

Overview

This poster describes a hybrid potentiometric/UV method that can accurately measure extreme pK_a s down to 0.7 (as well as pK_a s above 13).

Extreme pK_a values are difficult to measure by potentiometric methods or by capillary electrophoresis, and because of a scarcity of reliable measured pK_a values in the literature which can be used to train software, predicted pK_a values are also suspect.

As an example of their importance, pK_a s below 3 of drugs will cause basic groups to ionize in the stomach where pH is normally between 1 and 2, and when ionized, drugs will be more soluble. Knowledge of their pK_a values could be used for understanding dissolution properties, as well as for designing pharmaceutically relevant salts.

Method

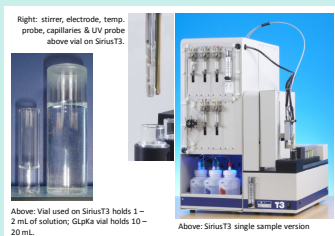
A stock solution of the compound is prepared in DMSO (10mM) and an aliquot (typically 5 μ L) is pipetted into a glass vial.

To measure a low pK_a , the sample is diluted with 1mL of 0.15M KCl solution, to which sufficient 0.5M HCl solution is added to lower the pH to below 1.0. The solution is then titrated with 0.5M KOH solution. To measure a high pK_a , the pH of the diluted sample is raised with 0.5M KOH, and the solution is titrated with 0.5M HCl.

Multiwavelength UV spectra are recorded after each titrant addition, and results are obtained from analyzing changes in the UV spectra as a function of pH.

Apparatus

Measurements were done using Sirius GLpKa and D-PAS instruments, or by the newer Sirius T3 (illustrated). The SiriusT3 features up to 6 automated dispensers for reagent addition, pH electrode, stirrer, in-situ UV probe and automatic calibration and cleaning procedures. The titration vessel is temperature controlled by a Peltier device and features a turbidity detection system for automated detection of precipitation events. An optional autoloader module allows for unattended analysis of up to 192 samples for pK_a , logP/D and solubility.



Results

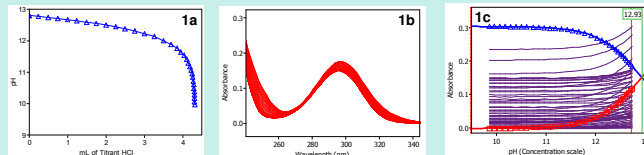
The table shows measured pK_a values for 12 compounds. Each of these compounds has at least one pK_a below 2 or above 12.

pK_a s associated with acidic groups are labelled (A); pK_a s associated with basic groups are labelled (B).

Even at the low concentrations used for pK_a measurement by UV, some compounds were poorly soluble in neutral form, and their pK_a values were determined by Yasuda-Shedlovsky extrapolation from values measured in water-methanol mixtures.

Compound	Extreme pK_a results (below 2, above 12)	Other pK_a s	Compound class
4-aminosalicylic acid	1.84 (B) 13.24 (A)	3.62 (A)	Ampholyte
Buspirone	1.67 (A)	7.61 (B)	Ampholyte
Dipyridamole	0.88 (B)	6.20 (B)	Base
Luminol	1.42 (B)	6.26 (A)	Ampholyte
Meloxicam	1.21 (B)	4.03 (A)	Ampholyte
Olsalazine	1.97 (A)	2.91 (A)	Acid
Phenazopyridine	12.12 (A)	11.05 (A)	Acid
Salicylic acid	0.67 (B)	5.13 (B)	Base
Sulfacetamide	12.98 (B)	2.78 (A)	Ampholyte
Sulfasalazine	1.59 (B)	5.24 (A)	Ampholyte
Tenoxicam	1.23 (B)	2.27 (A), 8.00 (A) _v	Ampholyte
Theophylline	1.07 (B)	10.84 (A)	Ampholyte
	0.99 (B)	5.22 (A)	Ampholyte
		8.53 (A)	Ampholyte

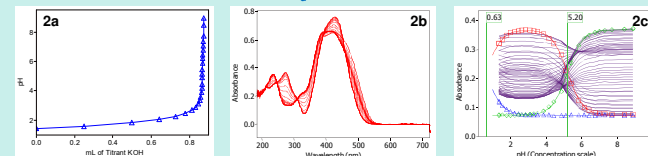
Case study 1: Salicylic acid high pK_a



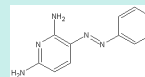
The carboxylic acid pK_a of salicylic acid (2.78) is easily measured by pH-metric or pH-UV methods, but the pK_a associated with the phenol is difficult to measure by classical techniques. In this experiment the pH of the starting solution was raised to 12.8 by adding strong KOH solution, and the solution was titrated with 0.5M HCl. Lines in fig. 1b represent absorbance vs. wavelength at each pH; lines in fig. 1c represent absorbance vs. pH at each wavelength. The curvature and crossover in fig. 1c at high pH indicate that the sample is converting between different ionizable forms. The pK_a result of 12.93 was obtained by target factor analysis applied to the pH-wavelength-absorbance data.



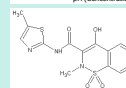
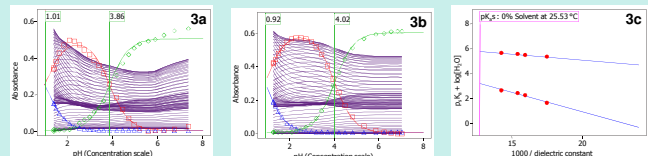
Case study 2: Phenazopyridine low pK_a



The pyridine pK_a of Phenazopyridine (5.20) is easily measured, but the pK_a s associated with the two amines are very low. We could measure one of the amine pK_a s; the other remains low to measure. The pH of the starting solution was lowered to 1.5 by adding strong HCl solution, and the solution was titrated with 0.5M KOH.



Case study 3: Meloxicam low pK_a



Meloxicam is an ampholyte. At pH between the basic pK_a and acidic pK_a s it is neutral, and poorly soluble in water. Figure 3a shows a poor fit when attempting to measure pK_a s in aqueous solution. Figure 3b shows an excellent fit when the sample is measured in aqueous solution containing 30% methanol. The pK_a results shown in Table 1 are obtained by Yasuda-Shedlovsky extrapolation, as shown in Figure 3c.

Conclusion

pH-metric instrumentation is unique in being able to automatically measure such extreme pK_a s. The high resolution of datapoints in the titrimetric method adds a level of reliability that other approaches cannot match.