

# The Central Role of $pK_a$ in Drug Discovery

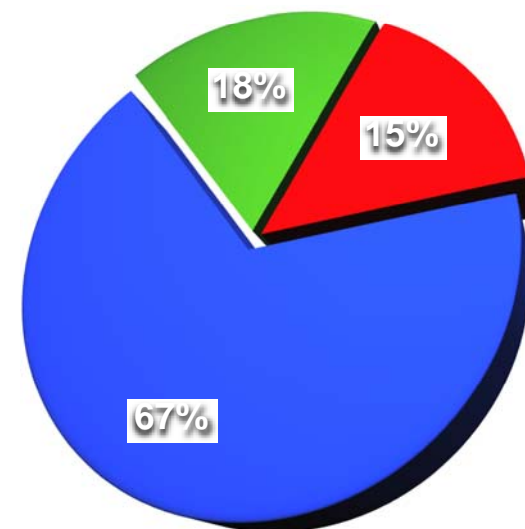
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UCB Celltech  
May 2013



Christer, living with Parkinson's disease

# Ionisable Drugs on the Market

- ② Approximately two-thirds of drugs on the market contain at least one group capable of ionisation in a pH range of 2 – 12
- ② A survey of the WDI in 1999 assessed ~52,000 compounds
- ② Of the ~32,500 that contained ionisable groups, most contained at least one basic centre
- ② Each ionisable centre in a molecule has an associated  $pK_a$  value
- ② Knowledge of the  $pK_a$  allows the percentage ionised to be calculated at any given pH



● Acidic  
● Basic  
● Amphotolytes and Zwitterions

# What is $pK_a$ ?

- ②  $pK_a$  refers to the extent of ionisation of a compound
- ② For practical purposes,  $pK_a$  can be defined as the pH at which a compound is 50% ionised

$$pK_a = pH + \log\left(\frac{[HA]}{[A^-]}\right)$$

- ② Where  $K_a$  is the ionisation constant:

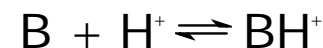
$$pK_a = -\log_{10} K_a$$

## Acids



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

## Bases

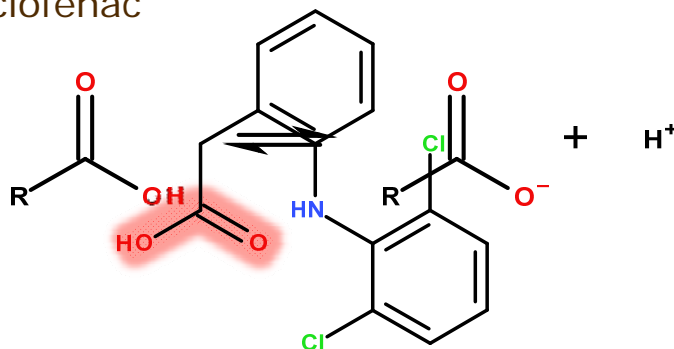


$$K_a = \frac{[H^+][B]}{[BH^+]}$$

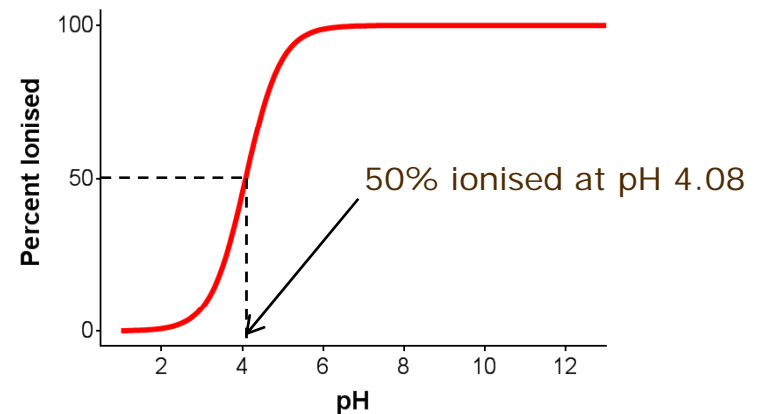
# Examples..

⊗ Acidic compounds:  $HA \rightleftharpoons H^+ + A^-$

Diclofenac

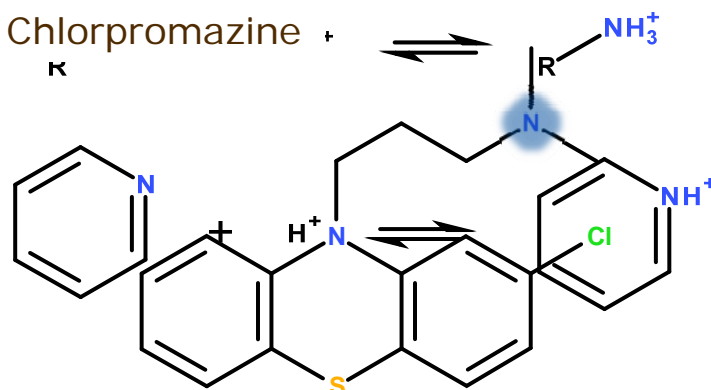


$pK_a = 4.08$  (acidic)

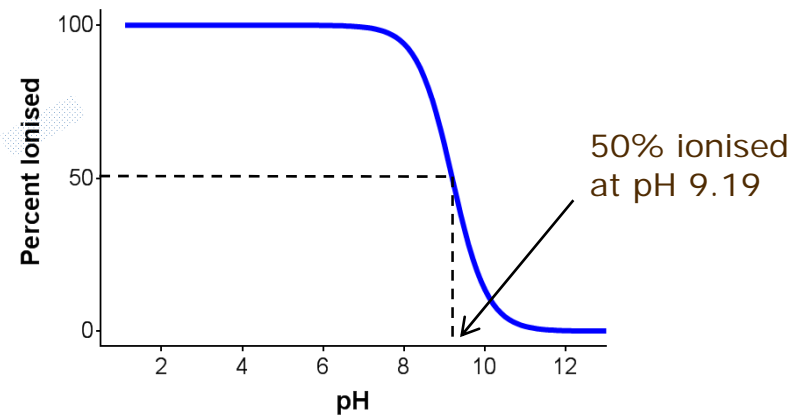


⊗ Basic Compounds:  $B + H^+ \rightleftharpoons BH^+$

Chlorpromazine



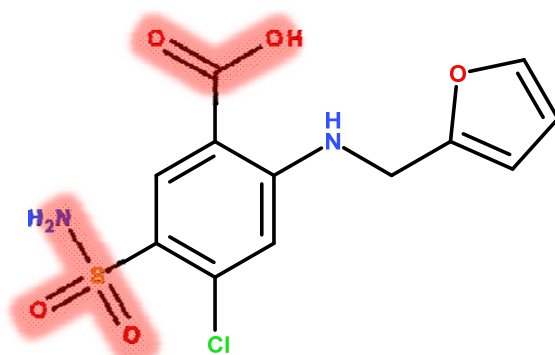
$pK_a = 9.19$  (basic)



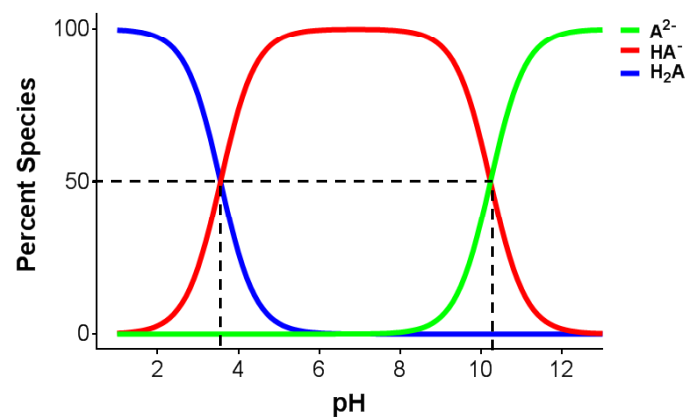
# Multiple Ionisable Centers

⊗ Compounds can have more than one  $pK_a$ :

