

# Fast pK<sub>a</sub> measurements for molecules with UV absorption

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## Summary

A method for fast measurements of pK<sub>a</sub> values has recently been developed. The method is based on the well established Dip Probe Absorption Spectroscopy (D-PAS), a hybrid method combining the traditional pH-metric method with spectroscopic detection. By conducting the titrations in a linear buffer solution with low UV absorbance whose pH was pre-adjusted to an initial pH of 2 or 12, the improved method allows pK<sub>a</sub> measurements in only 4 minutes.

Samples were selected to exemplify different properties commonly found in drug molecules, such as strong or weak UV absorbance, poor aqueous solubility, and instability at extremes of pH. Assays were performed at 25°C. Depending on the initial pH, the samples were titrated with HCl or KOH (0.5M) solutions and the UV spectra were recorded at each pH. Chlorpromazine was titrated in the presence of water-solvent mixtures.

## Experimental

The pK<sub>a</sub> determinations were made using a GLpKa titrator and D-PAS spectrometer, with RefinementPro 2 and Fast D-PAS pKa software (Sirius Analytical Instruments Ltd. East Sussex, UK). The UV absorption of the solution was continuously monitored in the titration vial by a fibre optic dip-probe. Acid and base titrants (0.5 M HCl and KOH) were delivered to the titration vessel through capillaries by precision dispensers capable of delivering reproducible aliquots of known liquid volume. Deionised water (>10<sup>14</sup> Ω cm) was used to prepare all the solutions. Samples were prepared as 10mM stock solutions in DMSO, and aliquots (typically 50µL) were pipetted for each assay into 10mL of 0.15 M KCl solution containing 0.25mL of linear buffer solution. The pH of the sample solution was automatically adjusted to 12 or to 2 before the start of the titration.

The linear buffer solution comprised a mixture of weak acids and bases with low UV absorbances (butylamine, citric acid, potassium dihydrogen phosphate, tris(hydroxymethyl)methylamine and ethanolamine). The precise concentration of each ingredient is proprietary information. With the buffer present in the sample solution, the buffer capacity of the solution was almost constant across the entire pH range of the titration.

During each titration, acid or base titrant was added in specified volume increments (typically 0.02 mL between pH 3 and 11, larger increments outside this pH range). Because the pH electrode reaches a stable value very quickly in buffer solution, the pH readings and UV spectra were taken after short, fixed time intervals (typically every 3 seconds). UV intensity was recorded at 256 wavelengths at each pH point. UV absorbances were calculated after subtracting reference. pK<sub>a</sub> values were calculated by Target Factor Analysis (TFA).

Reference spectra were obtained by titrating sample blanks (0.15M KCl + buffer) from low to high or high to low pH. They are saved in software and the appropriate reference (low – high pH, high – low pH) is subtracted from each sample data set.

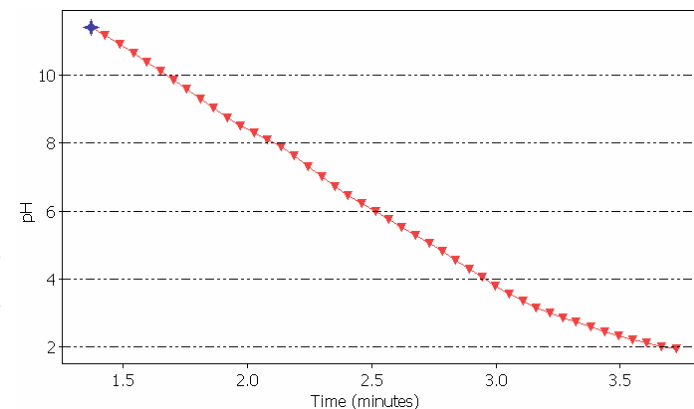


Figure 1. Titration curve obtained in the presence of linear buffer. Sample is furosemide.

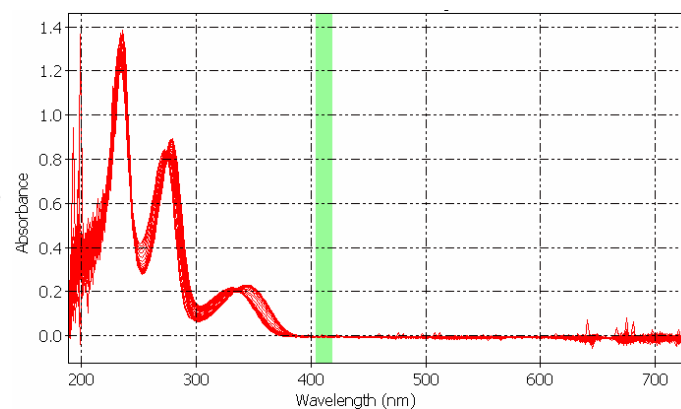


Figure 2. Absorbance vs. wavelength, based on spectra measured at each pH point in above titration.

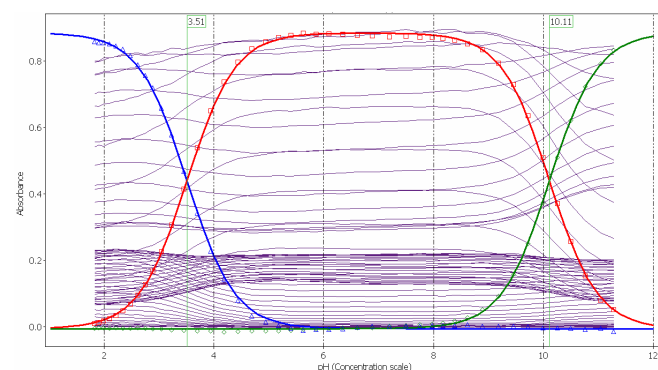


Figure 3. Absorbance vs. pH, with distribution of species superimposed. Points represent measured data; lines calculated from pKas determined by TFA.

