Validation of titrimetric measurement of pK_a, logP and solubility using sub-mg quantities of sample

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Purpose

Titrimetric methods for measuring pK_a, logP and intrinsic solubility (S_0) in drug development are powerful and accurate. However it has become increasingly difficult to apply them as the weight of sample available for analysis has decreased. To overcome these problems we developed an instrument (SiriusT3) with miniaturized sensors that performs automatic titration in 1 mL of solution. This enables titrimetric measurements using sub-mg sample weights, or samples pipetted from DMSO stock solutions. This poster validates the new instrument by measuring pK_a, logP and solubility of 20 drug molecules, and comparing results with previously validated measured results. This will be the first published study carried out under routine laboratory conditions.

Methods

20 ionizable drugs were chosen from the set of 24 physicochemically diverse molecules identified at Uppsala University [1]. The methods used were as far as possible identical to those described in the publication, but with sample weights on average 10 times lower. pK_a values of most of the samples were measured by Fast UV methods in four to five minutes using 0.01 mg of sample or less. LogP values, and pK_a values for drugs without chromophores were measured pH-metrically using typically 0.5 mg of sample. Solubilities were measured pH-metrically using typically 1 mg of sample.

Results

In this section we describe experimental optimization. pK_a, logP and $logS_0$ are all logarithmic terms, and final results are presented as log-log graphs fitted with linear functions showing slope, intercept and CV.

Conclusion

This poster shows that measurements of pK_a, logP and solubility of ionizable drugs in sub-mg weights by pH and UV titrimetric methods compare well with published values.

Reference

The compounds measured in this study were selected from: [1] *Sköld, C. et al. Presentation of a Structurally Diverse and Commercially Available Drug Data Set for Correlation and Benchmarking Studies. J. Med. Chem. 2006, 49(23), 6660-6671.* This paper proposed 24 easy-to-obtain drugs (20 ionizable, 4 non-ionizable) with diverse properties for testing assay procedures. Nearly all pK_a, logP and solubility values in the paper were measured by Sirius GLpKa and D-PAS instruments.

Right: stirrer, electrode, temp. probe, capillaries & UV probe above vial on SiriusT3.

Above: Vial used on SiriusT3 holds 1 – 2 mL of solution; GLpKa vial holds 10 – 20 mL.

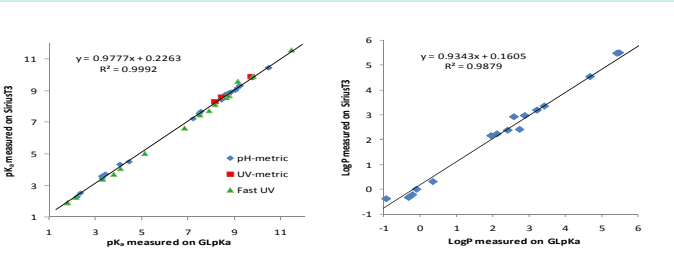
Above: SiriusT3 single sample version

Results

All pH-metric pK_a and logP measurements on SiriusT3 were made using less than 1.0 mg of solid sample.

UV-metric and Fast UV pK_a measurements used 3 µL aliquots of 10 mM stock solution in DMSO (typically about 0.01 mg of sample).

Solubility measurements require higher weights because samples must precipitate. Glipizide and bendroflumethiazide solubility required 0.94 mg and 2.18 mg of sample. Hydrochlorothiazide (a high solubility compound that supersaturates before precipitating) required 17.7 mg.