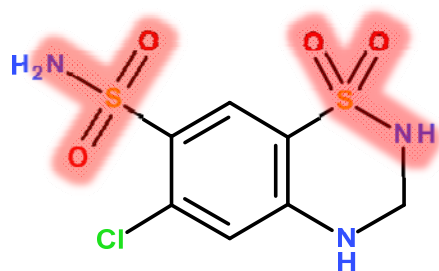
Furosemide



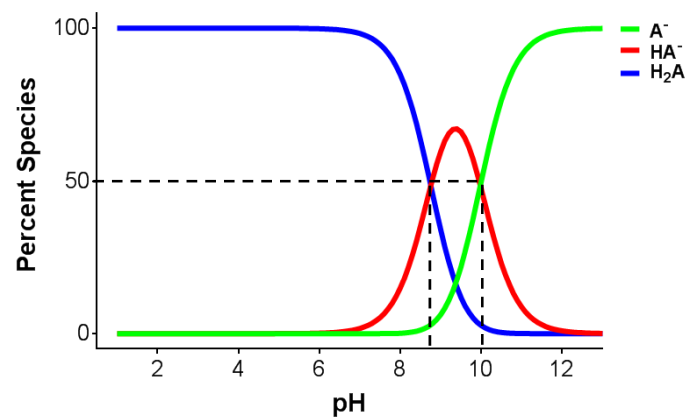
$pK_a = 10.23, 3.56$  (acidic)



Hydrochlorothiazide



$pK_a = 9.98, 8.76$  (acidic)

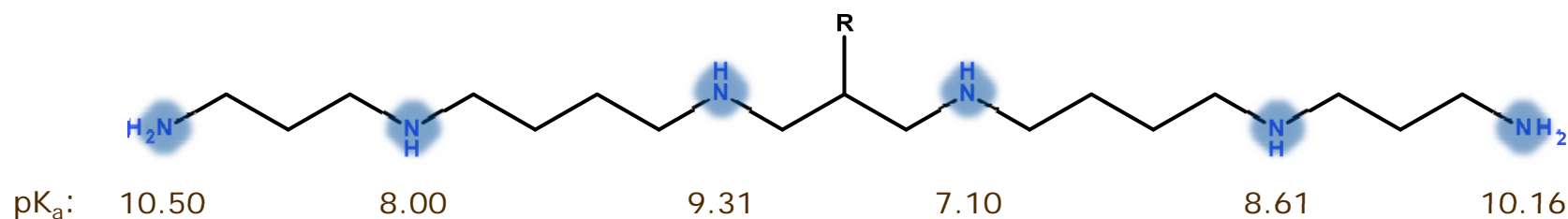
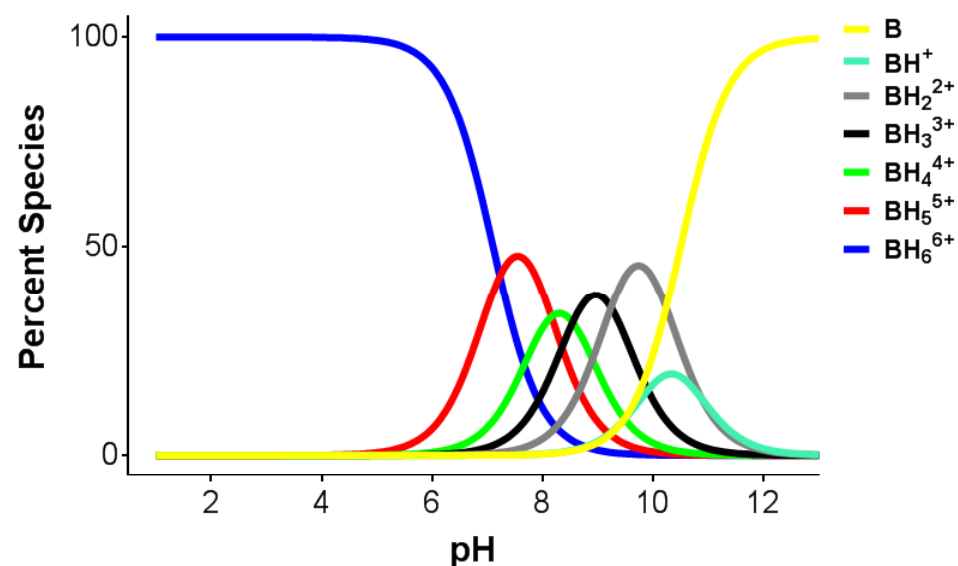


# Multiple Ionisable Centers

⌚ An extreme example from gene therapy:

⌚ Basicity decreases with increasing charge as it becomes more difficult to protonate each amine

⌚ Charges separate to minimise repulsion

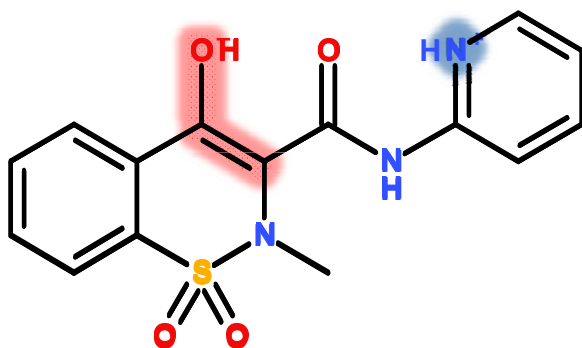




# Ampholytes and Zwitterions

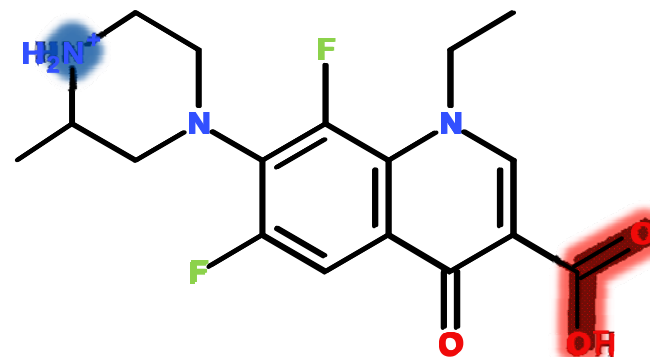
- Both an acidic and basic  $pK_a$  can exist within the same molecule:

Piroxicam – an **ampholyte**



$pK_a$ : 5.41 (acidic)  
1.89 (basic)

Lomefloxacin – a **zwitterion**



$pK_a$ : 8.95 (basic)  
5.96 (acidic)

- Ampholyte: Acidic  $pK_a \gg$  Basic  $pK_a$
- Zwitterion: Acidic  $pK_a \ll$  Basic  $pK_a$

# Why Should We Care?

- ④ The  $pK_a$  influences the key physical properties of a drug-like molecule:

Solubility

Lipophilicity

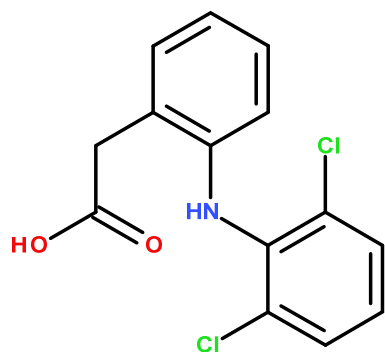
Permeability



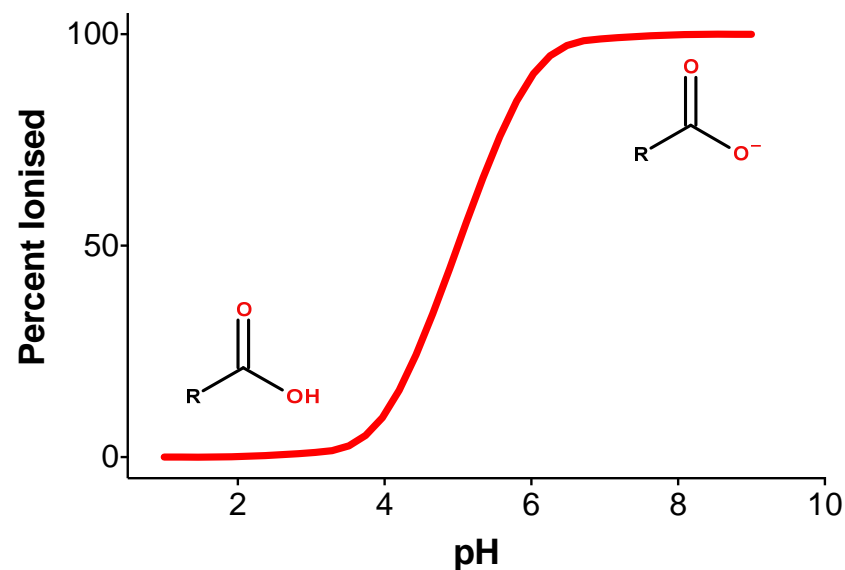
# Effect of $pK_a$ on Aqueous Solubility