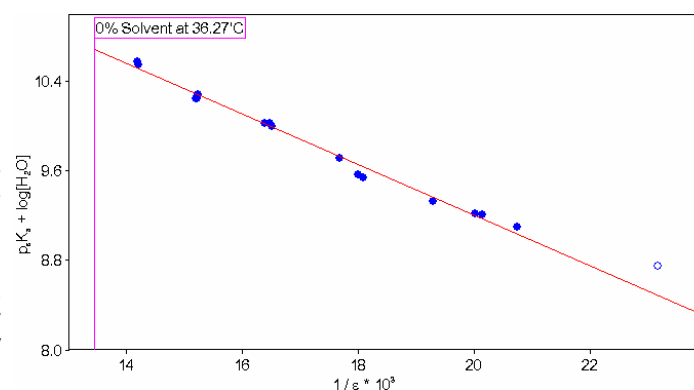


Figure 4. Poorly water-soluble molecule (chlorpromazine) measured at 37°C in water-methanol solutions (10% -60% methanol). Result by Yasuda-Shedlovsky extrapolation

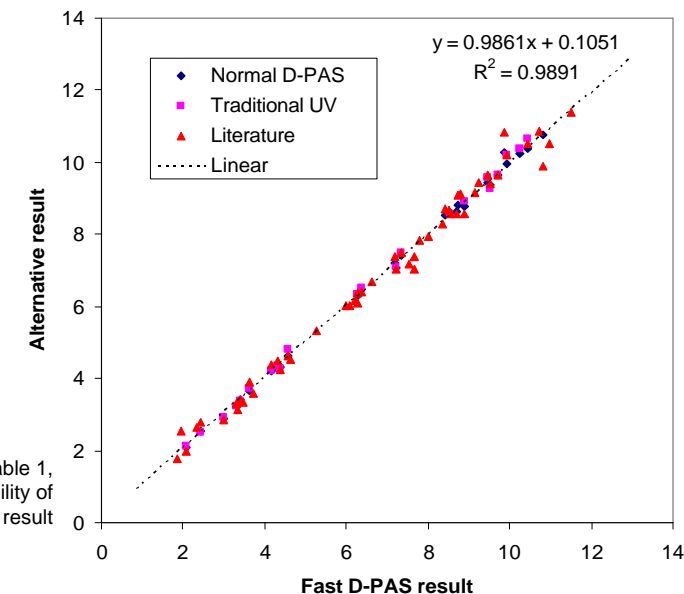


Figure 5. Results from Table 1, confirming the reliability of Fast D-PAS result

	Fast D-PAS	Normal D-PAS	Traditional UV	Literature	Ref.
3-Aminobenzoic acid	3.35 4.62			3.15 4.53	1
3-Chlorophenol	8.80			9.11	2
4-Aminosalicylic acid	1.86 3.73			1.79 3.58	3
4-Nitrocatechol	6.61 10.72			6.69 10.85	4
Amodiaquine	7.65 8.51 11.91			7.05 8.66	5
Benzocaine	2.43	2.53	2.50	2.78	6
Chlorpromazine*	9.14			9.17	7
Ciprofloxacin	6.20 8.59			6.16 8.62	8
Diazepam	3.42	3.42	3.38	3.40	6
Enrofloxacin	6.08 7.79			6.04 7.83	9
Ephedrine	9.70	9.66	9.65	9.64	10
Flumequine	6.26	6.31	6.35	6.38	10
Furosemide	3.63 10.25	3.65 10.24	3.74 10.37	3.90	6
Galic Acid	4.16 8.43 11.52 13.00	4.21 8.54	4.20	4.40 8.70 11.40	11
Hexachlorophene*	9.86	5.21 10.27		4.89 10.83	19
Hydrochlorothiazide	8.88 9.94	8.78 9.96	8.90 10.20	8.57 10.22	10
Labetalol	7.35 9.53	7.41 9.36	7.49 9.27	7.48 9.39	10
Nicotinic Acid	2.09 4.57	2.10 4.63	2.14 4.82	2.00 4.63	13
Nitrazepam	2.98 10.43	2.90 10.39	2.94 10.66	2.84 10.51	10
Ofloxacin	6.00 8.36			6.01 8.30	9
Oxytetracycline	3.30 7.19 8.74	3.23 7.22 8.82	3.25	3.30 7.40 9.10	6
Papaverine	6.38	6.47	6.51	6.40	6
Paracetamol	9.45	9.45	9.58	9.63	10
Pefloxacin	6.26 7.66			6.11 7.37	9
Phenylbutazone	4.31	4.30	4.34	4.50	6
Pilocarpine	7.23	7.06	7.12	7.05	6
Piroxicam	1.95 5.28			2.53 5.32	9
Quinine	4.39 8.53	4.33 8.59	4.30 8.56	4.24 8.55	10
Sulfasalazine	2.33 8.00 10.98			2.65 7.95 10.51	8
Terbutaline	8.69 10.83	8.64 10.76		8.57 9.89 11.00	14
Tetracycline	3.47 7.55 9.23			3.33 7.16 9.43	9

Table 1. Measurements of 31 compounds by Fast D-PAS pK<sub>a</sub> method

\* Aqueous result obtained by extrapolating apparent pK<sub>a</sub>s measured in water-methanol solutions, as in Figure 4 example

## Discussion

Results in Table 1 show good agreement between Fast D-PAS and other methods for measuring pK<sub>a</sub>.

Chlorpromazine pK<sub>a</sub> was measured at 37°C. The fast method minimizes volume changes caused by evaporation of methanol during experiments.

Galic acid decomposes at high pH, and the higher pK<sub>a</sub>s cannot be reliably measured in slower experiments.

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