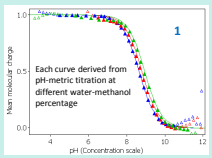


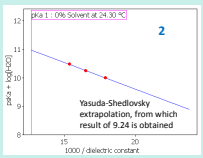
	pK_a (all methods)	pH-metric pK_a	UV-metric pK_a	Fast UV pK_a	LogP		Solubility	µg/mL
	GLpKa	SiriusT3	SiriusT3	SiriusT3	GLpKa	SiriusT3	GLpKa	SiriusT3
Amantadine	10.49	10.42			2.41	2.37	2119.0	2046.0
Amiloride	8.63	8.75	8.58	8.60	-0.32	-0.34		
Bendroflumethiazide	8.46, 9.74	8.39, 9.88	8.58, 9.88		1.95	2.15	19.6	27.8
Captopril	3.45, 9.80	3.71, 9.80			0.35	0.30		
Chlorprothixene	9.06	9.00			5.48	5.49	0.2	0.2
Chlorzoxazone	8.16		8.29	8.11	2.11	2.22	416.0	246.0
Erythromycin	8.87	8.89			2.88	2.96		
Flupenthixol	3.29, 7.57	3.58, 7.66			4.68	4.53	41.8	40.3
Folic acid	2.16, 3.79, 4.47, 7.90	4.49		2.24, 3.70, 7.72			2.3	1.4
Glipizide	5.13			5.02	2.58	2.91	1.4	1.5
Hydrochlorothiazide	8.77, 9.79	8.76, 9.93		8.68, 9.90	-0.10	-0.01	629.0	617.1
L-dopa	2.38, 8.75, 9.76	2.51, 8.82, 9.80						
L-thyroxine	6.84, 8.72			6.63, 8.69	3.21	3.18	42.8	43.0
Meclizine	2.23, 7.24	2.28, 7.20			6.20	5.48	0.1	0.1
Metoclopramide	9.25	9.29			2.74	2.40	75.5	82.0
Sulindac	4.08	4.32		4.06	3.42	3.34	10.8	10.7
Terfenadine	9.25	9.28			5.42	5.47	0.009	
Tetracycline	3.31, 7.50, 9.14	3.46, 7.53, 9.14		3.40, 7.37, 9.58	<0	<0	440.0	448.0
Thiamazole	11.45			11.58	-0.22	-0.23		
Tinidazole	1.8			1.9	-0.94	-0.39		

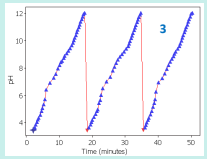
Case study: Metoclopramide pH-metric pK_a

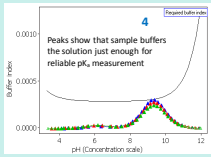
1, 2. 0.71 mg of sample titrated in water-methanol (52%, 42%, 32%), result 9.24 obtained by Yasuda-Shedlovsky extrapolation. Experiment time 66 min.

3,4. 0.16 mg of sample titrated in aqueous solution (0.15 M KCl), three titrations from low to high pH, result 9.29 obtained. Experiment time 51 min.



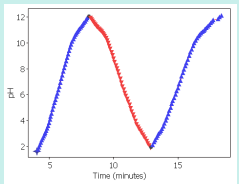


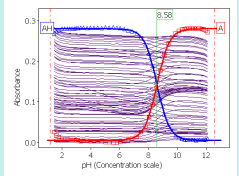




Case study: Amiloride Fast UV pK_a

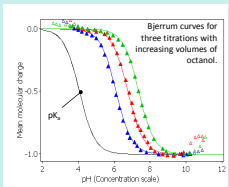
3 µL of 10 mM stock solution of amiloride in DMSO was placed in a vial with 25 µL of linear buffer solution and made up to 1.5 mL with 0.15 M KCl. Solution titrated with 0.5 M KOH, then with 0.5 M HCl, then again with KOH. pK_a obtained by analysis of multiwavelength UV spectra vs. pH. Each titration takes approx. 4 minutes; reported result 8.60 is mean of three.

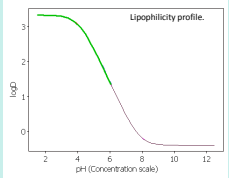




Case study: Sulindac pH-metric logP

0.72 mg of sample was titrated in 1.0 mL of 0.15 M KCl plus 0.05 mL of octanol. After the first titration, 0.2 mL of octanol was added before a second titration, and 1.25 mL of octanol was added before a third titration. logP result of 3.34 was obtained by curve-fitting. Performing three titrations enables logP of -0.38 for the anion also to be determined.





Case study: Hydrochlorothiazide CheqSol solubility

17.7 mg of hydrochloro-thiazide was dissolved at high pH in 1.0 mL of 0.15 M KCl and titrated with 0.5 M HCl until precipitation was detected. Thereafter, a solubility result of 617 µg/mL (2.07 mM) was measured by the CheqSol method.

1. Titration curve. Shaded areas denote pH where solid is present.

2. Neutral Species Concentration Profile, showing how sample supersaturates significantly before precipitation.