- ⊗ Aqueous solubility of an ionisable compound is highly pH dependent. The ionised form is usually more soluble, thus solubility increases with percentage ionised:

Diclofenac –  $pK_a$  4.08 (acidic)



pH 3	pH 5	pH 7.4	pH 9
17 $\mu$ M	>350 $\mu$ M	>350 $\mu$ M	>350 $\mu$ M

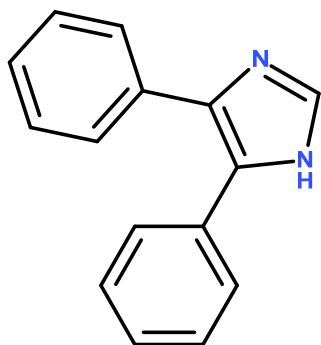


In house kinetic solubility assay (5% DMSO, 90 mins incubation at 25 °C)

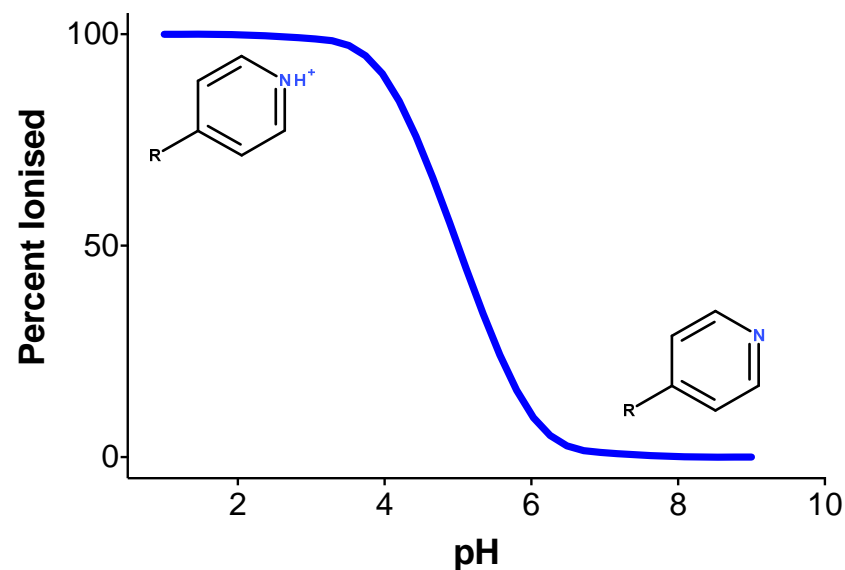
# Effect of $pK_a$ on Aqueous Solubility

- ⊗ Aqueous solubility of an ionisable compound is highly pH dependent. The ionised form is usually more soluble, thus solubility increases with percentage ionised:

4,5-diphenylimidazole –  $pK_a$  5.88 (basic)



pH 3	pH 5	pH 7.4	pH 9
> 350 $\mu$ M	> 350 $\mu$ M	84 $\mu$ M	63 $\mu$ M



In house kinetic solubility assay (5% DMSO, 90 mins incubation at 25 °C)

# Effect of $pK_a$ on Lipophilicity

- ⊗ Lipophilicity is a measure of how 'greasy' or lipophilic a compound is
- ⊗ A *more lipophilic* compound is able to better permeate a physiological membrane than a *less lipophilic* (or more hydrophilic) one
  - Orally dosed compound the membrane of interest is the gut endothelium
- ⊗ It is experimentally determined by partitioning a compound between two immiscible solvents, usually n-octanol and an aqueous buffer:

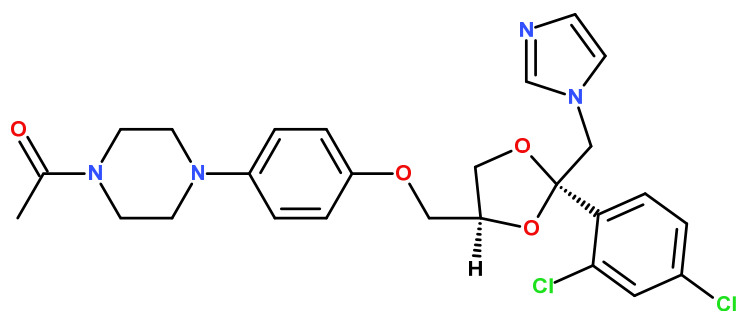
$$P = \frac{[\text{Neutral Species}]_{\text{Octanol}}}{[\text{Neutral Species}]_{\text{Aqueous}}} \quad D = \frac{[\text{Neutral + Ionised Species}]_{\text{Octanol}}}{[\text{Neutral + Ionised Species}]_{\text{Aqueous}}}$$

- ⊗ LogD is dependant on the pH of the aqueous buffer and therefore the  $pK_a$  of the analyte..

# Effect of $pK_a$ on Lipophilicity

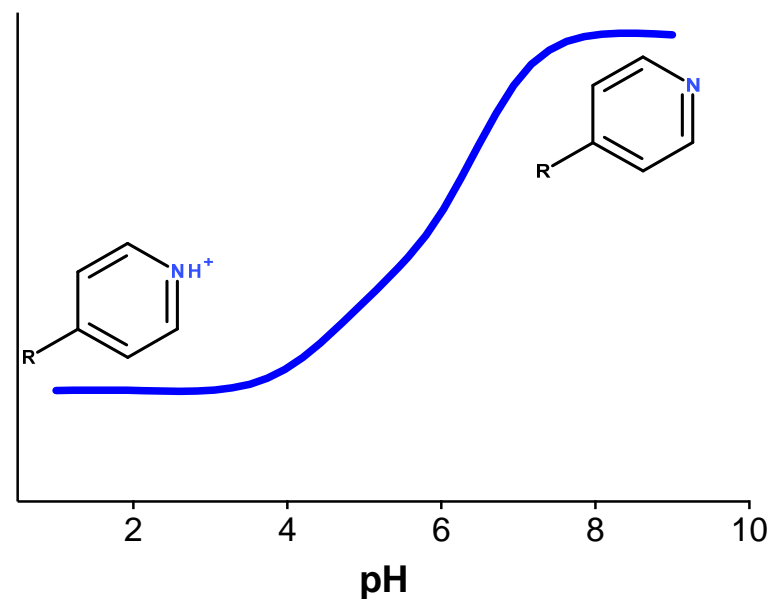
- ⊗ An ionised drug molecule is more hydrophilic (and therefore less lipophilic) than the neutral compound.
- ⊗ This is reflected in a lower LogD value at a pH where the compound is ionised:

Ketoconazole –  $pK_a$  6.43, 3.64 (basic)



