Fast pK_a screening starting from 3µL of 10mM DMSO stock

Karl Box[1], John Comer[1], Tom Gravestock[1], Jon Mole[2]

[1]Sirius Analytical, Forest Row, East Sussex, RH18 5DW, UK.
[2]Sirius Analytical Inc. Suite A, 1825 Swarthmore Avenue, Lakewood, NJ 08701

Abstract

Purpose: To develop a protocol for rapidly and successfully measuring pK_a values of a wide range of drugs using small weights of sample.
Methods: Because of the great diversity of structures of drug molecules, it is difficult to find a single method that can be relied on to measure any and all pK_as in just one attempt. An efficient approach has been developed in which pK_as are measured in aqueous solution at low concentration in experiments lasting 4 minutes, during which multiwavelength UV spectra are collected during acid-base titration in a buffered solution. Failed experiments are easily analysed to enable an alternative, successful method to be chosen and run on the same instrument.
Results: Samples were chosen to exemplify different classes of drug, with varying water solubility, UV absorbance and number of pK_as. All samples were initially screened by fast, aqueous pH-UV titration using 3 microlitres of 10mM stock solution in DMSO (typically 10µg of sample per aliquot). Successful samples are shown in table 1. Some samples were poorly water-soluble but remained in solution via supersaturation for the duration of the short experiment. There were two classes of failed measurements: either UV absorbance changed during the experiment but the sample precipitated, or there was no UV change during the experiment. Samples that precipitated were re-run successfully using 3µL of stock in three consecutive fast UV experiments in the same solution with three different ratios of water-methanol created by successive dilution and aqueous pK_a(s) obtained by extrapolation (table 2). A new instrument, **Sirius T3** enabled the remaining samples to be measured by potentiometric titration in 1mL of water-methanol using 50 microlitre aliquots of sample from 10mM DMSO stock solution (typically 0.2mg of sample per aliquot) (tables 3 and 4).
Conclusion: A two-step analytical approach enables pK_a(s) of ionizable drugs to be measured quickly and accurately using only 10µg of sample by the pH-UV approach and 0.2mg by the potentiometric approach.

Figure 1 (above). Sirius T3 automated PhysChem screening platform. The instrument comprises a dispenser module for reagent addition, titration module for measuring physicochemical properties of drugs in solution, and autoloader module with robotic arm for moving samples.

Figure 2 (right). Probes being lowered into vial containing 1mL of sample solution. Probes comprise pH electrode, thermometer, stirrer, UV dip probe and 5 reagent capillaries. Temperature of vial holder is controlled by Peltier system.

Figure 3 (above). The robotic arm of the autoloader module picks and places samples for analysis from 48-well position racks.

The Sirius T3 (figures 1,2 and 3) is a new instrument that has been developed for measuring pK_as using very small quantities of drug substance. Due to the great diversity of structures of drug molecules, traditionally it is difficult to find a single method that can be relied on to measure any and all pK_as in just one attempt. The new instrument uses a combination of approaches to assess and successfully determine these parameters. A suggested procedure is outlined in the flow-chart shown in figure 4.

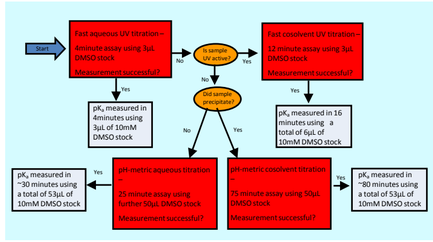

Figure 4 (left). Flow-chart with suggested work-scheme. Samples are first screened using the fast UV titration method. Successful compounds are identified and results reported. Those requiring further work are re-scheduled for further analysis, i.e., cosolvent UV or pH-metric.

(Note, >70% of samples are successful by a UV method.)

An efficient approach is first to use a Fast UV technique in aqueous solution. This four-minute experiment will often provide a valid result, but if it does not, the reason for failure will allow the analyst to choose an appropriate alternative method that will work. The Fast UV technique uses only 3µL of a 10mM DMSO stock solution in which the sample is dissolved in a multi-component buffer solution and titrated between pH 2 and 12 (figure 5) in only 1mL titration volumes.

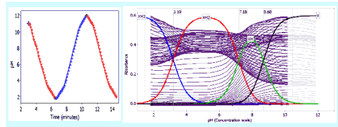

Figure 5 (above left). Three consecutive 4-minute Fast-UV titrations are performed on oxytetracycline.

Figure 6 (above right). The pK_as are fitted to the changes in UV absorbance data and overlaid with the distribution of species.

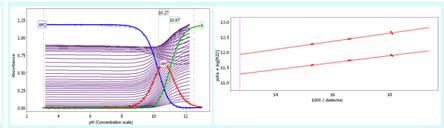

Figure 7 (above left). The p_cK_as in 30% methanol cosolvent solution are fitted to the changes in UV absorbance data and overlaid with the distribution of species for diethylstilbestrol.

Figure 8 (above right). The results from three consecutive 4-minute Fast-UV titrations in different percentages of methanol are extrapolated to aqueous conditions.

