0.05$$

Low salt

$$\log P_{\text{neutral}} = 3.92$$

$$\log P_{\text{ion}} = -0.97$$

★ Ionized species can be more lipophilic in different partition media, presumably because the ions bind to the media



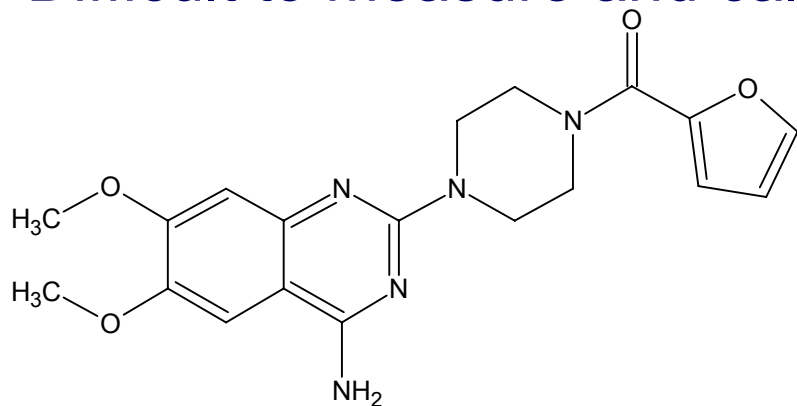
Octanol-water

$$\log P_{\text{ion}} = -0.05$$

Liposome-water

$$\log P_{\text{ion}} = 1.81$$

- ★ Compounds with strong capacity for hydrogen bond formation
- ★ May self-associate; often have high melting points
- ★ Difficult to measure and calculate!



Prazosin

Melting Point (MP) = 279°C

Calculated logP = 1.50 (Biobyte), -1.14 (ACD), 1.28 (SRC)

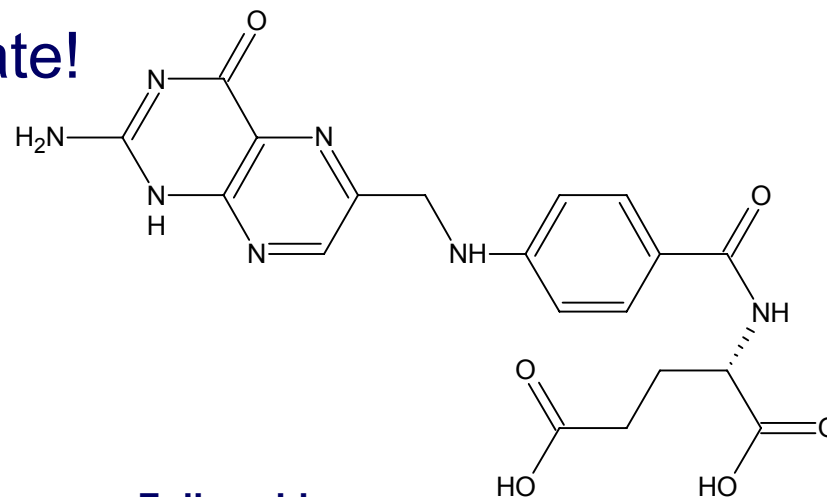
Couldn't measure logP

Calculated solubility

without MP = 17.2mg/mL,

with MP = 5.65mg/mL

Measured solubility 3.2µg/mL [1]



Folic acid

Melting Point (MP) = 250°C

Calculated logP = -2.32 (ACD), -2.81 (SRC)

Couldn't measure logP

Calculated solubility

without MP = 63.5mg/mL

with MP = 78.0mg/mL

CheqSol solubility = 2.6µg/mL

1. Bergström, C A S. Norinder, U. Luthman, K. Artursson, P. Experimental and computational screening models for prediction of aqueous drug solubility. Pharm. Res. 2002, 19(2), 182-188

- ★ Make sure you know what you're measuring
 - ★ Solubility and lipophilicity are highly dependent on pK_a for ionizable molecules
 - ★ Unless you really want to know about kinetic effects, stick to measurements made under equilibrium conditions
 - ★ Some compounds will break the rules
-
- ★ Thanks to
 - AT SIRIUS
 - > Karl Box, Jon Mole, Ruth Allen, Gabriela Cimpan, Carolyn Hibbert
 - COLLABORATORS
 - > Farah Huque, (Cardiff); Rebeca Ruiz (Barcelona); Gergely Völogi (Budapest); Derek Reynolds (Reytek)