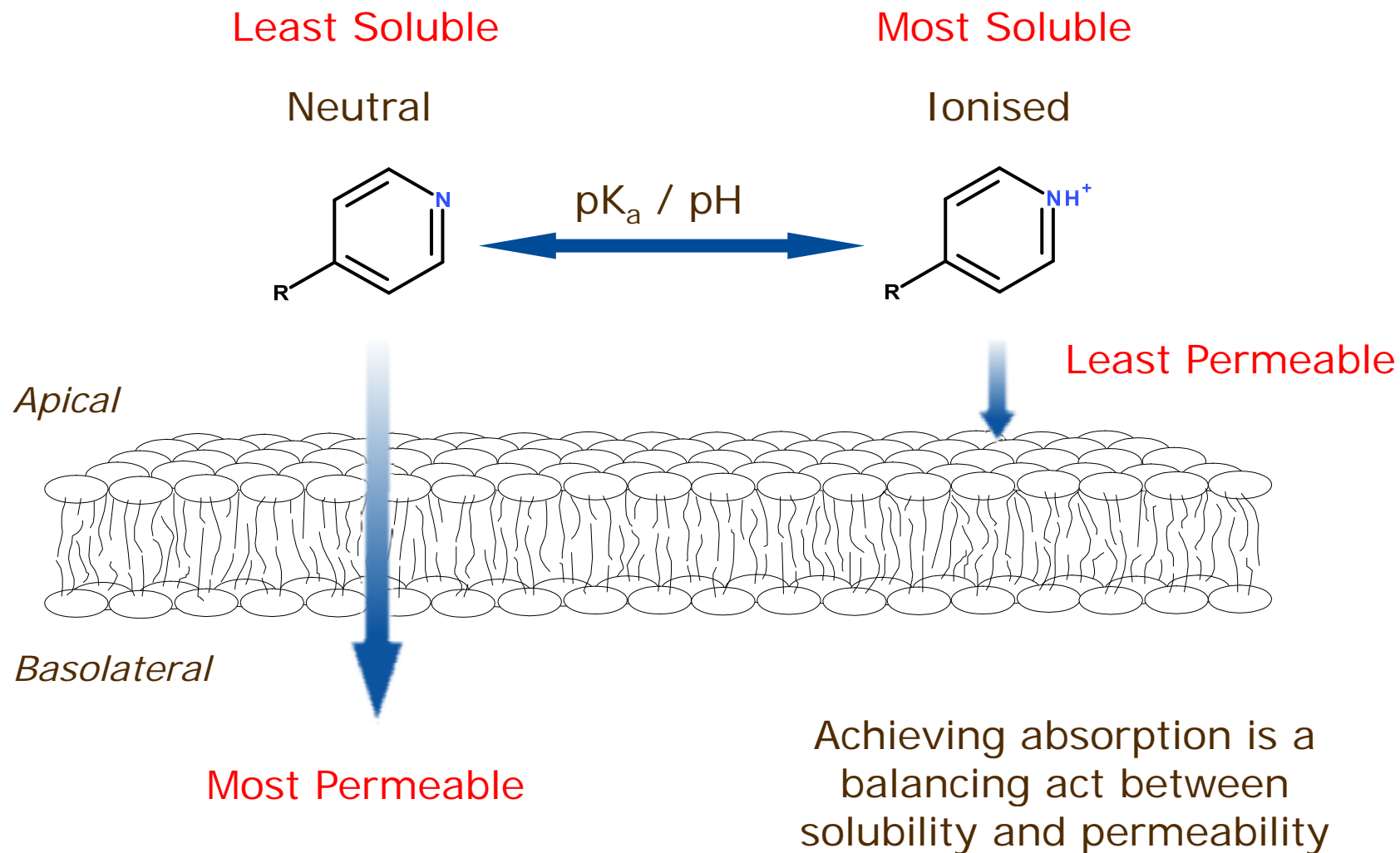
pH 3	pH 5	pH 7.4	pH 9
0.35	2.37	3.46	3.56

Lipophilicity



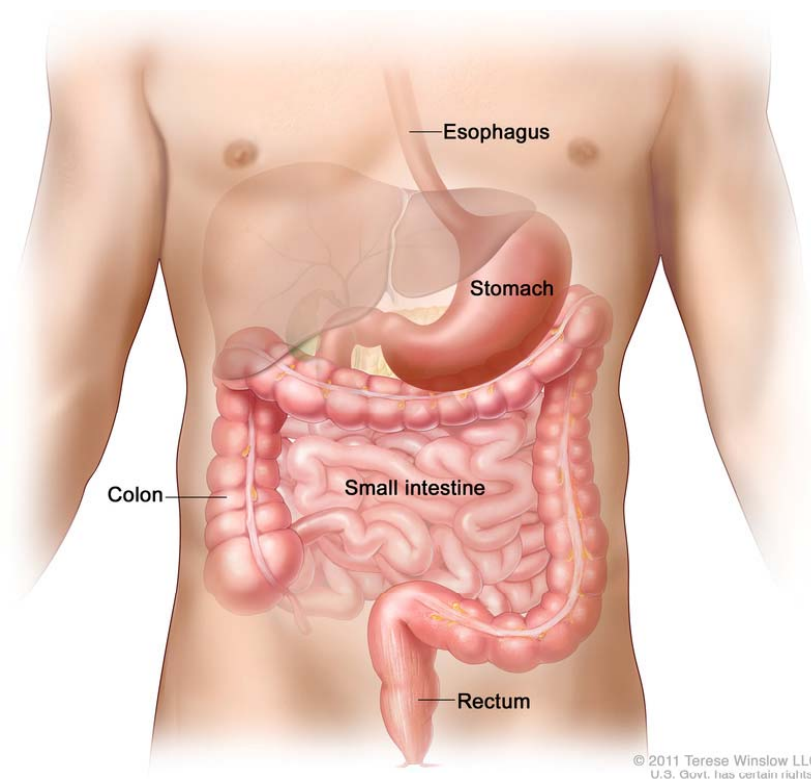
In house LogD assay (5% DMSO, 90 mins incubation at 25 °C)

# pH Partition Theory



# pH variation in the human GI-tract

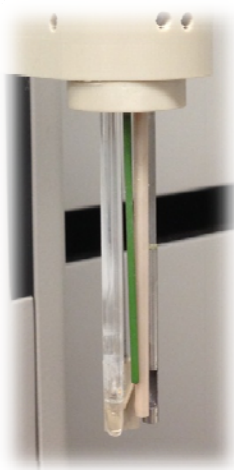
- ⊗ The pH of the environment an orally-dosed API experiences changes along the gastrointestinal tract.



	Surface area (m <sup>2</sup> )	pH (Fasted)	pH (Fed)
Stomach	0.11	1 - 3	4.5 - 5.5
Duodenum	0.09	5 - 6.5	4 - 6
Jejunum	60	6.5 - 7	5.5 - 6.5
Ileum	60	7 - 7.5	6.8 - 7.5
Colon	0.25	5.5 - 7	

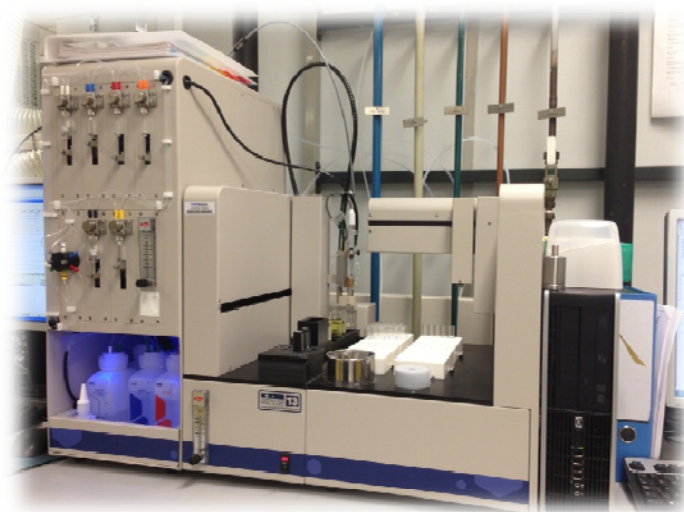
# Experimental Determination of $pK_a$

- ⌚ Prediction packages are available for  $pK_a$  values but are only as good as the databases behind them
- ⌚ There are a number of experimental methods for determining  $pK_a$ :
  - Potentiometric titration
  - UV-spectroscopy
  - NMR



Sirius T3 Probe:

Overhead Stirrer  
Thermometer  
UV-dip probe  
Titrant capillaries  
pH electrode



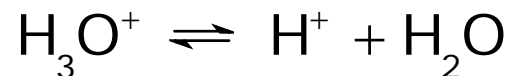
Sirius T3 instrument



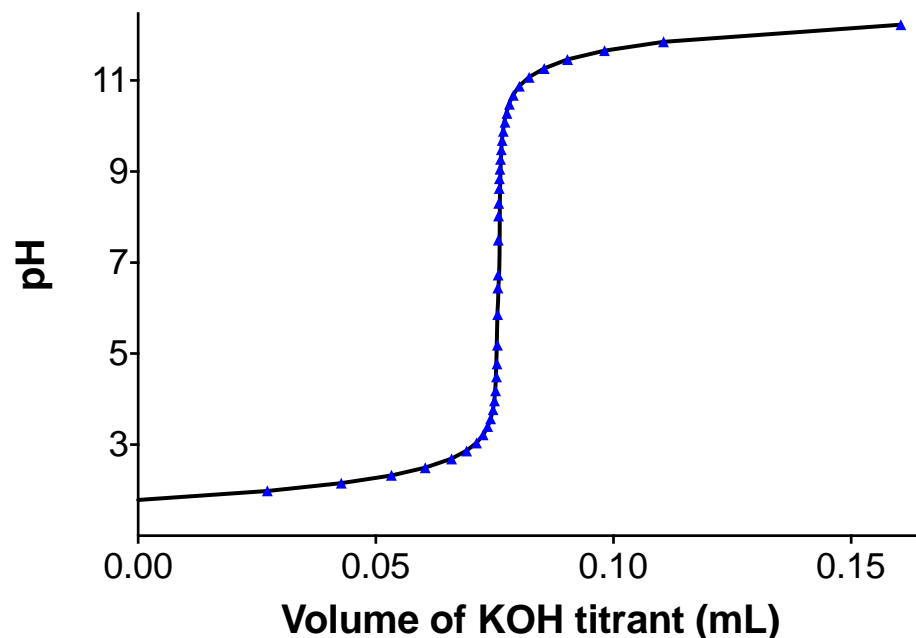
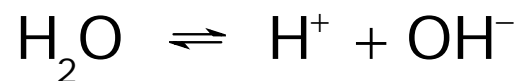
# Potentiometric Titration

