pK_a measurement using 50 - 100µL aliquots from DMSO stock, no chromophore required

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Abstract

Purpose: pH-metric pK_a measurement requires a weighed sample of pure drug. For titrations done in 1mL of solution, the amount required is so small that it is difficult to weigh accurately. A method was therefore sought for taking samples as aliquots of solution in DMSO stock.

Methods: 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. Though powerful, the method has been difficult to apply for drug samples from discovery because the weight required for titrating in 10mL volume (2 – 5 mg) may be too high. A new instrument has been developed for titrating in 1 mL 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 50-100µL aliquots of 10mM sample stock solution in DMSO.

Results: The goal of this study was to investigate the effect of volumes of DMSO introduced on the aqueous pK_a value. 100µL of DMSO represents 10% of the volume of a titration in 1mL, and it is known that the presence of DMSO changes the apparent pK_a (see pramoxine example in Figure 1a). This work shows that the average shift in pK_a caused by 10% DMSO varies between 0.03 and 0.26, and that the shift is similar when pK_a(s) are measured in aqueous solution (containing 10% DMSO), or when they are determined by extrapolation from successive measurements made in different ratios of water-methanol (also containing 10% DMSO). These shifts vary little from sample to sample, and could either be ignored, or applied to the measured result as a correction factor.

Conclusion: pK_a(s) can be measured by the pH-metric method using 50-100µL aliquots of 10mM DMSO sample stocks in 1mL volumes. The shifts in pK_a caused by the DMSO have been quantified.

Sirius T3 instrument, with dispenser module, titrator module and autoloader

Array of four 8 x 6 trays for **Sirius T3**. Each tray holds up to 48 glass vials

SiriusT3 is intended as a direct replacement for Sirius GLpKa and ProfilerSGA, but with all the critical hardware components miniaturized to fit into 1mL solution volumes, and many new features added to make the assays easier to run and more automated. It can measure pK_as, logP, logD, Kinetic Solubility and Equilibrium (thermodynamic) Solubility.

SiriusT3 uses less than 0.5mg of sample for most experiments, and solubility determination now requires 1-2mg instead of 10-20mg.

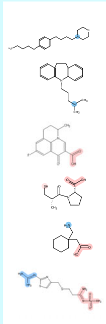
The instrument comprises three hardware modules. The unit on the left is the **Dispenser** module, which houses the precision micro dispensers for adding water, solvents and acid/base titrants from the reagent bottles stored in the drawer below. Also contained within this module is the UV/Vis spectrometer and light source, which is connected to a fibre optic dip probe.

To the right of the Dispensers, the **Titrator** module features a moving arm with the assay probes attached – pH electrode, UV dip probe, stirrer, temperature sensor and capillaries for reagent addition. There is a row of buffer and wash positions used for calibrating and cleaning the probes. This includes a flowing water wash which cleans the probes with fresh water after each experiment. At the front of the titrator is the sample position, which is temperature controlled with a Peltier device (fully controlled by computer), and an additional turbidity sensing device.

The unit on the right hand side, is the **Autoloader**, which has a worktable with four 48-position vial trays. It has a robotic arm which automatically picks up and moves vials to the sample position. At the front of the Autoloader is an ultrasonic bath, which can be automatically used to aid the dissolution of poorly soluble compounds.

Experimental

Six drugs were selected for study. The ionizable groups of four of these (imipramine, captopril, gabapentin and pramoxine) were remote from UV-absorbing chromophores, typical of drugs whose pK_a values cannot be measured by pH-UV methods. Samples were measured by the pH-metric method, under a variety of conditions shown in Table 1. A p_sK_a result is obtained from titration data collected in a water/solvent mixture. All experiments were done at 25±1°C, in solutions with ionic strength adjusted to 0.15M using KCl. As well as p_sK_a measured in water/methanol, pK_a was measured in 0.15M aqueous KCl (with and without 10% DMSO) for the water-soluble compounds (pramoxine, captopril, gabapentin and famotidine).