The presence of the buffer allows for a stable pH to be reached very quickly after each addition of titrant. The pK_a result is obtained from changes in multiwavelength UV spectra (220- 700nm) with respect to pH (figure 6), which occur if the disposition of electrons in chromophores containing conjugated double bonds, carbonyl groups and other UV-absorbing groups changes with the molecule's ionization state. It is obvious from the data quality if the Fast UV method has worked, and this data quality can be automatically monitored by software without need for manual inspection. If the method works, then the result will be the aqueous pK_a, which is the result required. Moreover, the result will have been obtained in about four minutes.

There are two principal reasons why the Aqueous Fast UV technique could fail: the sample could precipitate during the assay, or it could have no pH-active chromophore. If the sample has UV response but it precipitated, it can be re-run by Fast UV in the presence of cosolvent. An example is shown is figure 7 for diethylstilbestrol and results extrapolated to aqueous conditions (figure 8). Therefore, only 6µL of a 10mM DMSO stock solution was required to determine pK_as of compounds in this category.

In terms of throughput, the Fast UV method could measure 50 samples in aqueous solution or 18 samples in the presence of cosolvent in about 3.5 hours.

If the sample had no UV response but it did not precipitate, it can be re-run by a pH-metric method under aqueous conditions. If it had no UV response and it precipitated, it can be re-run by a pH-metric method in the presence of cosolvent.

Potentiometric titration is a powerful method for pK_a measurement because it is based only on pH measurement (hence the name pH-metric). It can therefore measure pK_a(s) of all drugs, including those such as aliphatic amines or carboxylic acids that show no significant UV absorbance associated with the ionizable group. The Sirius T3 has been developed for titrating in 1mL volume of solution, making it possible to do pH-metric titrations using 0.2mg of sample. Because such low amounts are difficult to weigh, samples were prepared using 50µL aliquots of 10mM sample stock solution in DMSO.

pH-metric methods take about 25 minutes to measure a sample in aqueous conditions or 75 minutes to measure one sample in the presence of cosolvent using three consecutive titrations in the same vial. An example of pH-metric data is shown for the aqueous titration of ofloxacin (figures 9-11).

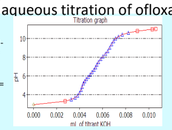

Figure 9. Aqueous pH-metric titration of of ofloxacin.

Dispenser resolution = 0.000005mL.

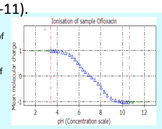

Figure 10. Ionisation of Ofloxacin showing average molecular charge as a function of pH. pK_as occur at charges of +0.5 and -0.5.

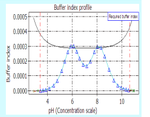

Figure 11. Buffer capacity plot for ofloxacin showing pK_as at 5.98 and 8.17.

Results in 1mL titration volumes using the Fast UV method are shown in tables 1 and 2 while tables 3 and 4 show results using the pH-metric method.

Sample	pK_a Result
Chloroquine	8.37, 10.49
Glipizide	5.15
Isoxicam	3.79
Nitrazepam	3.14, 10.71
Oxytetracycline	3.20, 7.22, 8.74
Phenazopyrine	5.13
Piroxicam	1.89, 5.32
Tenoxicam	1.12, 5.27
Theophylline	8.54
Warfarin	4.86

Table 1 pK_a results for compounds measured using the aqueous Fast UV method.

Sample	pK_a Result
5-chloro-7-iodo-8-quinolinyl	3.10, 7.58
Amodiaquin	7.49, 8.30, 11.65
Bifonazole	6.32
Clofazimine	9.82
Dicumarol	2.43, 8.69
Diethylstilbestrol	9.55, 10.19
Quinacrine	8.76, 9.49

Table 2 pK_a results for compounds measured using the cosolvent Fast UV method.

Sample	pK_a Result
Azithromycin	8.76, 9.49
Captopril	3.49, 9.81
Gabapentin	3.69, 10.70
Erythromycin	8.85
Lidocaine	8.01
Orphenadrine	9.03
Pramoxine	7.11
Propranolol	9.52

Table 3 pK_a results for compounds measured using the aqueous pH-metric method.

Sample	pKa Result
Chlorpromazine	9.20
Chlorprothixene	9.46
Dicyclomine	8.63
Diphenoxylate	6.71
Haloperidol	8.47
Imipramine	9.48
Loperamide	8.91
Verapamil	8.75

Table 4 pK_a results for compounds measured using the cosolvent pH-metric method.

Conclusion Decision-making and assay setup can now be automated entirely from DMSO stock solutions. A two-step analytical approach enables pK_a(s) of ionizable drugs to be measured quickly and accurately. By titrating in 1mL total solution volume it is possible to provide a full pK_a characterization over the pH range pH 2-12. Only 3µL of 10mM DMSO stock (~10µg sample) is required for the UV method and 50µL of 10mM DMSO stock (~0.2mg sample) for the pH-metric method.

Sirius - Instruments and PhysChem Analytical Services
www.sirius-analytical.com

Instrumentation

pK_a, logP & logD,
Solubility & dissolution
Surface Activity Profiling
Phospholipidosis

Analytical Services

PhysChem properties – pK_a, logP, logD, solubility , dissolution
Solid state assays – XRPD, DSC, TGA, Raman
Surface Tension – CMC, TSA, K_{AW}

For more information please contact
sirius@sirius-analytical.com

Sirius Analytical Ltd.
Riverside, Forest Row Business Park,
Forest Row, East Sussex, RH18 5DW, UK
Phone: +44 1342 820720 **Fax:** +44 1342 820725
Web: www.sirius-analytical.com