- ② Technique uses a pH electrode to measure the concentration of  $\text{H}^+$  ions in solution during a titration from low to high pH
- ② A blank titration is first carried out to characterise the pH electrode and derive a titration curve:

- ② At a pH below 3, water can be protonated:

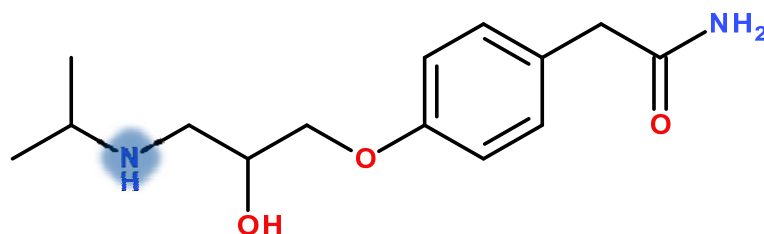


- ② At a pH above 10, water may be deprotonated to form the hydroxide ion:



# Potentiometric Titration

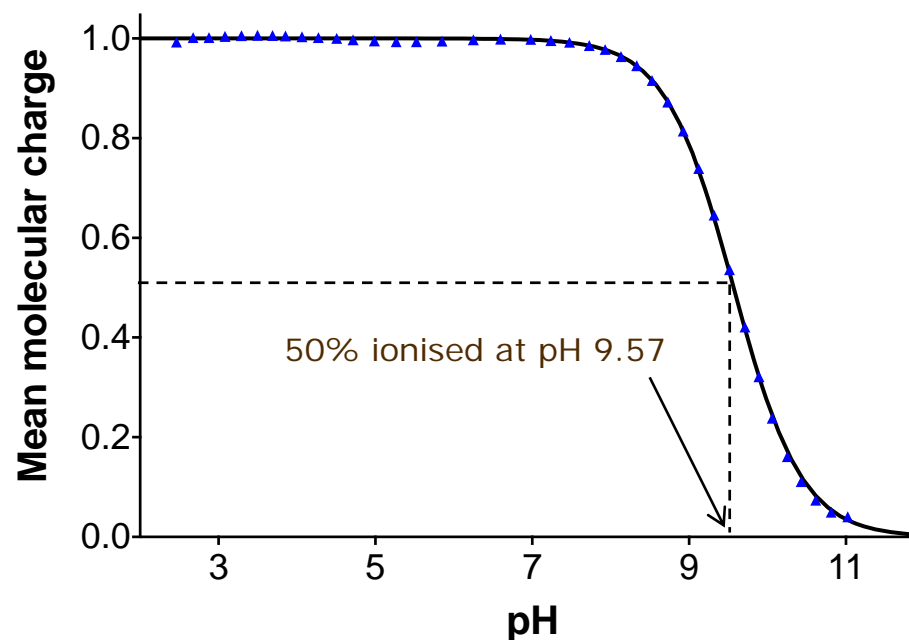
- ⌚ In the presence of an ionisable sample, such as atenolol below, the titration curve may display an additional horizontal region(s), indicative of pH buffering:



Atenolol – basic 2° amine

**Requires a relatively large sample quantity (2-5 mg)**

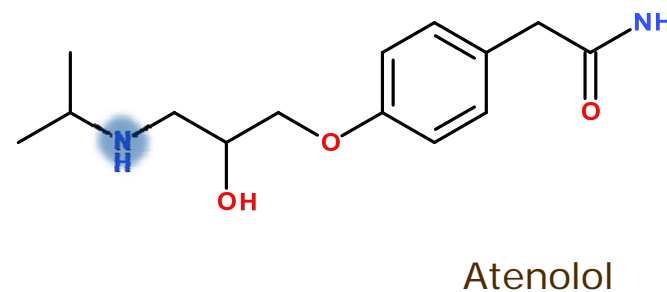
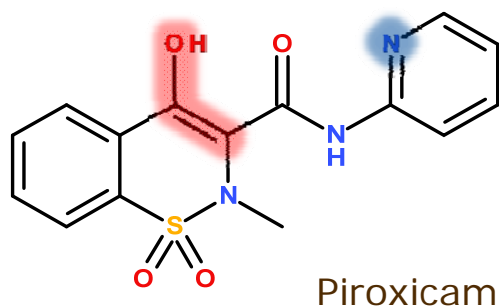
- ⌚ This is subtracted from the blank titration to reveal the ionisation curve
- ⌚  $pK_a$  is calculated at 50%



# UV-metric Titration

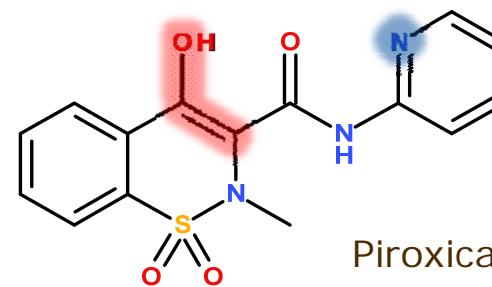
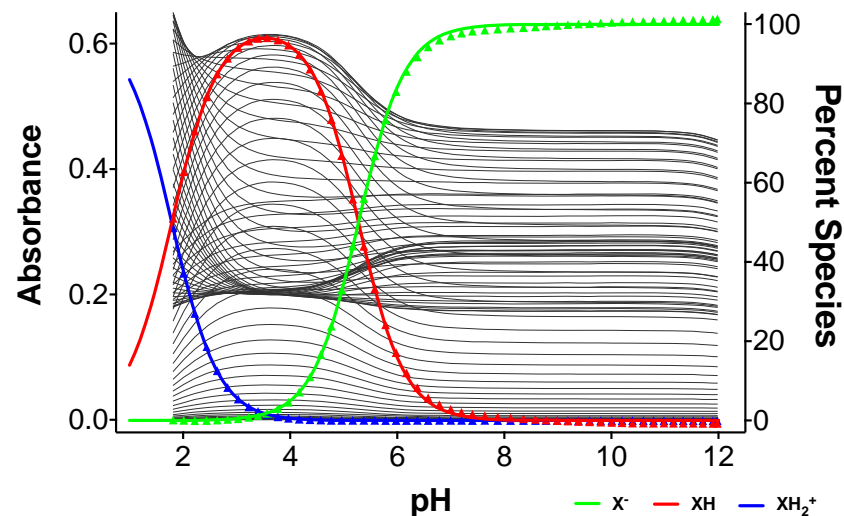
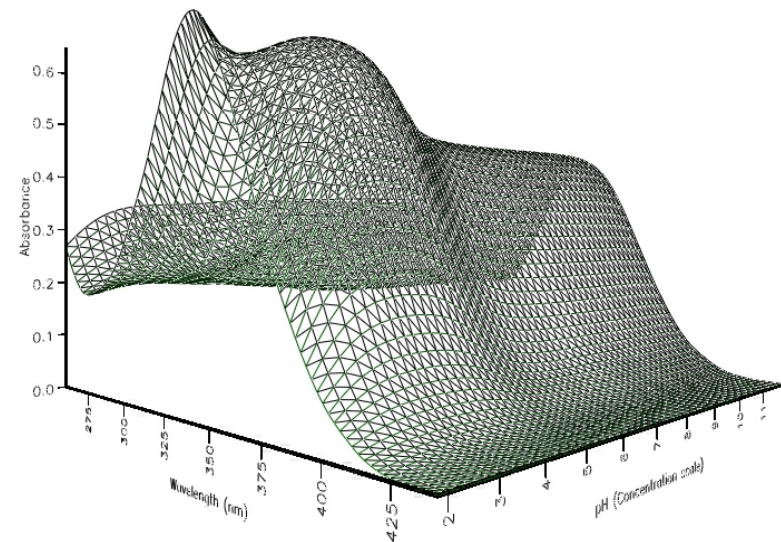
⌚ The UV method offers a more sensitive technique provided:

