

# $pK_a$ and log P

## Background Information



' $pK_a$  affects solubility, permeability, log D and oral absorption by modulating the distribution of neutral and charged species.'

<sup>1</sup>Di L and Kerns EH. (2003) *Current Opinion in Chemical Biology* 7; 402-408.

- The  $pK_a$  is the pH at which the molecule is 50% protonated.
- Log P (or partition co-efficient) is a measure of the lipophilicity of a compound.
- Cyprotex's  $pK_a$  and log P determination uses UV-metric and pH-metric technology developed by Sirius, which is considered to be a 'gold standard' method for determining these properties.
- In UV-metric methods, the  $pK_a$  is measured by analysing changes in multi-wavelength UV spectra during acid-based titration of the sample. UV-metric  $pK_a$  methods work for compounds with pH-sensitive chromophores.
- In pH-metric methods,  $pK_a$  is measured by titrating a solution of the sample in water or solvent with acid and base, and calculating the  $pK_a$  from the shape of the titration. pH-metric methods work for any ionisable compound, but require more sample than UV-metric methods.
- The pH-metric method is also used to measure log P in a two-phase acid-base titration in the presence of octanol.

### Protocol

#### Method

Fast UV titration for  $pK_a$   
UV-metric titration for  $pK_a$   
Potentiometric (pH-metric) titration for  $pK_a$  and log P

#### Instrument

SiriusT3

#### Test Article Requirements

3-5  $\mu$ L of 10 mM stock solution (UV-metric)  
1 mg solid compound (pH-metric)

#### Partition Solvent used for

##### Log P Determination

n-Octanol (others available on request)

#### Data Delivery

$pK_a$   
log P (optional)  
Standard error  
RMSD  
Calculated log D at  $pH_{7.4}$   
(based on  $pK_a$  and log P)

# log P is determined from the shape of titration curves obtained in dual-phase titrations.

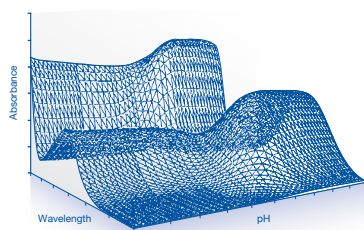


## pK<sub>a</sub> and log P

pK<sub>a</sub> measurements are determined using the SiriusT3 instrument from Sirius-Analytical using either a UV-metric or pH-metric approach.

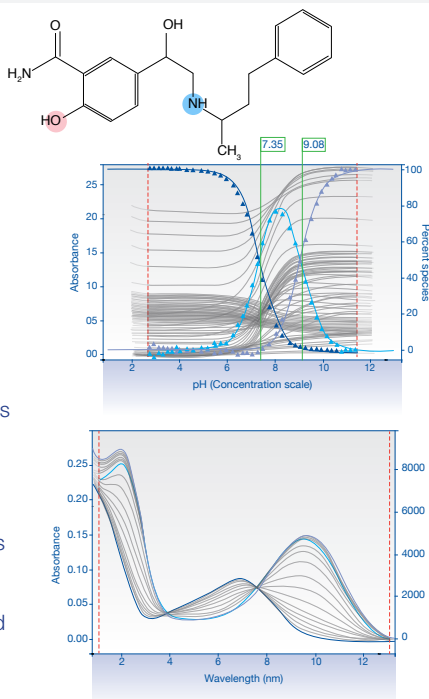
**Figure 1**

UV-metric method for measuring pK<sub>a</sub> values.



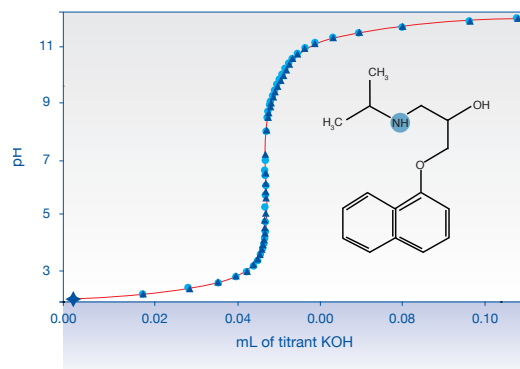
UV-metric methods provide pK<sub>a</sub> results for samples with chromophores whose UV absorbance changes as a function of pH.

On SiriusT3, the Fast UV method measures absorbance at 250 wavelengths and 54 pH values in a buffered solution in about 5 minutes. The slower UV-metric method in unbuffered solution extends the pH range below 1 or above 13. The 3D graph shows data from the measurement of labelalol pK<sub>a</sub>s. The other graphs are 2D projections showing change in absorbance vs. pH and vs. wavelength, with percent species and molar absorbance coefficients overlain.



**Figure 2**

pH-metric method for measuring pK<sub>a</sub> values.

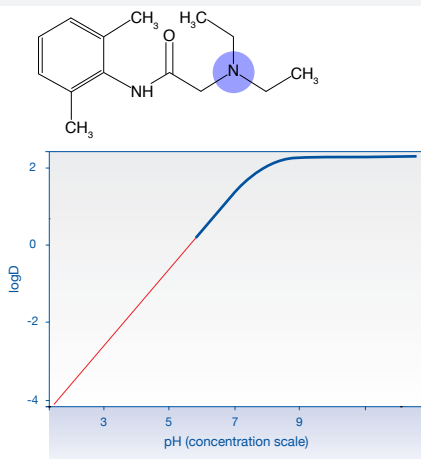
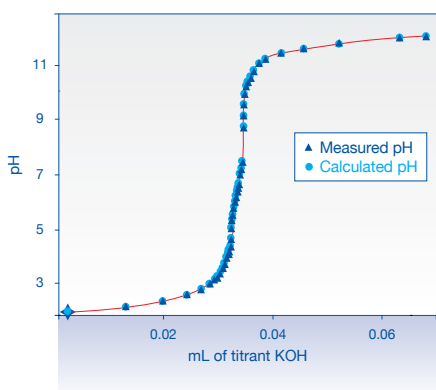


pH-metric methods are based on potentiometric acid-base titration. Results are obtained by a complex computational process. The pH of each point in the titration curve is calculated using equations that contain pK<sub>a</sub>, and the calculated points are fitted to the measured curve by manipulating the pK<sub>a</sub> value. The pK<sub>a</sub> that provides the best fit is taken to be the measured pK<sub>a</sub>. pH-metric methods will measure all pK<sub>a</sub>s between 2 and 12, provided the sample is in solution throughout the experiment.

**Figure 3**

pH-metric method for measuring log P values

log P of lidocaine = 2.30



In the pH-metric method for log P, a weighed sample is dissolved in a two-phase water-octanol system, and titrated over a pH range (typically 2 to 12 for bases and ampholytes, 12 to 2 for acids). Although the solution becomes opaque during stirring, the pH electrode continues to measure pH of the aqueous component of the solution. Results are obtained by a complex computational process. The pH of each point in the titration curve is calculated using equations that contain pK<sub>a</sub> and P, and the calculated points are fitted to the measured curve by manipulating the P value. The P that provides the best fit is taken to be the measured P value, which is reported as the logarithm, i.e. log P. As well as log P, the log D value as a function of pH is determined from the data.

## References

<sup>1</sup> Di L and Kerns EH. (2003) *Current Opinion in Chemical Biology* 7; 402-408.