Sample	pK_a (1)	pK_a (2)	Mean methanol Wt % per pair of points
Pramoxine: base with one pK_a			0 12 21 30 40 50
Extrapolated pK_a, no DMSO	7.123		
Extrapolated pK_a, 10% DMSO	6.906		
ΔpK_a	-0.217		
Imipramine: base with one pK_a			11 21 30 40 50 60
Extrapolated pK_a, no DMSO	9.467		
Extrapolated pK_a, 10% DMSO	9.133		
ΔpK_a	-0.334		
Flumequine: acid with one pK_a			12 21 30 40 50 60
Extrapolated pK_a, no DMSO	6.307		
Extrapolated pK_a, 10% DMSO	6.375		
ΔpK_a	+0.068		
Captopril: acid with two pK_as			0 12 21 31 40 50 60
Extrapolated pK_a, no DMSO	9.807	3.485	
Extrapolated pK_a, 10% DMSO	9.773	3.615	
ΔpK_a	-0.034	+0.13	
Gabapentin: zwitterion with two pK_as			0 12 21 30 40 49 60
Extrapolated pK_a, no DMSO	10.647	3.728	
Extrapolated pK_a, 10% DMSO	10.44	3.82	
ΔpK_a	-0.207	+0.092	
Famotidine: ampholyte with two pK_as			0 12 21 30 38 48 60
Extrapolated pK_a, no DMSO	11.279	6.777	
Extrapolated pK_a, 10% DMSO	11.162	6.512	
ΔpK_a	-0.117	-0.265	

Table 1 Compounds studied and extrapolated pK_a results.

The mean of all extrapolated acidic ΔpK_a values is +0.03 ± 0.10

The mean of all extrapolated basic ΔpK_a values is -0.26 ± 0.06

Results The aqueous pK_a values of poorly water-soluble drugs are determined by Yasuda-Shedlovsky (Y-S) extrapolation from p_sK_a values measured in solutions with different ratios of water to solvent (e.g. water/methanol). In these Y-S extrapolations, $p_sK_a + \log[H_2O]$ is plotted against $1/\varepsilon$, where ε is the dielectric constant of the water/solvent mixture. The aqueous pK_a is obtained by subtracting $\log[H_2O]$ of water (1.755) from the y-axis intercept at $1/\varepsilon$ for water, as illustrated in figures 1a and 1b.

Aqueous pK_a values determined by Yasuda-Shedlovsky extrapolation with and without DMSO present are shown in Table 1.

For each pair of points in Figure 1, the pK_a (or p_sK_a) measured without DMSO was subtracted from the equivalent value measured in the presence of 10% DMSO, and recorded as ΔpK_a, as plotted vs. mean methanol content in figure 2. Note that ΔpK_a values are negative for all basic groups, and smaller and more variable for acidic groups.

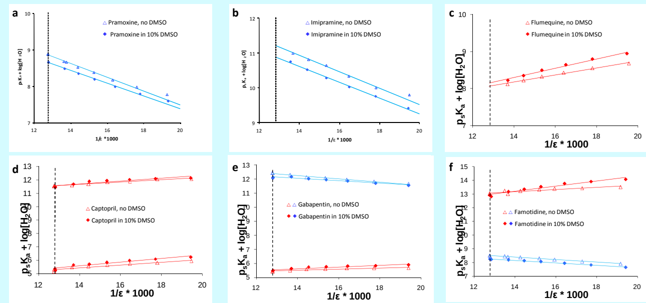


Figure 1 Six graphs showing Yasuda-Shedlovsky plots of data collected with and without 10% DMSO. p_sK_a values for acidic groups are denoted in red. p_sK_a values for basic groups are denoted in blue. Methanol content increases from left to right on the x-axis by approximately 10% between each pair of points.

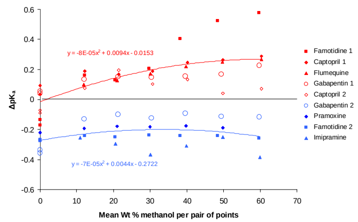


Figure 2 ΔpK_a per pair of measured p_sK_a points.

ΔpK_a values for acidic groups are denoted in red. ΔpK_a values for basic groups are denoted in blue.

The mean of all acidic ΔpK_a values is +0.15 ± 0.15

The mean of all basic ΔpK_a values is -0.23 ± 0.08

Conclusions The goal of this study was to quantify the differences between pK_a and p_sK_a values measured with and without 10% DMSO present. From the extrapolated pK_a values reported in Table 1 and the ΔpK_a values in Figure 2, we draw the following conclusions:

Average ΔpK_a for all acidic p_sK_a values	0.15 ± 0.15	Average ΔpK_a for all basic p_sK_a values	-0.23 ± 0.08
Average ΔpK_a for extrapolated acidic pK_a values	**0.03 ± 0.10**	**Average ΔpK_a for extrapolated basic pK_a values**	**-0.26 ± 0.06**

These differences caused by 10% DMSO are not very large, and may be within acceptable limits for many applications. Alternatively, a correction factor could be applied as above. The practical implications are that pH-metric pK_a measurements can be run in 1mL volumes using 100µL aliquots of 10mM sample in DMSO with the Sirius T3 instrument.

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