- The sample has a UV-chromophore
- The site of ionisation is close (typically 3-4 bond lengths) to the chromophore
- A change in ionisation will affect the extinction coefficient of the chromophore



# UV-metric Titration

- ⌚ A solution of the test compound is titrated over the chosen pH range and the UV absorbance is measured at each point
- ⌚ The data is displayed as a 3D matrix of pH vs. absorbance vs. wavelength
- ⌚ Evaluated mathematically using the Beer-Lambert law and Target Factor analysis



Piroxicam

pK<sub>a</sub>: 5.41 (acidic)  
1.89 (basic)

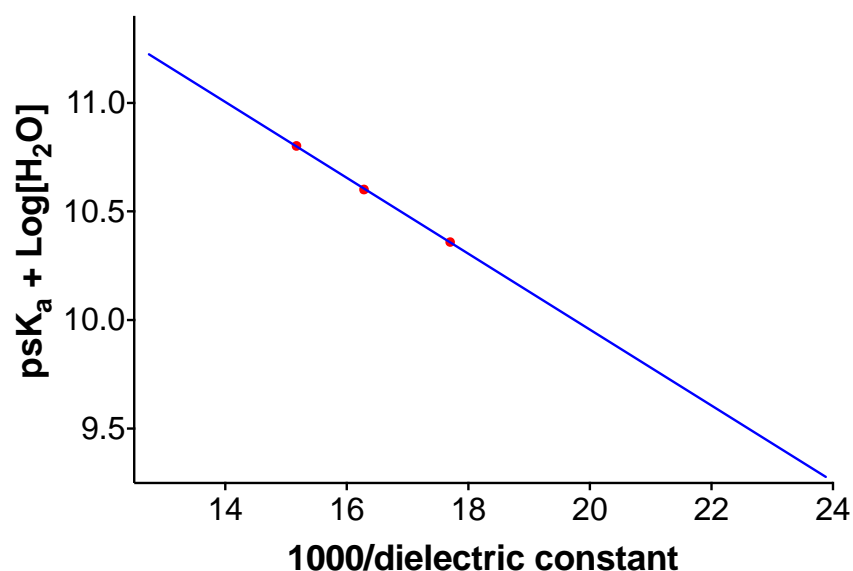
# Apparent $pK_a$ values and co-solvents

- ⌚ Experiments described thus far have all been performed in aqueous solution (0.15 M KCl)
- ⌚ The sample must remain in solution throughout the titration to generate reliable data:
  - pH-metric titrations require ~ **1 mM** sample concentration
  - UV-metric titrations require ~ **50  $\mu$ M** sample concentration
- ⌚ This concentration cannot be obtained for very poorly soluble materials so alternate media must be used
- ⌚ Titrations for such compounds are performed in varying ratios of a co-solvent:water and extrapolated to 0% co-solvent
  - Methanol is the most commonly used



# Apparent $pK_a$ values and co-solvents

- ⌚ Propranolol precipitated out of solution during a pH-metric titration at a pH value approaching its  $pK_a$
- ⌚ Performing three consecutive titrations in decreasing co-solvent ratios (50, 40 and 30 wt.% methanol in water) enabled extrapolation to the aqueous value

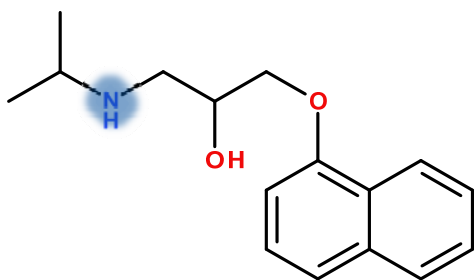


- ⌚ Most common extrapolation used is Yasuda-Shedlovsky
- ⌚ Plotted as the reciprocal of the dielectric constant against the apparent  $pK_a + \text{Log}[H_2O]$
- ⌚ Aqueous  $pK_a$  is calculated using the dielectric constant of pure water (78.31 at 25 °C)

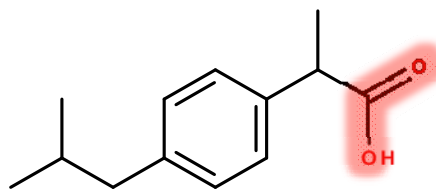
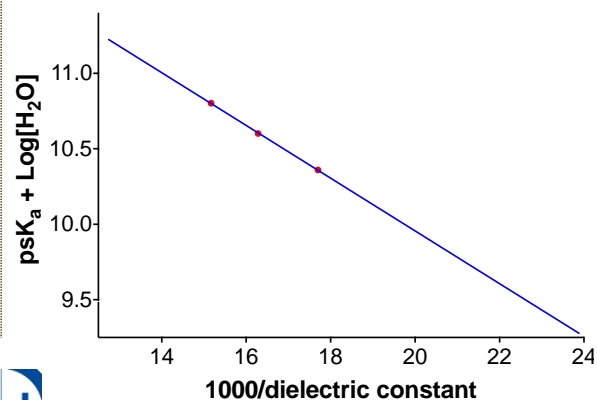
# Apparent $pK_a$ values and co-solvents

⊗ The gradient of the slope can indicate the type of measured  $pK_a$

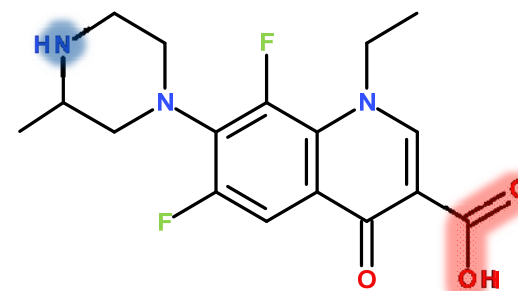
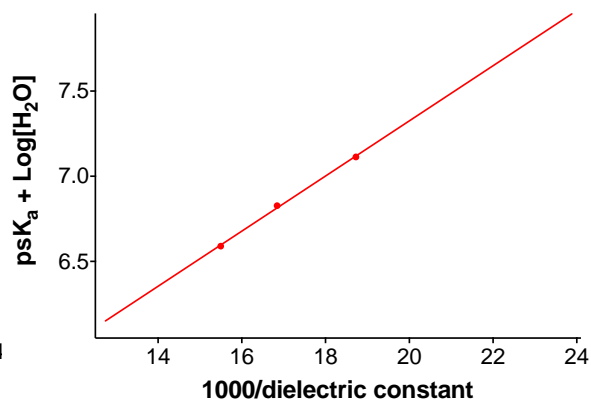
- The apparent  $pK_a$  of a base **decreases** with increasing co-solvent
- The apparent  $pK_a$  of an acid **increases** with increasing co-solvent



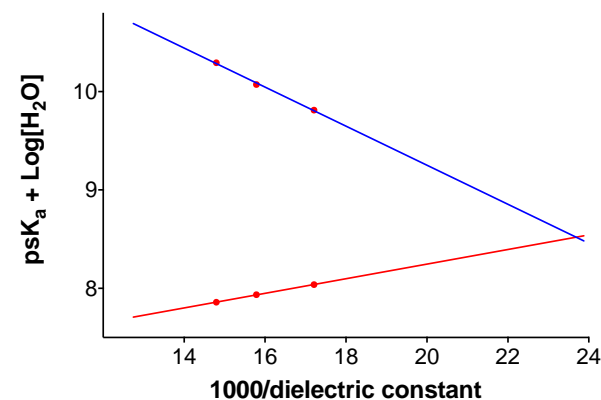
Propranolol - base



Ibuprofen - acid



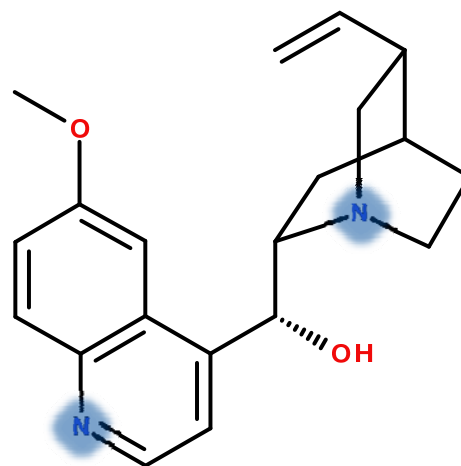
Lomefloxacin - ampholyte





# pK<sub>a</sub> determination by NMR

- ⌚ Potentiometric and UV-metric titrations are able to identify the type and value of a pK<sub>a</sub> but nothing about the location of the ionisation
- ⌚ In a compound with the multiple centres, the pK<sub>a</sub> of a specific site may be of interest
- ⌚ Ionisation of a molecule introduces a localised charge to the structure and thus affects the chemical shift of adjacent atoms
- ⌚ <sup>1</sup>H, <sup>13</sup>C, <sup>19</sup>F NMR spectroscopy can be used

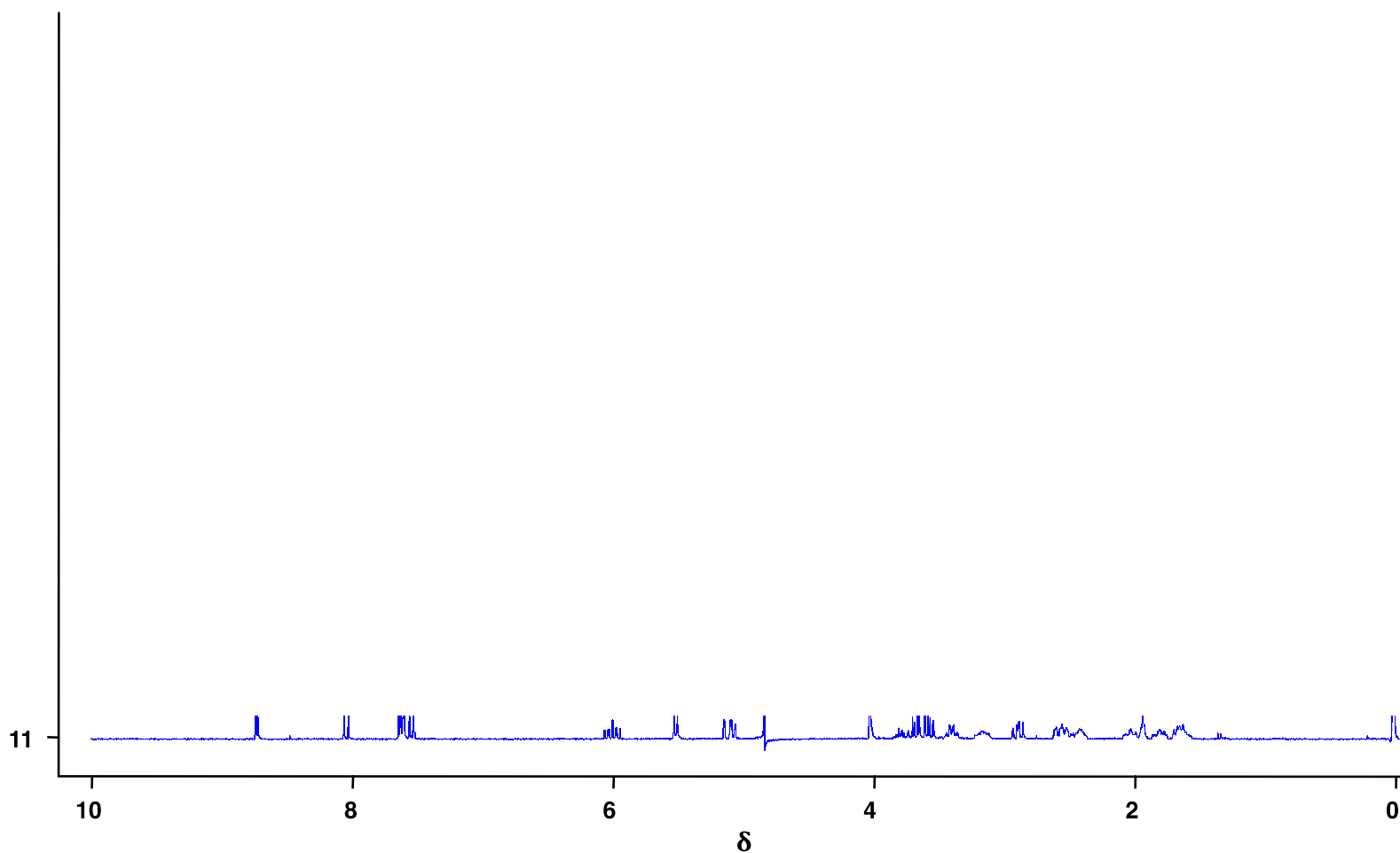


Quinine - dibasic



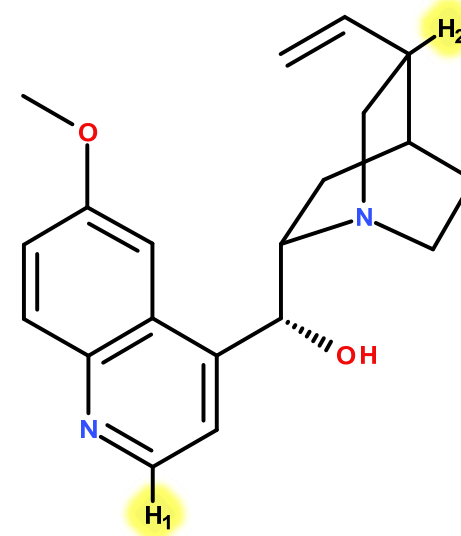
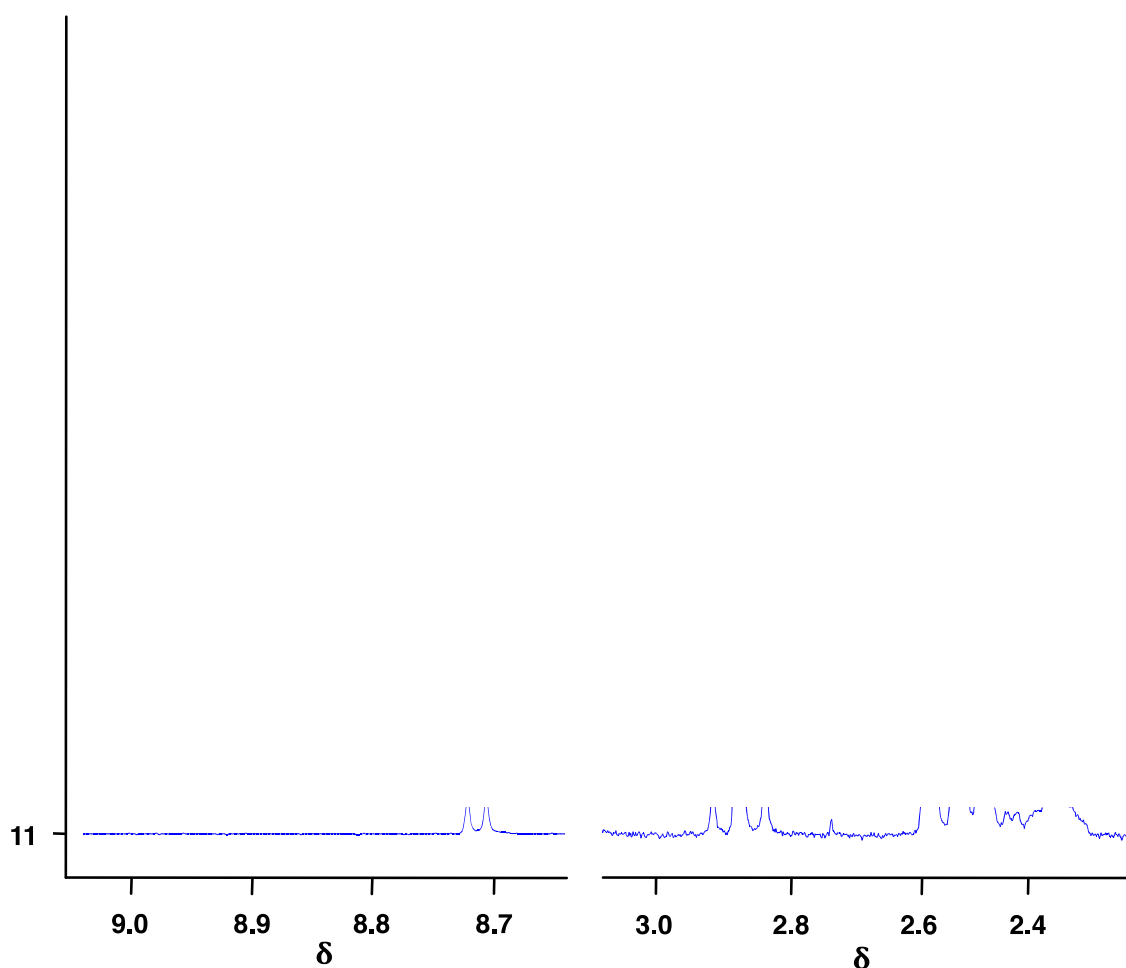
# pK<sub>a</sub> determination by NMR

- ② The compound is titrated across the desired pH range and an NMR spectrum is recorded at each pH point:



# pK<sub>a</sub> determination by NMR

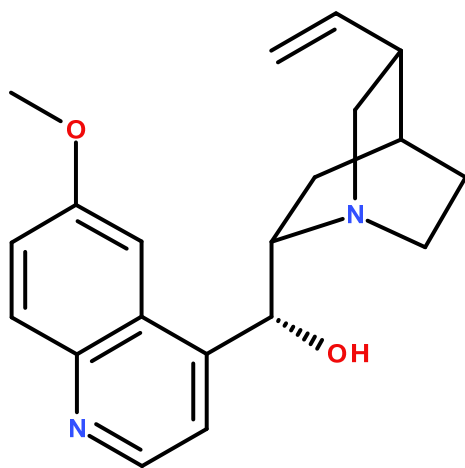
- ⊗ The shift in chemical shift is more pronounced on protons close to the site of ionisation:



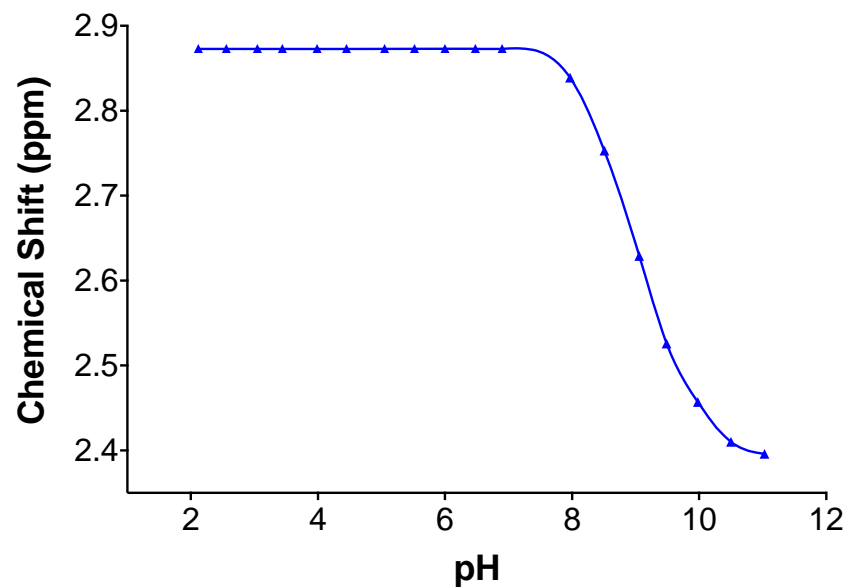
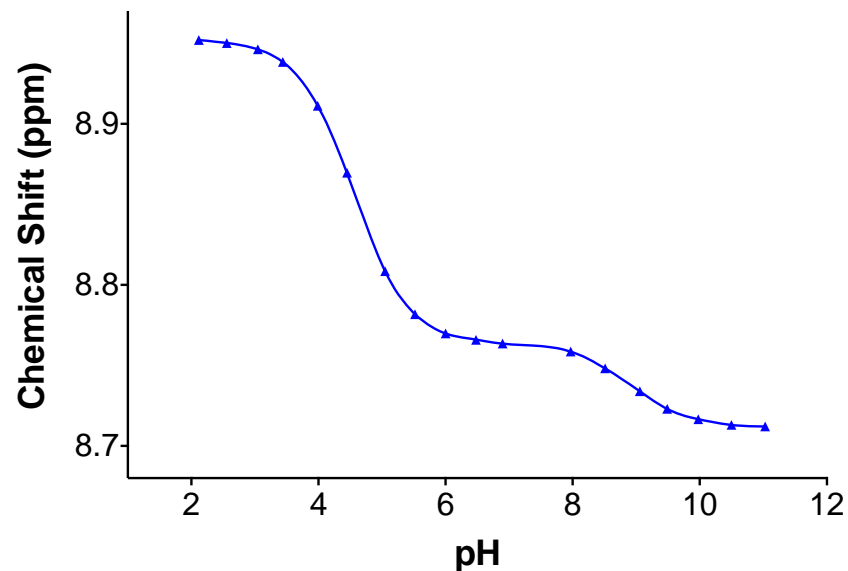
Quinine - dibasic

# pK<sub>a</sub> determination by NMR

- ② The chemical shift is plotted against the pH to construct the pK<sub>a</sub> curve
- ② pK<sub>a</sub> taken as the pH at the mid-point of the chemical shift difference

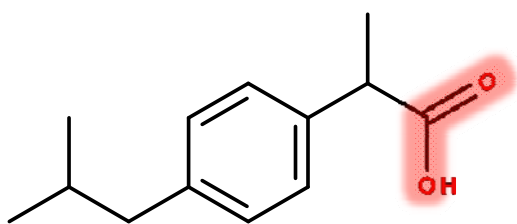


pK<sub>a</sub>: 4.50 (basic)  
9.00 (basic)

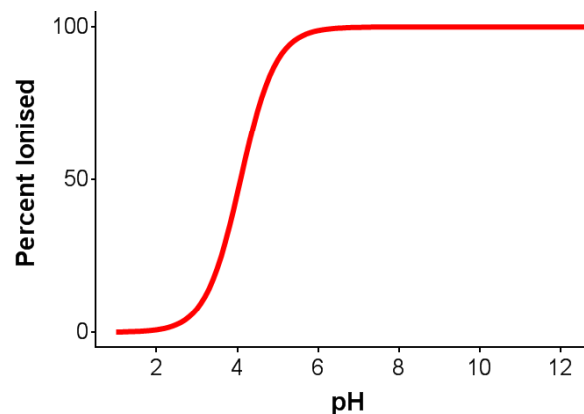


## A simple example..

- ⌚ Ibuprofen is an NSAID often used to treat headaches



$pK_a = 4.22$  (acidic)



- ⌚ Virtually insoluble in the fasted stomach (~pH 2)
- ⌚ Eating will raise the pH of your stomach to ~pH 5, thus increasing solubility
- ⌚ If you've had a heavy night: Fry-up first!



# Conclusions

- ④ The majority of compounds contain at least one ionisable centre
  - A knowledge of the extent of ionisation at these sites in the body leads to a greater understanding of physicochemical properties of a potential drug candidate
- ④ A variety of experimental methods for the determination of ionisation constants are available and can determine:
  - The value of the  $pK_a$
  - The type of the  $pK_a$  (acidic/basic)
  - The location of the ionisation event
- ④ The importance of the ionisation event can be seen in everyday life

# Acknowledgements

Richard Taylor  
Justin Staniforth  
Christine Prosser  
Hayley Roy  
Phil Gilbert  
Ian Whitcombe  
Matt Selby

Karl Box  
Robert Taylor





# Questions?



$$\text{pK}_a = -\log\left(\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}\right)$$

$$= -(\log [\text{H}^+] + \log [\text{A}^-] - \log [\text{HA}])$$

$$= -\log [\text{H}^+] - \log [\text{A}^-] + \log [\text{HA}]$$

$$\text{pH} = -\log [\text{H}^+]$$

$$\text{pK}_a = \text{pH} + \log\left(\frac{[\text{HA}]}{[\text{A}^-]}\right)$$

